Thermal Tolerances of Four Termite Species (Isoptera: Rhinotermitidae, Kalotermitidae)

by

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ABSTRACT

Upper thermal tolerances were determined for four species of termite pseudergates and nymphs using both a preset temperature method and a bioassay in which the temperature was increased at a known rate. Termites were acclimated at 28°C for a minimum of 24h prior to being placed into an oven preset to temperatures of 40°C to 49°C, or placed into an oven at room temperature (ca. 25°C) which was then raised at 1.0°C/min . In preset bioassays, the lowest temperature that caused 100% mortality in all replicates was 42°C for *Coptotermes formosanus* Shiraki and 45°C for *Incisitermes immigrans* (Light). In temperature rate bioassays, upper lethal limits (ULL) were 47.9°C, 51.0°C, 51.3°C and 51.3°C for *C. formosanus, I. immigrans, Neotermes connexus* Snyder, and *Cryptotermes brevis* (Walker), respectively. These elevated ULL values could be due to a time-lag between the body temperature and the chamber temperature, or result from increased thermotolerance; both of which are possibilities with gradually increasing temperatures. Actual lethal thresholds are likely to be lower than observed lethal tolerances generated by the ULL method.

INTRODUCTION

Thermal tolerances of termites are of more than academic interest because of the potential application of modified temperatures for termite control. The primary target of high temperature control in Hawaii is the West Indian drywood termite, *Cryptotermes brevis* (Walker) (Isoptera: Kalotermitidae). The high temperature control method as described by Ebeling (1994) involves isolating the area of termite infestation within a structure and introducing heated air to achieve and maintain an ambient temperature between 63°C and 70°C. These ambient temperatures are maintained for a period necessary for the internal wood temperatures to exceed a suggested thermal end-point for a minimum amount of time. To establish high temperature control procedures, Forbes and Ebeling (1987) studied the temperature tolerance of *Incisitermes minor* (Hagan) and several other insects. These

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authors utilized a thermal tolerance bioassay in which test insects were placed at preset temperatures and the time until death was determined at each temperature. Recent studies, however, have reported thermal tolerances in the form of critical thermal maxima (CTMAX) or upper lethal limits (ULL).

Determination of critical thermal maxima involves subjecting insects to rates of temperature increase as opposed to constant temperatures. Rates of temperature increase are more appropriate for testing the bioclimatology of insects because they simulate actual microclimatic conditions experienced by insects; temperatures never change instantaneously in nature. Test insects are observed throughout the process, removed when torpor is noted and observed for recovery for a predetermined period of time. The highest knockdown temperature which later results in recovery is termed a CTMAX and the lowest knockdown temperature without recovery is the ULL. Critical thermal maxima and ULL have been reported for a number of insects, including cockroaches (Appel 1991; Appel and Sponsler 1989), blister beetles (Cohen and Pinto 1977), and earwigs (Karboutli and Mack 1993) as well as termites (Sponsler and Appel 1991; Mitchell *et al.* 1993; Rust *et al.* 1979). CTMAX range from about 43°C to 51°C for most insect species with ULL being only slightly (ca. 1°C) higher.

In light of the differences between these two methods, the purpose of our study was to compare the temperature rate method and the constant temperature method of Forbes and Ebeling (1987) to determine if a definitive thermal death point could be produced and used to refine high temperature control recommendations. Preset temperature tolerances where determined for *Incisitermes immigrans* (Light) and *Coptotermes formosanus* Shiraki and upper lethal limits were determined for these same species in addition to *Neotermes connexus* Snyder and *C. brevis*.

MATERIALS AND METHODS

Preset temperature method

For the preset method, we chose to work with two termite species, *C. formosanus* and *I. immigrans* which were readily collected and easily cultured in the lab. *Coptotermes formosanus* was collected in wooden traps on the Manoa campus of the University of Hawaii (Tamashiro *et al.* 1973). Time and temperature were varied over the ranges of 10 min. to 90 min. and 40°C to 49°C, respectively. The experimental units consisted of covered 100 x 20mm glass petri plates containing 50 pseudergates each. Three replicate units were chosen at random to receive each time-temperature combination. Petri plates were placed

into a Fisher model 655F Isotemp oven (±0.5°C) (Fisher Scientific, Pittsburgh, PA) at time zero and quickly removed at their respective treatment times (this methodology precluded the control of relative humidity). The number of dead, moribund and live individuals were then counted and held in separate petri dishes containing a single piece of filter paper and 1ml of distilled water. The plates were covered and placed in a Precision model 815 incubator (Precision Scientific Inc., Chicago, IL) set to 28°C (±0.5°C) for a period of 24h, then the number of dead, moribund and live individuals were again assessed. The values reported here represent 24h mortality.

Incisitermes immigrans test subjects were at least third instar nymphs (determined by size) selected from several different colonies collected from dead standing Kiawe (*Prosopis pallida* [Lam.]) near the University of Hawaii at Manoa campus. Each colony extracted from wood samples was placed into an individual 21 X 34.3 X 8.9cm plastic container (Consolidated Plastics Company, Inc., Twinsburg, Ohio) along with hardwood tongue depressors as a food source. The covered containers were placed at 28°C (±0.5 C) and allowed to acclimate for a period of no less than 24h prior to testing. Because of the difficulty in obtaining large numbers of this species, treatments consisted of three replicates of twenty nymphs each placed in smaller (60 X 15 mm Kimax) glass petri plates. Treatments were conducted as previously described for *C. formosanus*, with mortality assessed 24h after removal from each time-temperature regime.

Temperature rate method

Coptotermes formosanus, *I. immigrans*, *N. connexus*, and *C. brevis* pseudergates were tested using the temperature rate bioassay. *Coptotermes formosanus* and *I. immigrans* were collected as previously discussed. *Neotermes connexus* was collected from infested Koa Haole (*Leucocaena leucocephala* [Humb. & Bonpl. ex Wild]) and *C. brevis* from infested plywood taken from a warehouse on the Honolulu seaboard. Extracted termites were placed into plastic boxes along with tongue depressors and paper towels and held at 28°C (±0.5 C) for no less than 48h prior to treatment. Ten pseudergates were arbitrarily selected from cultures and placed into a 15cm diameter glass petri dish lined with a Whatman no. 2 filter paper. The dishes were placed on inverted 50ml beakers inside of a 500ml Qorpak jar which contained 200ml of distilled water to maintain saturated conditions during the test. Temperatures within the petri dishes were monitored with a 20 gauge type-T thermocouple attached to the petri plate with tape and threaded through a 2mm diameter hole in the lid of the Qorpak jar and connected to a Cole Parmer model 8110-25 thermocouple thermometer (Cole Parmer In-

strument Company, Vernon Hills, IL). The jar containing the termites was then placed into a Hewlett Packard model 5700A gas chromatograph. The gas chromatograph oven was programmed to increase the temperature of the oven at 1°C/min; actual rate of increase within the test chamber was determined to be ca. 0.70°C/min. Termites were removed from the oven at 0.5°C increments starting at 45°C up to the point of no recovery. Once removed from the oven, 0.2ml of distilled water was added and the plates were covered and incubated at 28°C. Mortality was assessed 24 h post-treatment.

In order to determine the difference between the test chamber and the body temperature of test insects, a 33 gauge hypodermic temperature probe (Omega Engineering Inc., Stamford, CT) was inserted through the anus into the haemolymph of a third instar *C. brevis* nymph. The probe with termite and a second identical bare probe were affixed with 1.4cm masking tape to the edge of a small glass petri plate containing a 5.5cm Whatman #2 filter paper. The petri plate was placed on top of an inverted 50ml beaker inside of a 500ml Qorpak jar containing 100ml distilled water. Singly, the jars were firmly capped and placed onto the center shelf of a Salvis Thermocenter Top Oven. The oven was programmed to equilibrate at 30°C for 15 min. prior to increasing the temperature at 0.5°C/min to 70°C. Test chamber and body temperature were manually monitored with by way of a Cole Parmer model 8110-25 type-T thermocouple thermometer (Cole Parmer Instrument Company, Chicago, IL) (accuracy: ± 0.25°C). Once the test chamber reached 52°C, the jars were removed. The test was replicated 8 times and the data analyzed in a one-sample T-test (SAS Institute 1985) to test the null hypothesis that the difference between body and chamber temperatures equaled zero ($\mu=0$ or $\mu>0$).

RESULTS AND DISCUSSION

Tables 1 and 2 present the mean (n=3) mortality for *I. immigrans* and *C. formosanus* exposed to each preset time/temperature combination. At the low end of the temperature scale, an upward trend in the mortality of *C. formosanus* can be seen at 40°C from 70 min. onward; while with *I. immigrans*, this same trend is apparent at 44°C from 60 minutes onward. Results obtained with *I. immigrans* (Table 2) were converted to a form comparable to those for *I. minor* reported by Forbes and Ebeling (1987) (Table 3). The lowest temperature exposure producing total (100%) mortality for *C. formosanus* was 42°C at 90 min. and 45°C at 70 min. for *I. immigrans*. In our tests, the minimum exposure times required for 100% mortality of *I. immigrans* at 46°C and 49°C were 30 min. and 20 min., respectively. Minimum exposure times reported

Table 1. Mean percent mortality of *Coptotermes formosanus* pseudergates at various combinations of temperature and time.

Time (min)	Temperature (°C) 40	41	42	43	44	45	46	47
90	5 (0.67)	47 (5.5)	100	—	—	—	—	—
80	3 (1.3)	22 (4.7)	98 (1.2)	—	—	—	—	—
70	1 (0.67)	14 (8.3)	74 (3.2)	100	—	—	—	—
60	0	33 (1.2)	56 (16)	100	—	—	—	—
50	0	0	14 (4.2)	100	100	—	—	—
40	—	0	3 (1.3)	67 (17.7)	100	100	—	—
30	—	—	0	27 (13.5)	100	100	100	100
20	—	—	0	5 (1.3)	77 (14.7)	44 (26.6)	100	100
10	—	—	—	0	0	0	1 (0.67)	1 (0.67)

Values in parentheses represent standard errors of the means (n=3) greater than 0.
— = No data collected

Table 2 . Mean percent mortality of *Incisitermes immigrans* nymphs at various combinations of temperature and time.

Time (min)	Temperature (°C) 42	43	44	45	46	47	48	49
90	2 (1.7)	5	36 (8.3)	—	—	—	—	—
80	2 (1.7)	3 (1.7)	20 (5.0)	100	—	—	—	—
70	0	5	30 (9.0)	100	—	—	—	—
60	0	0	12 (1.7)	80 (12.6)	—	—	—	—
50	—	0	0	26 (13.4)	100	—	—	—
40	—	—	0	14 (6.3)	100	100	—	—
30	—	—	—	5 (2.9)	100	100	100	100
20	—	—	—	0	3 (1.7)	90 (10)	100	100
10	—	—	—	0	0	2 (1.7)	7 (4.4)	17 (6.7)

Values in parentheses represent standard errors of the means (n=3) greater than 0.
—= No data collected.

for *I. minor* (Forbes and Ebeling 1987) were considerably higher: 256 min. and 30 min., respectively, suggesting that *I. minor* is more heat tolerant than *I. immigrans* (Table 3).

Upper lethal limits developed with the temperature rate method are presented in Table 4 for the four termites species tested. The ULL for *C. formosanus* pseudergates (47.9°C) was close to that of 48.0°C reported by Sponsler and Appel (1991) while the ULL of *C. brevis* was somewhat similar to that generated by Scheffrahn *et al.* (1997) for this species. The other two termite species have not been previously studied. However, the ULLs for congeners *Incisitermes fruticavus* Rust and *I. minor* were

Table 3. Exposure times (minutes) producing 100% (n=3) mortality at various constant temperatures.

Temperature(°C)	Species *Incisitermes immigrans*	*Coptotermes formosanus*
42	90+	90
43	90+	50
44	90+	30
45	70	30
46	30	20
47	30	20
48	20	—
49	20	—

—= No data collected.

reported to be in the range of 51°C to 53°C (Rust *et al.* 1979), comparable to the value we generated for *I. immigrans* (51.3°C). The similarity of *I. immigrans* to *I. minor* in the ULL method is interesting considering the results of the preset bioassays which revealed a distinct difference in lethal exposure times at 46°C between *I. minor* (256 min.) (Forbes and Ebeling 1987) and *I. immigrans* (30 min.).

The ULL values generated for the drywood termites in our study, *I. immigrans*, *N. connexus* and *C. brevis*, are similar to CTMAX values reported for xeric adapted beetles in the family Meloidae (Cohen and Pinto 1977), while the ULL for the rhinotermitid *C. formosanus* is similar to values reported for mesic-adapted cockroaches (Appel 1991), earwigs (Karboutli and Mack 1993) and other subterranean termites (Mitchell *et al.* 1993; Sponsler and Appel 1990). These observations are consistent with the biology of these taxa; drywood termites, as their name suggests live in relatively warm and dry habitats, whereas *C. formosanus* exists in a cool moist subterranean habitat.

The differences between the two bioassay methods were striking. ULL values generated in the thermal rate bioassay (Table 4) were 47.9°C and 51.3°C for *C. formosanus* and *I. immigrans*, respectively, while results of the preset method (Tables 1 and 2) indicated that temperatures as low as 42°C and 45°C were lethal to these two species. These differences, however, are consistent with previous work. Using the temperature rate procedure, Rust *et al.* (1979) produced knockdown with recovery at 52°C for *I. minor*, while in the constant temperature experiments with this species significant mortality was produced at temperatures as low as 46°C (Forbes and Ebeling 1987). The question posed by these results is whether the two methods produced different values due to a physical artifact of the tests or an actual change in

Table 4. Mean (n=3) upper lethal limits of 4 species of termites collected in Hawaii.

Species	ULL (°C)	Standard error
Coptotermes formosanus	47.9	0.49
Neotermes connexus	51.0	0.14
Incisitermes immigrans	51.3	0.14
Cryptotermes brevis	51.3	0.37

thermotolerance of the insect. The later possibility would caste some doubt on existing high temperature control recommendations.

Biologically, a possible explanation for the different values could be an increase in thermotolerance in the ULL procedure. This is likely since the insects are subjected to a gradually increasing temperature and presumably have some time to make physiological adjustments as the temperature increases. The preset method, on the other hand, does not allow for any acclimatory adjustment because the change of temperature is instantaneous. However, recent work by Scheffrahn *et al.* (1997) with *C. brevis* did not find any effect of rate of temperature increase on thermotolerance down to 0.5°C/min. However, nothing is known about acclimation at rates of temperature increase below 0.5°C/min and such low rates are not uncommon in commercial practice (Woodrow and Grace 1998).

Since humidity was not controlled during the preset temperature study, the effects of desiccation on mortality during extended exposures warrants discussion. Rust *et al.* (1979) demonstrated that low humidity had a detrimental effect on the survival of both *I. minor* and *I. fruticavus* in long-term thermal exposure tests. This effect, however, was only evident with exposure times of 4h or greater, while the longest exposure in the current study was 90 minutes. Thus, it is unlikely that the degree of difference between the two thermotolerance methods is entirely attributable to desiccation.

Given the lack of evidence for desiccation effects, the most likely possibility is a body temperature time-lag produced by a widening gradient between internal body temperature and rapidly increasing ambient temperature in the test chamber. The study of body temperature of *C. brevis* nymphs produced a significant mean gradient of 0.41°C ($T=5.05$; 7 d.f.; $p=0.0008$) between the body and the test chamber air at a rate of increase of 0.5°C/min (1-sample T-test of $m=0$ or $m>0$; SAS Institute 1985). The variation between the body temperature and the test chamber was not consistent, with a standard deviation of 0.23°C and a range from 0.004 to 0.7°C. This degree of time lag at 0.5°C/min provides sufficient evidence to conclude that body temperature time-lag

is a factor which should be considered when conducting thermal assays using rates of temperature increase.

The thermal rate concept with respect to establishing CTMAX and ULL values in insects originated from research with poikilothermic vertebrates such as fish and salamanders. In rate bioassay work conducted by Hutchinson (1961) a rate of temperature increase of 1°C/min was chosen to determine thermal tolerances of salamanders because this rate was slow enough to minimize any time-lag effect between body and aqueous test-chamber temperatures. The 1°C/min rate of temperature increase has since become the conventional rate for thermal studies on terrestrial insects, but may not be entirely appropriate given that the thermal conductivity of water is much greater than that of air. Despite the initial assumption that the conventional 1°C/min rate of temperature increase would not lead to a body temperature time-lag, our observations provide compelling evidence that this occurs. As well as observed body-temperature time-lags, lethal effects were seen for *I. immigrans* at constant preset temperatures as low as 45°C, while at a rate of 0.7°C/min, a temperature of 6.3°C higher was required. Thus, it is likely that the true lethal body temperatures are somewhat lower than the thermal thresholds measured by the rate method. In addition, slower rates of thermal increase may be more appropriate for testing the thermal tolerance of terrestrial organisms and may have to be further adjusted according to thermal characteristics (i.e., mass, volume, conductivity, etc.) of the test animal.

In commercial high temperature termite control practice, the original recommendation of 49°C for a 30min exposure (Ebeling 1994) was selected to maximize the effect on termites while minimizing any possible damage to heat sensitive materials such as plastics and paints (Forbes and Ebeling 1987). However, this recommendation has recently been raised to 54.4°C for a minimum 60 minute duration (Ebeling 1997). Based on our results, the true lethal body temperature of *C. brevis* is somewhat lower than the ULL of 51.3°C and the current recommendation should be more than adequate for the control of this drywood termite. These data also suggest that a lower treatment temperature held for a longer exposure period could be equally effective with less probability of thermal damage to heat-sensitive materials in the structure.

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