Modification of Cuticular Hydrocarbons of *Cryptotermes brevis* (Isoptera: Kalotermitidae) in Response to Temperature and Relative Humidity

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ABSTRACT To assess their ability to modify cuticular hydrocarbon (CHC) composition and survive adverse conditions, *Cryptotermes brevis* (Walker) nymphs were subjected to various combinations of temperature and relative humidity. Cuticular hydrocarbon profiles of *C. brevis* were consistent with previous studies. Alkenes were the most prevalent in the CHC mixture, comprising 54.5% of the total hydrocarbon (n = 12), whereas n-alkanes and branched alkanes comprised 24.8 and 6.3%, respectively. Sixteen compounds yielded >2% of the total hydrocarbon and were subsequently tested for temperature and humidity effects in two successive experiments. In both experiments, temperature effects were found: n-C29 increased and n-C25 decreased with increasing temperature. Similarly, five compounds (n-C25, n-C27, n-C29, C39:2, and C41:2) were analyzed for relative humidity effects in experiment 2, based on the results of experiment 1; only C41:2 indicated a significant positive relative humidity effect. The remaining 11 compounds comprising >2% of the total hydrocarbon were tested, and a single statistically significant increase was found with C45:3 with increasing temperature. Significant positive effects were found with total alkenes, dienes, and trienes; relative humidity had the opposite effect on total n-alkanes. In both experiments warm, damp conditions were detrimental to survival. Overall, CHC modification was minor; and given that *C. brevis* has numerous adaptations for dealing with desiccation and an inability to tolerate high relative humidity it suggests that this species may not vary widely from a highly desiccation-tolerant state.

KEY WORDS drywood termite, cuticular hydrocarbons, temperature, relative humidity, control

THE WEST INDIAN DRYWOOD TERMITE *Cryptotermes brevis* (Walker) has a worldwide distribution and is primarily restricted to artificial structures (Williams 1977a). This termite's ability to exploit the urban environment has made it one of the most ubiquitous and pestiferous termites worldwide (Gay 1969, Edwards and Mill 1986). Apart from dispersal facilitated by humans, termite distributions are predominantly shaped by temperature and relative humidity (Emerson 1955, Calaby and Gay 1959, Rudolph et al. 1990). Because insects, including termites, have a large surface to volume ratio and an exoskeleton made of chitin, a material permeable to water, they are therefore particularly susceptible to desiccation (Hadley 1985, Blomquist et al. 1987). In fact, the cuticle represents the most significant potential source of water loss affecting the survival of terrestrial arthropods (Edney 1977).

The outermost layer of the exoskeleton, the epicuticle, is the primary barrier to water, and underlying layers of the cuticle confer structural stability and strength (Kuhnelt 1928, Beamont 1945, Edney 1977, Noble-Nesbitt 1991). Numerous filaments of wax penetrate the epicuticle, which extend to pore canals in the procuticle and then continue into the epidermis (Locke 1965, Noble-Nesbitt 1991). Various researchers have hypothesized that if not covered by this lipid layer, the porous cuticle would allow free diffusion of water out of the body, leading to rapid desiccation. Beamont (1945) and Wigglesworth (1945) demonstrated that abrasive dusts disrupt the wax covering and cause rapid desiccation. Locke (1965) used peanut oil as a solvent for cuticular wax and found that treated insects rapidly lost water.

Similar desiccation experiments have been performed with termites. Collins (1969) studied the epicuticle of drywood termites (Kalotermitidae) treated with alumina (an abrasive) and peanut oil and found marked and dramatic increases in rates of water loss, as high as ≈40 mg/cm^2/h in comparison to untreated termites. Sponsler and Appel (1990) measured the cuticular permeability of *Coptotermes formosanus* Shiraki and *Reticulitermes flavipes* (Kollar) before and after immersion in hexane to remove surface lipids. In that study, cuticular water losses were as much as 1.8 times greater in individuals without cuticular lipids.

The environment can also affect epicuticular permeability. Relative humidity affects the saturation deficit, or potential for water loss, whereas temperature

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alters the phase of the lipid component of the epicuticular wax layer (Edney 1977). It is well established that insects are able to acclimate to changes in microclimate by modifying the gross amount of the cuticular wax layer. In a study of the tenebrionid *Eleodes armata* (Blaisdell) Hadley (1977) found seasonal differences in the quantity of cuticular lipid. Beetles collected during the summer months had greater amounts of cuticular lipids than those collected during the winter. Hadley also found that winter-collected beetles, when placed in warm desiccating conditions, developed increased amounts of the hydrocarbon component of the cuticular lipid, whereas amounts in summer-collected individuals decreased. McClain et al. (1985) also found that as the average mean temperature increased and relative humidity decreased, the amount of surface wax increased, causing lower transpiration rates in beetles that were acclimated.

The cuticular lipid contains a wide range of organic compounds including fatty acids, alcohols, esters, ketones, glycerides, sterols, aldehydes, and hydrocarbons (Blomquist et al. 1987, Lockey 1988). However, hydrocarbons are the primary components of the lipid layer that are responsible for waterproofing the cuticle (Hadley 1978, 1985). Hydrocarbons predominate in the insect cuticle, with abundance >90% in some cases (Blomquist et al. 1987, Lockey 1988). Numerous authors have documented acclimation effects upon the relative amounts of cuticular hydrocarbons (CHC) in response to changes in temperature and relative humidity (Hadley 1977, 1978; Toolson and Hadley 1977, 1979; Gibbs and Mousseau 1994; Howard et al. 1995).

The extreme desiccation tolerance of the genus *Cryptotermes* Banks is well documented (Minnick et al. 1973; Steward 1981, 1982; Rudolph et al. 1990). The desiccation-tolerance of this species could be caused by any number of physiological mechanisms, including modified rectal pads (Noirot and Noirot-Timothee 1977), metabolic water production (Williams 1977a, Steward 1983), and water imbibition (Rudolph et al. 1990). In addition, a number of behavioral mechanisms have been discussed in the literature, including avoidance (Steward 1981, 1982; Cabrera and Rust 1996), clumping (Sen-Sarma 1964, Collins 1969, Minnick et al. 1973, Steward 1982), altruistic trophallaxis (Sen-Sarma 1964, Minnick et al. 1973), altruistic sacrifice (Collins 1991), and spiracular regulation (Sen-Sarma 1964). Given these acclimatory abilities, it is also possible that, like many other xerically adapted insects, *Cryptotermes* spp. are able to modify the epicuticular hydrocarbon composition to minimize water loss under xeric conditions.

Acclimation to extremes of temperature and relative humidity is of particular interest with *C. brevis* because of the commercial use of heat to control this species and other drywood termite species (Lewis and Haverty 1996, Woodrow and Grace 1998a). Woodrow and Grace (1998b) reported that there was no evidence for a short-term acclimation effect from slow rates of thermal increase typical during commercial heat treatments. However, little or nothing is known about potential increases in thermal tolerance as a result of long-term acclimation effects.

There has also been a great deal of interest in the characterization of termite CHC profiles for taxonomic purposes (Howard et al. 1978, 1982; Blomquist et al. 1979; Haverty et al. 1988, 1990a, 1990b, 1991, 1992, 1996; 1997; Haverty and Thorne 1989; Watson et al. 1989 Bagneres et al. 1990; Brown et al. 1990; Collins et al. 1997; Haverty and Nelson 1997). If changes in CHC composition are induced by environmental conditions, this could have profound implications on the use of CHC data in phylogenetic reconstruction.

Despite a lack of information on CHC acclimation in termites, there have been some comparative field studies and observations. Collins et al. (1997) recently compared the CHC profiles of various termites collected under different climatic conditions and found a predominance of long chain hydrocarbon components in xerically adapted species. They hypothesized that desiccation tolerance could be attributable to higher proportions of long-chain compounds found in these species.

The current studies were initiated to determine the effect of various combinations of temperature and relative humidity on the CHC profiles and survival of *C. brevis* nymphs. In both studies, termites were allowed to acclimate to various combinations of temperature and relative humidity for up to 3 wk, after which CHC compounds were extracted, identified, and quantified using gas chromatography-mass spectrometry (GC-MS). Resulting relative quantity data were analyzed to determine the impact of temperature and relative humidity on individual CHC compounds and classes of compounds and to examine possible correlation with termite survival.

Materials and Methods

Extraction and Preparation of Termites. Lumber infested with *C. brevis* was collected from a warehouse in Foreign-Trade Zone No. 9, Honolulu, HI. Termites were extracted from infested wood by splitting the wood with hatchet and hammer and separating termites from debris using an aspirator. Termites were placed into small plastic jars with Kimwipes (Kimberley-Clark, Roswell, GA) and hardwood tongue depressors (Fisher, Pittsburgh, PA) as food sources. The jars were placed in a Precision model 815 incubator (Precision Scientific, Chicago, IL) at 28 ± 0.5°C and allowed to acclimate for a minimum of 1 mo before testing.

Experiment 1. Groups of 20 third-instar, or older, nymphs, as determined by size, were placed into each of 24 small (15 cm) glass petri plates, each containing a 5.5-cm-diameter Whatman #2 (Whatman, Hillsboro, OR) filter paper disk. The petri plates were placed on top of a 50-ml beaker inside of 500-ml glass Qorpak jars, 12 of which contained 100 ml of saturated NaCl solution, and the remaining contained 100 g Drierite ($CaSO_4$) to maintain either 75 or 0% RH, respectively. After tightly covering with lids, half of the jars in each relative humidity treatment were held in one of two

Fisher model 146A low temperature incubators (Fisher, Pittsburgh, PA) at either 28 or 33°C (±1.0°C) for 3 wk. The jars were opened once each week to introduce fresh air. At the end of the 3-wk acclimation period, mortality was assessed. Dead individuals were removed from the jars and the six replicates were combined into three replicates of each treatment combination to have sufficient numbers for solvent extraction. Data were analyzed using an analysis of variance (ANOVA) model (PROC GLM, SAS Institute 1985) to determine the effects of temperature and relative humidity on percent mortality. Before testing, model residuals were analyzed to verify that model assumptions had been met.

Experiment 2. Groups of 30 nymphs were placed into petri plates in Qorpak jars as described for experiment 1. In this experiment we used saturated salt solutions to maintain four variations of relative humidity. In addition to the previously described NaCl and Drierite, we used saturated MgCl2 for 33% RH and distilled water for 100% RH. As in the previous experiment we used two separate, but different, temperatures, 25 and 35°C, maintained in the same incubators as in experiment 1. There were four replicates of each combination of temperature and relative humidity, each assigned to different 1-wk-exposure blocks. Each week, temperature was randomly assigned to the two incubators. After the 1-wk exposure, termites were removed from the jars, mortality was assessed, and the still-living termites in units with >20 individuals were prepared for CHC analysis.

CHC Analysis. Specimens were first killed by freezing, then dried in a desiccator. Cuticular lipids were extracted from the specimens by placing them in 10 ml *n*-hexane (EM-Science "OmniSolv") for 10 min. The extracts were pipetted through 4 cm of activated BioSil A (silica gel, 100–200 mesh, Bio-Rad Laboratories, Hercules, CA) in 5-mm (i.d.) Pasteur pipette minicolumns to isolate CHC components. The extracts were then evaporated to dryness under nitrogen and redissolved in 60 μl of *n*-hexane for GC-MS analysis.

Gas chromatographic-mass spectrometric analysis was performed as described by Haverty et al. (1996). Integration of the total ion chromatogram was performed by data analysis software (HP59974J Rev. 3.1.2) in a Hewlett-Packard Chemstation computer. GC-MS peak areas were converted to percentages of the total hydrocarbon fraction. Compounds representing <1% of the total were deleted from the data before statistical analysis. Percentages of each hydrocarbon were analyzed by ANOVA (PROC GLM, SAS Institute 1985) to determine the effect of the experimental factors, temperature, and relative humidity (and interactions) on the relative abundance of each compound, as well as upon combined classes of compounds: alkanes, alkenes, internally branched monomethylalkanes, monoenes, dienes, and trienes, and combinations of size classes of alkene (C37, C39, C41, and so on). Tests were done to determine if any relationship existed between the CHC compounds and classes and pooled (n = 3) percentage survival. An overall alpha level of P = 0.05 was chosen for the various hypotheses made during the first study. Individual alpha levels were derived by dividing the overall alpha by the total number of individual compounds and combined classes of compounds being tested (Haverty et al. 1996). In the second study more selective hypotheses were tested based on the results of the first study.

In the text and tables, a shorthand is used to denote individual CHC compounds. A descriptor is used to denote the total number of carbons in the parent chain (CXX), the location of methyl groups (X-me), and the number of double bonds (CXX:Y). Under this shorthand, *n*-octane becomes *n*-C8, 3-methyl octane becomes 3-meC8, and octadiene becomes C8:2.

Results

Cuticular hydrocarbon profiles of *C. brevis* were consistent with the results of Haverty et al. (1997) and contained a mixture of *n*-alkanes, terminally and internally branched monomethylalkanes, terminally branched dimethylalkanes, and olefins (alkenes, alkadienes, alkatrienes) (Table 1; Fig. 1). Alkenes were most prevalent in the 12 CHC samples, comprising 54.5%, whereas *n*-alkanes and branched alkanes comprised 24.8 and 6.3%, respectively. Compounds eluted in GC-MS chromatograms in two predominant groups. The low molecular weight (C23 to C29) group which consisted of *n*-alkanes with some terminally branched monomethylalkanes and dimethylalkanes. A second group of late eluting high molecular weight compounds from 37–45 carbons in length, which contained mostly alkenes with some internally branched monomethylalkanes that co-eluted with even-numbered dienes (C38:2, C40:2, C42:2, C44:2).

Experiment 1. There were 16 compounds that yielded >2% of the total hydrocarbon (Table 1); thus, alpha levels for individual hypotheses were set at P = 0.003 (overall alpha level/number of concurrent hypotheses 0.05/16). Based on this alpha level, there were no significant effects on any of the compounds; although for purposes of further study some notable changes did occur. Although there was no effect of temperature and relative humidity on the presence or absence of individual compounds, quantitative differences were apparent with some compounds. Based on a decision level of P = 0.10, there were apparent relative humidity effects on *n*-C25, *n*-C27, *n*-C29 and C41:2, and C39:2, whereas the effect of temperature was only evident for *n*-C25 and *n*-C29 (PROC GLM, SAS Institute 1985) (Table 2). There were increases in the mean percentage of *n*-C25, *n*-C27, *n*-C29, and C39:2, whereas the mean percentage of C41:2 increased, with decreased relative humidity (Table 2). Increased temperature resulted in an increase in the mean percentage of *n*-C25, whereas *n*-C29 decreased (Table 2).

Another series of seven hypotheses were tested when compounds were combined into the classes total *n*-alkanes, total alkenes, monoenes, dienes, trienes, branched alkanes, as well as combinations of size classes: C39, C41, C43, C45. An overall alpha level of 0.05

Table 1. Mean (n = 3) percentages of cuticular hydrocarbon compounds with abundance greater than 2% extracted from *C. brevis* nymphs maintained for 3 wk at four combinations of relative humidity and temperature

Hydrocarbon[a]	Mean ± SEM			
	28°C / 0% RH	33°C / 0% RH	33°C / 75% RH	28°C / 75% RH
2MEC24	1.89 ± 0.16	2.03 ± 0.18	1.85 ± 0.13	1.90 ± 0.27
n-C25	11.68 ± 1.14	7.08 ± 0.77	6.61 ± 0.89	7.94 ± 0.16
3MEC25	4.02 ± 0.11	4.49 ± 0.06	4.57 ± 0.44	4.57 ± 0.43
n-C27	14.10 ± 1.09	14.70 ± 1.75	10.80 ± 1.32	10.99 ± 0.47
n-C29	2.40 ± 0.42	3.87 ± 0.54	2.02 ± 0.29	1.52 ± 0.08
C37:2	3.90 ± 0.85	4.35 ± 0.71	4.71 ± 0.74	4.62 ± 0.33
C39:2	9.20 ± 0.81	10.66 ± 1.37	12.36 ± 0.18	11.93 ± 0.03
C39:1	3.68 ± 0.16	3.48 ± 0.23	5.94 ± 2.27	3.73 ± 0.14
C41:3	4.33 ± 1.1	5.56 ± 0.61	6.24 ± 0.92	6.58 ± 0.27
C41:2	7.36 ± 1.13	8.32 ± 0.72	10.14 ± 0.84	9.56 ± 0.40
C41:1	3.86 ± 0.36	3.44 ± 0.18	3.57 ± 0.20	3.38 ± 0.14
C43:3	2.49 ± 0.50	2.66 ± 0.10	2.70 ± 0.33	3.24 ± 0.08
C43:2	3.95 ± 0.47	3.50 ± 0.09	3.90 ± 0.22	4.04 ± 0.18
C43:1	3.78 ± 0.68	3.42 ± 0.55	2.72 ± 0.32	2.71 ± 0.24
C45:3	2.03 ± 0.39	1.89 ± 0.16	1.56 ± 0.13	2.17 ± 0.12
C45:2	3.17 ± 0.55	2.54 ± 0.20	2.32 ± 0.13	2.76 ± 0.25

[a] A descriptor is used to denote the total number of carbons in the parent chain (CXX), the location of methyl groups (X-me), and the number of double bonds (CXX:Y). Under this shorthand, *n*-octane becomes *n*-C8, 3-methyl octane becomes 3-meC8, and octadiene becomes C8:2.

was chosen for this series of hypotheses, thus individual tests could not exceed $P = 0.005$ (0.05/10). As in the first series of hypothesis tests, no significant differences were found; however, notable differences were found for both alkenes and alkanes with changes in relative humidity. The mean for the total alkane decreased from 28.47 to 21.21% ($P = 0.008$), whereas the total alkene increased from 51.2 to 57.69% ($P = 0.008$), at the 0 and 75% humidity levels, respectively.

Percent mortality data indicated that temperature had a significant effect ($F = 6.61$; df = 1, 21; $P = 0.018$) on percent survival, whereas relative humidity effects were not significant ($F = 2.18$; df = 1, 21; $P = 0.154$), with 44.58% mean survival for termites held at 33°C and 64.17% for those at 28°C.

Experiment 2. The ANOVA model of the percent survival data, including the factors temperature, relative humidity, and their interaction, was highly significant ($F = 7.89$; df = 7, 24; $P = 0.0001$), although the interaction was not significant (type III F-test; $F = 2.63$; df = 3, 24; $P = 0.073$). Without the interaction, the model remained significant ($F = 10.01$; df = 7, 24; $P = 0.0001$) with relative humidity and temperature having significant effects on survival in added last F-tests ($F = 10.19$; df = 3, 24; $P = 0.0001$) and ($F = 9.5$; df = 1, 24; $P = 0.005$), respectively. Mean survival was significantly higher at 25°C (76.87%) than at 35°C (53.50%). The only significant difference, as evident from individual mean comparisons (Tukey–Kramer mean separation, SAS Insitute 1985), was the 100% RH condition, which was significantly different than the other relative humidity levels ($P < 0.005$) (Fig. 2). Mortality within the 100% RH treatment was so great that it had to be dropped from the CHC analysis altogether.

Regression models (PROC GLM, SAS Institute 1985) containing temperature and relative humidity indicated that temperature had effects on some CHC compounds; however, the interaction of relative humidity and temperature was not significant. This model was consistent with the previous experiment. Based on a decision level of $P = 0.10$ from the results of experiment 1 we tested *n*-C25 and *n*-C29 for tem-

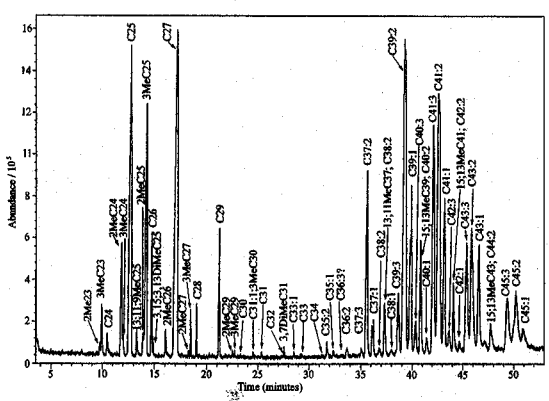

Fig. 1. Total ion chromatogram of the cuticular hydrocarbons from *C. brevis* nymphs maintained for 3 wk at 75% RH and 28°C.

Table 2. Mean (n = 6) percentages of individual cuticular hydrocarbons extracted from *C. brevis* nymphs maintained for 3 wk under all possible combinations of two temperatures and two relative humidities

Compound	Relative humidity			Temp, °C		
	0%	75%	P^a	28	33	P^a
n-C25	9.38	7.27	0.053	9.81	6.85	0.012
n-C27	14.39	10.90	0.011[b]	12.55	12.75	0.878
n-C29	3.14	1.77	0.007	1.96	2.95	0.031
C39:2	12.15	9.93	0.042[b]	10.57	11.51	0.344
C41:2	7.84	9.85	0.026[b]	8.46	9.23	0.371

[a] Probability generated in type III F-test (PROC GLM, SAS Institute 1985).

[b] Probability generated in ANOVA model without temperature.

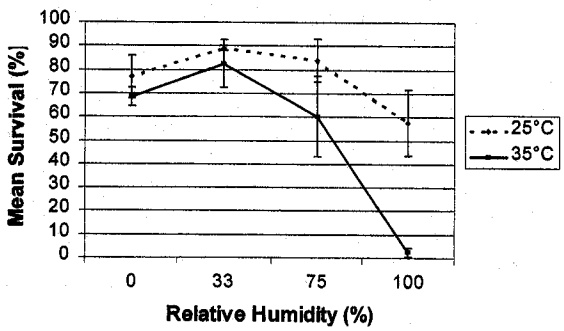

Fig. 2. Mean percentage mortality of *C. brevis* nymphs maintained for 1 wk at various combinations of temperature and relative humidity. The combination of 35°C and 100% RH was significantly different from all other combinations ($P < 0.05$; Tukey–Kramer mean separation test; SAS Institute 1985).

perature effects. Based on an individual alpha level of 0.025 or 0.05/2) an increase in temperature significantly increased *n*-C29 ($F = 30.03$; df = 1, 23; $P = 0.0001$), whereas *n*-C25 was marginally decreased ($F = 5.47$; df = 1, 23; $P = 0.029$) (Table 3). Similarly, we examined *n*-C25, *n*-C27, *n*-C29, C39:2, and C41:2 for relative humidity effects based on the results of experiment 1. For these tests the individual alpha level was set to 0.01 (0.05/5) for determining significance. Based on this significance level, only a single compound, C41:2, indicated a significant relative humidity effect; the highest relative amount was found at 75% RH (Table 4).

The remaining 11 compounds comprising >2% of the total fraction were tested using an individual alpha level of 0.005 (0.05/11). A statistically significant relative increase in was found with C45:3 ($F = 10.82$; df = 1, 23; $P = 0.004$) with increased temperature. Although nonsignificant, a notable decrease in C45:2 was found at the higher temperature (Table 3), whereas C41:3 increased and C45:2 decreased with increasing relative humidity (Table 4). Individual compounds were combined into classes of compounds as previously described in experiment 1. In relation to increasing relative humidity, significant increases were found in total alkenes as well as including dienes and trienes ($F = 8.34$, 5.83, and 8.44; df = 1, 23; $P = 0.008$, 0.024, and 0.008, respectively) in addition to a negative effect

Table 3. Effects of temperature on the mean ($n = 16$) percentages of cuticular hydrocarbons of *C brevis* nymphs that were allowed to acclimate for 1 wk

Compound	P^a	Mean ± SEM	
		25°C	35°C
n-C25	0.029[b]	10.20 ± 0.52	8.69 ± 0.26
n-C29	0.000[b]	1.15 ± 0.12	2.71 ± 0.24
C45:3	0.004[c]	2.24 ± 0.09	1.61 ± 0.14
C45:2	0.011[c]	3.11 ± 0.23	2.44 ± 0.22

[a] Probability generated in type III *F*-test (PROC GLM, SAS Institute 1985).
[b] Individual alpha level of 0.025.
[c] Individual alpha level of 0.005.

Table 4. Effects of relative humidity on the mean ($n = 8$) percentages of cuticular hydrocarbons of *C. brevis* nymphs that were allowed to acclimate for 1 wk

Compound	P^a	Mean ± SEM		
		0%	33%	75%
n-C25	0.211[b]	9.90 ± 0.41	8.99 ± 0.32	9.03 ± 0.34
n-C27	0.126[b]	10.94 ± 0.44	9.86 ± 1.71	9.62 ± 0.32
n-C29	0.029[b]	2.30 ± 0.34	1.96 ± 0.21	0.83 ± 0.20
C39:2	0.041[b]	10.00 ± 0.35	10.92 ± 0.33	11.29 ± 0.23
C41:2	0.006[b]	8.24 ± 0.26	8.96 ± 0.27	9.72 ± 0.24
C41:3	0.009[c]	5.51 ± 0.34	5.82 ± 0.29	6.74 ± 0.31
C45:2	0.074[c]	2.99 ± 0.28	2.86 ± 0.22	2.45 ± 0.22
Total *n*-alkane	0.046[d]	24.85 ± 1.21	22.30 ± 1.24	21.70 ± 1.00
Total alkene	0.008[d]	49.51 ± 1.50	53.37 ± 1.40	54.28 ± 0.69

[a] Probability generated in type III *F*-test (PROC GLM, SAS Institute 1985).
[b] Individual alpha level of 0.01.
[c] Individual alpha level of 0.005.
[d] Individual alpha level of 0.05.

on total *n*-alkanes ($F = 4.47$; df = 1, 23; $P = 0.046$). As in the previous experiment, *n*-alkanes decreased with increased relative humidity, whereas alkenes increased (Table 4).

Discussion

In experiment 1, the most dramatic changes in CHC composition occurred with *n*-C27 and *n*-C29, which increased ≈30 and 80%, respectively, under the lower relative humidity condition (Table 2). Differences in CHC composition in the follow-up experiment, although significant, were not as dramatic as the first study; most likely due, in part, to the lessened period of acclimation (from 3 to 1 wk). Small changes in composition, however, do not necessarily equate to small changes in the biophysical properties of the cuticular lipid layer. The most significant observation from both experiments is the increase in total *n*-alkanes relative to the longer chain olefins and methylalkanes under decreased relative humidity conditions.

The majority of studies on CHC acclimation to temperature and relative humidity have reported increased proportions of higher molecular weight compounds under xeric conditions. Toolson and Hadley (1976) and Hadley (1977, 1978) found increased proportions of long-chain branched alkanes in various arthropod species collected in xeric habitats and in individuals that had been xerically stressed. In a more recent study, Howard et al. (1995) reported an increase in long-chain alkenes, especially dienes and monoenes in CHC mixtures when adult sawtoothed grain beetles, *Oryzaephilus surinamensis* (L.), were placed under high temperature desiccation stress. Although no reports of similar CHC modification have been reported for termites, Collins et al. (1997) reported a predominance of long chain CHCs in xerically adapted termites collected in the Caribbean region. Although these previous reports might lead one to believe that xeric tolerance may be positively correlated with CHC chain length, our findings seem to suggest a more complex relationship.

The results of our study showed that, although late eluting olefins and methyl-alkanes predominated throughout, there was a decrease in the proportion of these compounds relative to shorter-length *n*-alkanes when termites were held under low relative humidity conditions, regardless of temperature. These results are very similar to those reported by Howard et al. (1995), who also found that larval sawtoothed grain beetle, contrary to their adult counterparts, increased the proportion of short-chain *n*-alkanes relative to high molecular weight alkenes under high temperature and desiccation stress.

Although Howard et al. (1995) reported a 35-carbon *n*-alkane, hydrocarbons longer than 34 carbons are most often branched or double/triple bonded (Hadley 1981, 1985). Although branching and unsaturation decrease Van der Waals bonding between molecules in hydrocarbon mixtures, these high molecular weight compounds (>35 carbons) tend to increase fluidity and permeability of the cuticular lipid mixture (Toolson 1982, Lockey 1988). These statements are supported by the results of a study by Gibbs and Mousseau (1994) who showed that cuticular lipid melting points of *Melanoplus sanguinipes* (F.) increased with greater proportions of *n*-alkanes during acclimation to high temperature and low relative humidity. In addition, Gibbs et al. (1995) found that not only did saturated *n*-alkane fractions have higher melting points but that they also had shorter chain lengths than methyl-branched and unsaturated CHC fractions.

Given that long-chain compounds, because of their branching and unsaturation, may not improve the waterproofing ability of the epicuticle, they may have other roles. Lockey (1988) and Noble-Nesbitt (1991) hypothesized that these compounds might be used to regulate the viscosity of the cuticular lipid and allow the lipid to evenly coat the epicuticular surface. This rapid spreading of the lipid may be particularly important when rapid acclimation to changing environmental conditions is required, as in the study by Howard et al. (1995) who observed cuticular lipid modification in as little as 24 h in adult sawtoothed grain beetle. This phenomenon may also be particularly important in the case of the desert-inhabiting arthropods discussed by Toolson and Hadley (1976) and Hadley (1977 1978).

Some CHCs seem to have roles, totally aside from the biophysical properties, that may also complicate the functional relationship of the CHC mixture. Howard et al. (1982) suggested that sympatric termite species, *Reticulitermes flavipes* and *Reticulitermes virginicus* (Banks), used internally branched monomethylalkanes and 6,9 dienes as epicuticular species recognition cues. They also went further in hypothesizing that, similarly, ratios of compound classes (unsaturated/saturated; monomethylalkanes/dimethylalkanes; branched/unbranched) could be used in caste recognition in these termite species. Gibbs et al. (1995) found similar pheromone activities of unsaturated and methyl-branched compounds in female *Musca domestica* L. The most interesting observation they made in reference to this discussion was a depression of melting point concomitant with the production of sex pheromone in recently matured females. It was hypothesized that a consequence of sexual maturity might be increased cuticular transpiration rates, i.e., increased cuticular permeability (Gibbs et al. 1995). If such artifacts exist it could explain why CHC mixtures are so different between immature and adult insects.

Given the complexity of the CHC mixture and the various roles that individual compounds can play, it is difficult to generalize about the biophysical utility of individual compounds or what role classes play in cuticular waterproofing. However, it appears that the smaller *n*-alkanes are most prevalent during high desiccation stress in the epicuticle of *C. brevis* nymphs as well as in the immature forms of other xerically adapted insects. It is possible that longer-chained *n*-alkanes (>35 carbons) would be more prevalent under extreme desiccant conditions were it not for the fact that insects are unable to synthesize these compounds. Given this, higher molecular weight methylalkanes and olefins might also be important for waterproofing. Regardless of their degree of branching and unsaturation, these lipids will be equally impermeable to water at some distant chain length beyond their *n*-alkane analogues. This might be the reason that compounds on the order of 45 or greater carbons in length predominate in the epicuticles of *C. brevis* and other xerically adapted termites.

Another question that was posed before these experiments was whether taxonomic conclusions using CHC profiles might be obscured by environmentally induced changes in CHC composition. Although we found significant differences in the quantity of individual compounds, no qualitative differences were evident. Given that the majority of data used to differentiate species is qualitative, i.e., present absence of compounds, this data does not appear to negate any previous work on CHC compounds as taxonomic characters.

Our termite survival data were entirely consistent with previous studies. The observation of high mortality of the 100% RH/35°C treatment confirms previous reports of "water poisoning" (Steward 1981, 1983; Rudolph et al. 1990). These data are also consistent with previous research, indicating that this species is able to resist desiccation even under extreme conditions, and actually prefers cool and dry conditions (Collins 1969, Williams 1977b, Steward 1983) to the warm and moist conditions preferred by most termites.

Cryptotermes brevis is interesting because it appears to be wholly unequipped to deal with high relative humidity, further suggesting that it may not vary widely from a highly desiccant-tolerant state. This should not imply, however that this species does not make adjustments in desiccation tolerance. Although the changes observed in this study were relatively minor, they were consistent with previous studies on CHC modification. Changes from a relatively high to a higher desiccation tolerance are bound to be smaller in their overall magnitude than those observed in

other insects that, overall, tolerate a broader range of relative humidity.

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