Survey of Metabolic Reserves, Stored Uric Acid, and Water Content from Field Populations of Subterranean Termites (Isoptera: Rhinotermitidae) from Georgia

TIMOTHY J. ARQUETTE AND BRIAN T. FORSCHLER

Department of Entomology, University of Georgia, Athens, GA 30602

J. Econ. Entomol. 99(3): 873–878 (2006)

ABSTRACT Total levels of uric acid, soluble proteins, lipid, glycogen, and body water were determined for *Reticulitermes flavipes* (Kollar) workers 24–72 h after field collection. In addition, levels of biological molecules were compared for termites collected from the same inspection ports within 24 h after field collection, and after laboratory captivity of 6 or 9 mo. Uric acid content, described in a previous study as steadily accumulating in *R. flavipes* termites in captivity, was found to be low or undetectable in workers tested after 6 or 9 mo in the laboratory.

KEY WORDS *Reticulitermes*, vigor, body water, uric acid, biological molecules

Subterranean termites are periodically collected from inspection ports on Sapelo Island, Georgia, as part of long-term studies being conducted by the Household and Structural Entomology Research Program at the University of Georgia, Athens, GA. The termites are cultured at room temperature in petri dishes containing wet filter paper, stacked inside of a clear plastic box with a lid. Several hundred *Reticulitermes flavipes* (Kollar) termites can be maintained in petri dishes with wet filter paper for months at a time without other food resources (Becker 1969). However, termites in such conditions decline in vigor soon after field collection (Su and LaFage 1984), with changes in metabolism occurring from artificial diet and the laboratory environment (Arquette et al. 2006).

This study determined levels of uric acid, soluble proteins, glycogen, lipid, and body water as they occur in *R. flavipes* workers immediately after field collection. These results can be compared with previous reports for levels of these molecules of *R. flavipes* and other termite species (Mauldin and Smythe 1973, Mauldin 1977, Mauldin et al. 1978, Potrikus and Breznak 1980, Nazarczuk et al. 1981, Lovelock et al. 1985, Sponsler and Appel 1990, Waller and Curtis 2003) as well as the results from Arquette et al. (2006) that identified body water percentage as a measure of the vigor of termite populations. This study also determined amounts of stored biological molecules both at the time of collection and after 6 or 9 mo in captivity, by using termites collected from the same inspection ports. Results of this study compliment findings of Arquette et al. (2006) that described changing levels of biological molecules in *R. flavipes* workers in captivity.

Materials and Methods

Insects

R. flavipes workers were collected from different areas of Georgia in July, August, and November 2003 (Table 1). Collection sites were four inspection ports in the yard of the Reynolds Mansion, Sapelo Island, designated BH10 (BH short for Big House), BH18, BH28, and S (Sapelo); inspection ports from Morehouse College, Atlanta (MH) and next to the University of Georgia chapel, Athens (CH); and from pine logs and bark found at Mistletoe state park (MT), near Tallulah Gorge and Fort Mountain state parks (TG and FM), and two sites at Whitehall Forest, University of Georgia, Athens (WH1 and WH2) (Table 1). Insects were frozen within 24 h after collection at −70°C, except for MH workers, which were frozen within 72 h. Termites also were collected in February and May 2003 from BH10, BH18, and BH28 inspection ports. These termites were cultured in the laboratory in petri dishes (100 × 20 mm) containing damp filter paper for 6 mo (BH28) or 9 mo (BH10 and BH18) before freezing (Table 3). Levels of each biological molecule were determined for one termite equivalent from three pooled groups of 10 termites from each population. Units of micrograms of biomolecule per milligram of termite were used except for body water content, which was expressed as a percentage of total live body weight. The mean weight of one termite was estimated from six groups of 10 termites per population ± the standard deviation.

Assay Procedures

Uric Acid. Uric acid content was measured following the procedure of Potrikus and Breznak (1980) by

0022-0493/06/0873–0878$04.00/0 © 2006 Entomological Society of America

Table 1. Natural environments of termites used in this study

Collection site	Abbreviation	Environment	Where found	When collected	Additional information
Whitehall Forest, site 1	WH1	Woods	Log	July 2003	
Whitehall Forest, site 2	WH2	Woods	Log	July 2003	
UGA Chapel	CH	Urban landscaped	Inspection port	July 2003	Cardboard bait
Morehouse College	MH	Urban landscaped	Inspection port	Aug. 2003	Cardboard bait
Fort Mountain State Park	FM	Woods	Log	Aug. 2003	5 miles NW of park
Tallulah Gorge State Park	TG	Woods	Bark	Aug. 2003	3 miles N of park
Mistletoe State Park	MT	Woods	Log	Aug. 2003	
Sapelo Island big house	S	Yard of house	Inspection port	Aug. 2003	Wood block bait
Sapelo Island big house, site 10	BH10	Yard of house	Inspection port	Nov. 2003	Wood block bait
Sapelo Island big house, site 18	BH18	Yard of house	Inspection port	Nov. 2003	Wood block bait
Sapelo Island big house, site 28	BH28	Yard of house	Inspection port	Nov. 2003	Wood block bait

using a diagnostic kit (Sigma 292, Sigma-Aldrich, St. Louis, MO). Termites were dried in a convection oven (VWR, Buffalo Grove, IL) at 85°C for 8 h and then held at room temperature in a desiccation chamber containing Drierite crystals (W. A. Hammond, Xenia, OH) for 5 min before weighing. The dried termites were placed in a 1.5-ml centrifuge tube (Eppendorf Scientific, Westbury, NY) and ground into a powder with a plastic pestle, followed by addition of 1.5 ml of lithium carbonate solution [0.6% (wt:vol)] (Sigma-Aldrich). Known amounts of uric acid (Sigma-Aldrich) were added to 1.5 ml of lithium carbonate as a standard. Standards and unknown samples were suspended in a water bath at 60°C for 10 min, followed by centrifugation for 15 min at 3000 × g. Supernatant (either 40 μl, or 400 μl for very dilute samples) was added to 1.5-ml centrifuge tubes containing 0.2 ml of glycine buffer solution (0.7 M; pH 9.4) (Sigma 292) and 1.2 ml of Nanopure water and pulsed for 10 s on an Eppendorf microcentrifuge. Supernatant (0.6 ml) was added to 1.5-ml centrifuge tubes labeled "test" and "blank," followed by 10 μl of uricase (Sigma 292) added to the test vials, and 10 μl of Nanopure water added to the blank vials. Tubes were vortexed and left at room temperature for 30 min to allow for complete digestion of uric acid in the tubes containing uricase. Both test and blank samples were read simultaneously at 292 nm (Spectronic Genesys model 5, Spectronic Instruments, Milford, MA). The difference between test and blank absorbance values was used to determine uric acid concentration. The absorbance value for an unknown sample was defined as the fraction of termite extract in supernatant at the time the absorbance was measured. An initial 10 termite extract was diluted to an equivalent of 0.093 termite extract for reading on the spectrophotometer.

Soluble Proteins. The Bradford method (Bradford 1976) was used to determine levels of soluble proteins (Bio-Rad, Hercules, CA) with a standard of bovine serum albumin. Termites were added to 1.5-ml centrifuge tubes (Eppendorf) containing 1 ml of distilled water and then sonicated on ice (Branson sonifier model 250, VWR). After centrifuging at 14,000 rpm for 5 min, 0.8 ml of supernatant was added to empty centrifuge tubes followed by 0.2 ml of reagent. Sample supernatant, standards, and distilled water for a blank were added at 175 μl per microplate well (BD Biosciences, Franklin Lakes, NJ), and protein concentration was determined at 595 nm (Spectra Max 340 microspectrophotometer, Molecular Devices, Sunnyvale, CA).

Glycogen. Glycogen content of whole termites was determined based on a procedure by Van Handel (1965). Pooled groups of live termites were placed in 1.5-ml microcentrifuge tubes (Eppendorf) containing 0.4 ml of sodium sulfate solution [2% (wt:vol)] (J. T. Baker, Phillipsburg, NJ) and sonicated on ice (Branson sonifier model 250, VWR). To each homogenized termite sample was added 1 ml of 100% ethanol (J. T. Baker). Tubes were vortexed and samples were frozen at −70°C for at least 24 h to break cells and release glycogen. As termite samples thawed, glycogen standards (Sigma-Aldrich) were prepared. Termite samples and glycogen standards were heated 10 min in a water bath at 60°C and then centrifuged at 4000 × g for 5 min. Supernatant was then poured off and discarded, with traces of liquid removed from around the remaining pellet with a pipetter. To each tube containing a homogenized termite pellet was added 750 μl of amyloglucosidase/sodium acetate solution [stock solution: 3.2 mg of amyloglucosidase (wt:vol) (Sigma-Aldrich) mixed with a 5 ml of sodium acetate solution (0.2 M, pH 5.2) (Fisher, Pittsburgh, PA)]. To standard pellets, and 50 μl distilled water to be used as a blank for the spectrophotometer reading, was added 50 μl of the amyloglucosidase/sodium acetate solution. Microcentrifuge tubes were taped to rotators in a mini hybridization oven (Bellco Glass, Vineland, NJ) and spun at 55°C for 2 h at medium speed. After centrifugation for 5 min at 12,000 rpm, between 25 and 200 μl of solution containing termite sample (depending on glycogen concentration) and 50 μl of blank and standard solution were transferred to empty microcentrifuge tubes. After the addition of 0.5 ml of glucose trinder solution (Sigma-Aldrich), tubes were vortexed and allowed to stand for 18 min at room temperature. Supernatant was transferred to microplate wells (0.15 ml/well) (BD Biosciences), and absorbances were determined at 505 nm (SpectraMax model 340).

Lipid. Total lipids were extracted based on the procedure of Zera and Larsen (2001). Pooled groups of live termites were weighed on a 0.01-mg scale (model AB104, Mettler Toledo, Columbus OH) and placed in 1.5-ml microcentrifuge tubes containing 0.66 ml of chloroform (J. T. Baker) with 0.05% buty-

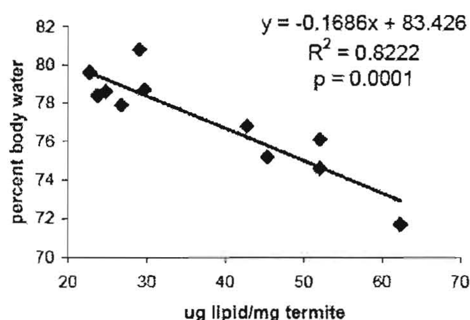

Fig. 1. Linear regression for body water percentage and lipid content (micrograms of lipid per milligram of termite) measured from 11 field populations of *R. flavipes* workers.

lated hydroxytoluene [BHT; (wt:vol)] (Sigma-Aldrich) added. This was followed by addition of 0.33 ml of methanol (J. T. Baker) containing 0.05% BHT (wt:vol). Termites were sonicated on ice (Branson sonifier model 250, VWR) and centrifuged 5 min at 14,000 rpm. All supernatant was transferred into empty 1.5-ml centrifuge tubes with a pipette, and the pellet was discarded. Samples were vortexed after adding 0.34 ml of aqueous KCl (Sigma-Aldrich) [0.88% (wt:vol)] to the supernatant, resulting in two liquid layers. Nonlipid contaminants, isolated in the upper hydrophilic layer, were suctioned off with an aspirator. Dissolved lipids remained in the lower chloroform layer. The chloroform with lipid was poured onto a preweighed aluminum foil bowl and evaporated overnight. Lipid content was determined from the difference in weight between the foil bowl with lipid residue and the initial weight of the bowl.

Body Water. Insects dried for the uric acid assay were those used to determine water content. After weighing, groups of 10 live termites were dried at 85°C for 8 h in a convection oven (VWR). Dried termites were held at room temperature in a desiccation chamber containing Drierite crystals for 5 min before reweighing. Percentage of body water was determined by obtaining the difference between live and dry weights, divided by the live weight.

Statistical Analysis. Levels of biological molecules measured from BH termites in captivity as well as just after collection from the same inspection ports were compared for significant differences using the Student's paired *t*-test and one-way analysis of variance (ANOVA) (Microsoft Excel, Microsoft, Redmond, WA). Duncan multiple range test (SAS Statistical Software version 8, SAS Institute 2000) was used to identify significant differences in levels of biological molecules between all field populations. Correlation analysis was used for comparison of lipid content and body water percentage of all field populations (Microsoft Excel) (Fig. 1).

Results

Uric Acid. Differences in uric acid readings among field populations were significant ($P \leq 0.05$; Duncan multiple range test) (Table 2). CH and MH termites had significantly higher levels of uric acid compared with other populations ($P \leq 0.05$; Duncan multiple range test) (Table 2). Readings of 40.4 ± 2.1 μg uric acid/mg termite were recorded for CH, 51.3 ± 2.0 μg/mg for MH, and between 1 and 10 μg/mg for remaining populations (Table 2). CH and MH, respectively, measured 40- to 50-fold higher for uric acid content than BH18, the group with the lowest levels ($P = 0.001$ for BH18 versus either CH or MH; paired *t*-test. Uric acid content of BH termites was low both from the field and after 6 or 9 mo in captivity, with no significant difference in levels between field and laboratory termites (paired *t*-test). In captivity, BH10 workers measured 1.0 μg uric acid/mg termite, whereas 3.5 μg/mg was recorded from BH18 and

Table 2. Levels of biomolecules and water content measured from workers of freshly collected *R. flavipes* field populations ± the standard deviation

Termite pop	Soluble protein[a]	Lipid[a]	Glycogen[a]	Water content[b]	Uric Acid[a]
WH1[c]	46.7 ± 3.4a[d]	24.7 ± 1.0c	2.6 ± 1.3e	78.6bc[e]	9.6 ± 0.9c
WH2	44.7 ± 2.0a	26.7 ± 3.2c	3.2 ± 0.1e	77.9 ± 0.81cd	2.5 ± 0.3d
CH	40.2 ± 2.7ab	52.0 ± 10.6ab	4.1 ± 0.6de	74.6 ± 0.81g	40.4 ± 2.1b
MH	39.6 ± 3.9ab	62.3 ± 13.4a	3.3 ± 0.5e	71.7 ± 0.8h	51.3 ± 2.0a
FM	38.7 ± 6.7ab	45.3 ± 1.0b	4.0 ± 1.0de	75.2 ± 1.0fg	4.5 ± 1.2cd
TG	37.6 ± 8.4abc	49.0 ± 7.1b	ND	76.1 ± 0.9ef	2.4 ± 1.5d
MT	ND	42.7 ± 5.5b	3.9 ± 0.6de	76.8 ± 0.4de	ND
S	28.2 ± 3.4cd	29.0 ± 2.8c	9.0 ± 0.5cd	80.8 ± 0.5a	ND
BH10	24.7 ± 3.2d	29.7 ± 3.2c	25.4 ± 6.8a	78.7 ± 0.8bc	6.1 ± 7.9cd
BH18	32.5 ± 3.4bcd	23.7 ± 4.0c	12.7 ± 1.7c	78.4 ± 0.1bc	1.1 ± 0.6d
BH28	30.8 ± 7.9bcd	22.7 ± 8.5c	19.5 ± 4.3b	79.6 ± 0.6ab	5.8 ± 0.2cd

ND, no data.
[a] Micrograms of uric acid, soluble protein, lipid, or glycogen per milligram of termite.
[b] Percentage of live weight.
[c] Indicates collection site: WH1 and WH2, Whitehall forest sites 1 and 2; CH, UGA Chapel; MH, Morehouse College; FM, Fort Mountain park; TG, Tallulah Gorge park; MT, Mistletoe park; SAP, Sapelo Island, Reynolds mansion, August 2003; and BH10, BH18, and BH28, Reynolds mansion, Nov. 2003.
[d] Means followed by the same letter within each column are not significantly different from each other by Duncan multiple range test at the 0.05 level of probability.
[e] One reading only.

Table 3. Levels of biological molecules from *R. flavipes* workers measured within 24 h of collection or after 6 or 9 mo in captivity, ± the standard deviation

Test	Field	Captivity
	BH10[a]	BH10[b]
Uric acid	6.1 ± 7.9a[c]	1.0 ± 0.1b
Soluble proteins	24.7 ± 3.2a	36.8 ± 3.2a
Lipid	29.7 ± 3.2a	140.3 ± 9.5b
Glycogen	25.4 ± 6.8a	22.7 ± 1.8a
Water content	78.7 ± 0.78a	68.0 ± 1.7b
	BH18	BH18[b]
Uric acid	1.1 ± 0.6a	3.5 ± 2.8a
Soluble proteins	32.5 ± 3.4a	38.2 ± 4.2a
Lipid	23.7 ± 4a	145.3 ± 18.5b
Glycogen	12.7 ± 1.7a	22.9 ± 3.9b
Water content	78.4 ± 0.1a	67.7 ± 0.4b
	BH28	BH28[d]
Uric acid	5.8 ± 0.2a	0.07 ± 0.1b
Soluble proteins	30.8 ± 7.9a	29.3 ± 2.6a
Lipid	22.7 ± 8.5a	127.3 ± 8.5b
Glycogen	19.5 ± 4.3a	29.4 ± 4.6b
Water content	79.6 ± 0.6a	70.2 ± 1.0b

[a] BH, Big House inspection port at Reynolds Mansion, Sapelo Island.
[b] Measured after 9 mo in the laboratory.
[c] Measurements followed by the same letter across a row are not significantly different by the Student's paired *t*-test.
[d] Measured after 6 mo in the laboratory.

0.07 μg/mg from BH28 (Table 3). Uric acid was too low to measure for BH28 from two of three pooled samples.

Soluble Proteins. Soluble protein measurements were determined to be significantly different between field populations only for high and low extremes (paired *t*-test, $P = 0.02$, and Duncan multiple range test) (Table 2). Among the measurements performed for this study, soluble protein levels were the most consistent across populations; intermediate levels recorded between field groups were not significantly different (Table 2). Sapelo Island termites had significantly lower soluble protein content than the two Whitehall Forest populations ($P < 0.0001$; ANOVA) (Table 2). Protein levels of BH10, BH18, and BH28 workers were not significantly different between groups measured just after collection to those maintained in the laboratory (paired *t*-test). The BH groups measured from 24.7 ± 3.2 to 32.5 ± 3.4 μg/mg just after collection from the field and from 29.3 ± 2.6 to 38.2 ± 4.2 μg protein/mg termite after 6 or 9 mo in captivity (Table 3).

Glycogen. Glycogen levels were determined to be significantly different between field populations ($P \leq 0.05$; Duncan multiple range test) (Table 2) with up to a 10-fold difference in measurement (2.6 ± 1.3–25.4 ± 6.8 μg glycogen/mg termite; Table 2). Workers collected from Sapelo Island had significantly higher glycogen levels than those from other areas ($P < 0.0001$; ANOVA), measuring from 9.0 ± 0.5 to 25.4 ± 6.8 μg glycogen/mg termite compared with 2.6 ± 1.3 to 4.1 ± 0.6 μg/mg from other sites (Table 2). BH10 and BH18 termites had significantly more glycogen just after collection than any other field group ($P \leq 0.05$; Duncan multiple range test (Table 2). BH10, BH18, and BH28 termites in captivity also had high glycogen levels, ranging between 22.7 ± 1.8 and 29.4 ± 4.6 μg glycogen/mg termite. Glycogen content of BH termites in captivity was not significantly different compared with BH field termites ($P = 0.07$; paired *t*-test) (Table 3).

Lipid. Differences in lipid levels were significant among field populations ($P \leq 0.05$; Duncan multiple range test) (Table 2). Lipid content ranged between 22.7 ± 8.5 and 62.3 ± 13.4 μg lipid/mg termite just after collection (Table 2). Readings were lowest for workers from Sapelo Island and Whitehall Forest, ranging between 22.7 ± 8.5 and 29.7 ± 3.2 μg lipid/mg termite; termites from remaining sites measured significantly higher at between 42.7 ± 5.5 and 62.3 ± 13.4 μg lipid/mg termite ($P \leq 0.05$; Duncan multiple range test) (Table 2). Mean lipid content of BH10, BH18, and BH28 was up to six-fold higher in captivity compared with freshly collected workers (Table 3). BH10 measured 29.7 ± 3.2 μg lipid/mg termite from the field, compared with 140.3 ± 9.5 μg/mg in captivity ($P = 0.004$); BH18, 23.7 ± 4.0 versus 145.3 ± 18.5 μg/mg ($P = 0.009$); and BH28, 22.7 ± 8.5 versus 127.3 ± 8.5 μg/mg ($P = 0.002$; paired *t*-test) (Table 3).

Body Water. Body water percentages differed significantly among field populations ($P \leq 0.05$; Duncan multiple range test) (Table 2). Body water percentage was highest in workers collected from Sapelo Island and Whitehall Forest, measuring 77.9 ± 0.8–80.8 ± 0.5%. MH, CH, and FM readings were significantly lower, with readings of 71.7 ± 0.8% for MH, 74.6 ± 0.8% for CH, and 75.2 ± 1.0% for FM ($P \leq 0.05$; Duncan multiple range test) (Table 2). Intermediate levels were determined for termites collected from the remaining areas: 76.1 ± 0.9% for TG and 76.8 ± 0.4% for MT (Table 2). Termites in the laboratory 6 or 9 mo measured 12–15% lower for body water compared with field termites collected from the same sites, with the mean of all BH field termites significantly different than those in captivity ($P < 0.0001$; paired *t*-test) (Table 3).

Discussion

This article reports total levels of lipid, uric acid, soluble proteins, glycogen, and body water of *R. flavipes* workers from up to 11 field sites (Table 2). There was a 50-fold range in uric acid content among workers of different field populations, a 10-fold range in glycogen, and a three-fold range in lipid. Soluble protein levels were similar among the groups surveyed, ranging less than two-fold. A difference of 11% separated the highest and lowest body water measurements (Table 2).

A surprising finding was that uric acid levels were low or absent in workers in captivity (Table 3). This is contrary to past studies that reported uric acid consistently accumulating in termites of various species in laboratory culture (Potrikus and Breznak 1980, Nazarczuk et al. 1981, Lovelock et al. 1985). Potrikus and Breznak (1980) reported that uric acid content of

R. flavipes workers increased at a steady rate from 1.3 to 45% dry weight over 18 mo. Insects measured for the Potrikus and Breznak (1980) study were extracted from a log stored in a laboratory in a metal trash container. The current study, in contrast, found two of three pooled groups of BH28 termites did not have detectable levels of uric acid after 6 mo in a petri dish with filter paper (Table 3). BH10 and BH18 uric acid content was low after 9 mo in captivity, measuring 1.0 and 3.5 μg uric acid/mg termite, respectively (Table 3). The assumption from previous studies that uric acid consistently or regularly accumulates in termites in captivity clearly needs to be reconsidered.

This study also showed uric acid accumulations from field termites, as reported previously (Chappell and Slaytor 1993). Freshly collected CH and MH workers measured 40.4 ± 2.1 and 51.3 ± 2.0 μg uric acid/mg termite, respectively, compared with 1–10 μg uric acid/mg termite for the nine other field groups, and 13 μg/mg reported by Potrikus and Breznak (1980) for freshly collected *R. flavipes* workers. Uric acid accumulation in termites has been described as resulting from starvation (Slaytor and Chappell 1994, Korb and Lenz 2004), perhaps as a result of digestion of body proteins when food is not available (Slaytor and Chappell 1994).

Glycogen levels have not been described for termite workers previously. This is understandable because flying insects are ordinarily used for glycogen studies (for reviews, see Chapman 1998 and Nation 2002). Glycogen content has been reported as 15 mg/g for blow flies, a strong flyer, as well as the American cockroach, *Periplaneta americana* (L.), a weak flyer, with levels depleted in blowflies after flight (for review, see Downer 1982). In comparison, termites collected in November from Sapelo Island measured 12.7–25.4 mg glycogen/g termite (Table 2). An increase in glycogen levels in flying insects has been attributed to conversion from trehalose during resting periods (Downer 1982). Because termites in late fall are less active than in the summer, higher glycogen levels for BH10, BH18, and BH28 termites could have resulted from low activity level. Further study could establish whether seasonality plays a role in levels of glycogen or other biomolecules stored by termites.

Soluble protein levels were mostly similar in both field and laboratory-cultured termites (Table 2). Abnormal levels of soluble proteins could indicate starvation (Slaytor and Chappell 1994); consistent soluble protein readings across populations did not indicate that nutritional stress was a problem.

Lipid content ranged threefold between high and low extremes among field groups (Table 2). Within this range were levels reported by Mauldin (1977) of 53 μg lipid/mg termite for *R. flavipes* workers. The same study reported defaunated workers dropping to as low as 13 μg lipid/mg termite, well below the lowest reading for the current study. Lipid content increased in BH termites up to six-fold after 6 or 9 mo in captivity, comparable with results reported in Arquette et al. (2006) with lipid levels in captivity increasing up to five-fold. In each case, lipid increase may have resulted from an artificial diet of pure cellulose or filter paper, because termites fed wood in the Mauldin (1977) study did not increase in lipid content from field levels.

As mentioned previously, workers collected from CH, MH, and FM showed a low body water percentage (Table 2). Workers at the opposite extreme for percentage of body water came from Whitehall Forest and Sapelo Island, with readings significantly higher than the three groups with the lowest percentages (Table 2). In previous studies, 75 to 76% water content was noted for freshly collected *R. flavipes* workers (Sponsler and Appel 1990, Waller and Curtis 2003). Mean water percentage was 12–15% higher for BH field termites compared with BH groups in captivity for 6 or 9 mo (Table 3). This similarly was seen in Arquette et al. (2006) with up to 16% lower body water readings in captivity. Also, in Arquette et al. (2006), body water percentage correlated with lipid levels for each of the three populations of the study, as described previously for *R. flavipes* alates by Shelton and Appel (2001). Field termites for this study also showed a correlation between water percentage and lipid content (R^2 = 0.822; Fig. 1). However, in Arquette et al. (2006) lipid content and percentage of water changed differently early in captivity. In that study, lipid levels of each population increased two- to three-fold by 2 wk in captivity and then stabilized through 8–12 wk, whereas water percentage steadily declined across populations through 6–12 wk. Therefore, an additional effect besides change in lipid content was apparent for declining water percentage. A switch in diet to pure cellulose may have been responsible for the initial increase in lipid content from week 0 levels. Further study could establish whether different foods affect body water percentage, perhaps from the water content of the food itself.

Current termite control strategies, for example, placement of bait stations around structures, rely on a zero-tolerance approach to termite control, rather than taking into consideration integrated pest management techniques such as an action threshold (Su and Scheffrahn 1998, Thorne and Forschler 2000).

Geographical region and environment influence the vigor of termite field populations (Lenz et al. 1982), and both factors may similarly influence levels of biological molecules for individual insects. Termites measured for this study were collected from different parts of Georgia in varying environmental conditions (Table 1). Similar readings were measured for termites from CH and MH (Table 2), with insects collected from inspection ports in landscaped areas of urban college campuses. Also, comparable readings were seen for the two populations of termites collected from Whitehall Forest as well as the four from Sapelo Island, a barrier island well south of other collection sites (Table 2). Repeating the current study with a larger sampling of termites from different geographic areas, field environments, and times of year could determine how each influences levels of biological molecules in termites.

Acknowledgments

We thank Glenn Ware and Darold Batzer (University of Georgia) for assistance and suggestions for statistical treatments and Mark Brown and Donald Champagne (University of Georgia) for assistance with bioassay procedures.

References Cited

Arquette, T. J., D. E. Champagne, M. R. Brown, and B. T. Forschler. 2006 Evaluation of novel and traditional measures for vigor of laboratory-cultured termites, *Reticulitermes flavipes* (Kollar). J. Insect Physiol. 52: 51–66.

Becker, G. 1969. Rearing of termites and testing methods used in the laboratory, pp. 351–385. *In* K. Krishna and F. M. Weesner [eds.], Biology of termites, vol. 1. Academic, New York.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein using the principle of protein dye binding. Anal. Biochem. 72: 248–254.

Chapman, R. F. 1998. The insects: structure and function, 4th ed. Cambridge University Press, Cambridge, United Kingdom.

Chappell, D. J., and M. Slaytor. 1993. Uric acid synthesis in freshly collected and Laboratory-maintained *Nasutitermes walkeri* Hill. Insect Biochem. Mol. Biol. 23: 499–506.

Downer, R.G.H. 1982. Fat body and metabolism, pp. 151–173. *In* W. J. Bell and K. G. Adiyodi [eds.], The American cockroach. Chapman & Hall, New York.

Korb, J., and M. Lenz. 2004. Reproductive decision-making in the termite *Cryptotermes secundus* (Kalotermitidae) under variable food conditions. Behav. Ecol. 15: 390–395.

Lenz, M., R. Barrett, and E. R. Williams. 1982. Influence of diet on the survival and wood consumption of *Porotermes adamsoni* (Froggatt) (Isoptera: Termopsidae) at different temperatures. Bull. Entomol. Res. 72: 423–435.

Lovelock, M., R. W. O'Brien, and M. Slaytor. 1985. Effect of laboratory containment on the nitrogen metabolism of termites. Insect Biochem. 15: 503–509.

Mauldin, J. K. 1977. Cellulose catabolism and lipid synthesis by normally and abnormally faunated termites, *Reticulitermes flavipes*. Insect Biochem. 7: 27–31.

Mauldin, J. K., and R. V. Smythe. 1973. Protein-bound amino acid content of normally and abnormally faunated Formosan termites, *Coptotermes formosanus*. J. Insect Physiol. 19: 1955–1960.

Mauldin, J. K., N. M. Rich, and D. W. Cook. 1978. Amino acid synthesis from 14-C acetate by normally and abnormally faunated termites, *Coptotermes formosanus*. Insect Biochem. 8: 105–109.

Nation, J. 2002. Insect physiology and biochemistry. CRC, Washington, DC.

Nazarczuk, R. A., R. W. O'Brien, and M. Slaytor. 1981. Alteration of the gut microbiota and its effect on nitrogen metabolism in termites. Insect Biochem. 11: 267–275.

Potrikus, C. J., and J. A. Breznak. 1980. Anaerobic degradation of uric acid by gut bacteria of termites. Appl. Environ. Microbiol. 40: 125–132.

SAS Institute. 2000. SAS Statistical software, version 8. SAS Institute, Cary, NC.

Shelton, T. G., and A. G. Appel. 2001. Cyclic CO_2 release and water loss in alates of the eastern subterranean termite (Isoptera: Rhinotermitidae). Ann. Entomol. Soc. Am. 94: 420–426.

Slaytor, M., and D. J. Chappell. 1994. Nitrogen metabolism in termites. Comp. Biochem. Physiol. 107B: 1–10.

Sponsler, R. C., and A. G. Appel. 1990. Aspects of the water relations of the Formosan and eastern subterranean termites (Isoptera: Rhinotermitidae). Environ. Entomol. 19: 15–20.

Su, N.-Y., and J. P. LaFage. 1984. Differences in survival and feeding activity among colonies of the Formosan subterranean termite (Isoptera, Rhinotermitidae). Z. Ang. Entomol. 97: 134–138.

Su, N.-Y., and R. H. Scheffrahn. 1998. A review of subterranean termite control practices and prospects for integrated pest management programmes. Integr. Pest Manage. Rev. 3: 1–13.

Thorne, B. L., and B. T. Forschler. 2000. Criteria for assessing efficacy of stand-alone termite bait treatments at structures. Sociobiology 36: 245–255.

Waller, D. A., and A. D. Curtis. 2003. Effects of sugar-treated foods on preference and nitrogen-fixation rates in *Reticulitermes flavipes* (Kollar) and *R. virginicus* (Banks) (Isoptera: Rhinotermitidae). Ann. Entomol. Soc. Am. 96: 81–85.

Van Handel, E. 1965. Estimation of glycogen in small amounts of tissue. Anal. Biochem. 11: 256–265.

Zera, A. J., and A. Larsen. 2001. The metabolic basis of life history variation: genetic and phenotypic differences in lipid reserves among life history morphs of the wing-polymorphic cricket, *Gryllus firmus*. J. Insect Physiol. 47: 1147–1160.

Received 7 June 2005; accepted 23 December 2005.