Effect of Temperature and Humidity on Survival of *Coptotermes formosanus* and *Reticulitermes flavipes* (Isoptera: Rhinotermitidae)

by

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ABSTRACT

Two subterranean termite species were subjected to combinations of six temperatures (10°, 15°, 20°, 25°, 30°, or 35°C) and five relative humidities (RH) (55, 65, 75, 85, or 99%) to determine optimum conditions for survival. When small groups of the Formosan subterranean termite *Coptotermes formosanus* Shiraki or the eastern subterranean termite *Reticulitermes flavipes* (Kollar) were exposed to all 30 combinations of temperature and RH, survival times were significantly influenced by temperature, RH, and their interaction. For both species, survival times were longest at low temperatures and high RH. Maximum survival of small groups of *C. formosanus* and *R. flavipes* workers and soldiers occurred at the combination of 10°C and 99% RH C (LT_{50}= 28.2 d, LT_{50} = 18.1 d, respectively). Survival of paired *C. formosanus* dealates was evaluated at combinations of 20°, 25°, or 30°C and 55, 65, 75, 85, or 99% RH. Survival was strongly influenced by temperature and humidity. Longest survival times until 50% mortality occurred at 99% RH and 20° or 25°C (LT_{50}= 2.5 d, LT_{50} = 3.0 d, respectively). At all temperatures, mortality occurred too quickly for LT_{50} values to be determined when RH was 55% or 65%.

INTRODUCTION

Temperature and moisture are key factors affecting termite survival, activity, and geographic distribution. Effects of soil moisture on feeding and tunneling activity have been extensively studied (Su and Puche 2003, Arab and Costa-Leonardo 2005, Green *et al.* 2005, McManamy *et al.* 2008). In addition, several studies have linked relative humidity to the survival and

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activity of arid climate species (Smith and Rust 1993a,b, Cabrera and Rust 1994). Nakayama *et al.* (2004) determined optimum temperature-humidity combinations for feeding by two Japanese subterranean termite species and Kulis *et al.* (2008) investigated the effects of moisture and relative humidity at a single temperature (28°C) on survival and feeding of the Asian subterranean termite, *Coptotermes gestroi*.

Termites are well adapted to regulating moisture within the nest, thus limiting the possibility for direct effects of RH on colony survival. Sponsler and Appel (1990) found that nest materials from two subterranean termite species had moisture contents ranging from 16.3% – 67.7% and interstitial spaces near saturated RH levels. Humidity may indirectly influence subterranean termite success by affecting the ability of soil and wood to retain moisture. Changes in moisture content of wood occur much more slowly than changes in air temperature and relative humidity, but wood moisture eventually stabilizes at an equilibrium moisture content dictated by average relative humidity (Smulski 1996). Outdoors, daily fluctuations in temperature and relative humidity do not have much affect on wood moisture. However, inside homes, the relative humidity of outdoor air is altered by heating and cooling, resulting in seasonal changes in wood moisture content. More directly, humidity and temperature can affect the survival of alates, dealates, aerial populations, and colony fragments transported to new locations, thus having important implications for termite dispersal.

In most of its current United States range, the Formosan subterranean termite *Coptotermes formosanus* Shiraki is sympatric with the eastern subterranean termite *Reticulitermes flavipes* (Woodson *et al.* 2001, Messenger 2003) and both are important economic pests. The purpose of this study was to determine the combined effects of temperature and relative humidity on survival of these two species.

MATERIALS AND METHODS

Termites

Termites from two field colonies each of *C. formosanus* and *R. flavipes* were used in laboratory bioassays. *C. formosanus* were collected from colonies located in McNeill, MS and New Orleans, LA using underground open-bottom bucket traps (Su and Scheffrahn 1986). *R. flavipes* were collected from logs in Picayune, MS. Termites were maintained on stacked, moistened spruce (*Picea* spp.) (15.5 x 2.5 x 0.5 cm) in plastic containers (12 x 17 x 6.5 cm) in constant darkness and tested within 30 d of collection. *C. formosanus* alates and dealates were collected on the evening they swarmed in 6 locations in New Orleans and Metairie, LA. Following their flights, termites were collected from surfaces using soft forceps or damp paper towels.

Temperature and Humidity Treatments

For each termite species, survival and feeding assays were conducted at each combination of six temperatures (10, 15, 20, 25, 30, and 35° C) and five relative humidities (55, 65, 75, 85, and 99%). Humidity chambers were created using covered plastic boxes (12 x 17 x 6.5 cm) containing a 2 cm layer of one of five saturated salt solutions: $Mg(NO_3)_2 \cdot 6H_2O$ (55% RH), NaCl + sucrose (65% RH), NaCl (75% RH), KCl (85% RH), or K_2SO_4 (99% RH). Salts were selected for their stability across a range of temperatures and relatively low toxicity and solutions were prepared according to the methods of Winston and Bates (1960). A data logger (model 42270, Extech Instruments, Waltham, MA) was attached inside the lid of each box to record temperature and humidity and monitor conditions without opening the humidity chambers. Covered Petri dishes (55 mm diameter) containing pea pebbles were placed in the bottom of each box to elevate assay dishes from the salt solutions. Before starting experiments, humidity chambers were placed in incubators (Model 1-36 VL, Percival Scientific) at the appropriate temperature and the salt solutions were adjusted when necessary to achieve the desired humidity.

Survival Assays

For assays conducted on small groups of termites, filter paper circles (Whatman #4, 42.5mm) were oven-dried for 24 h, weighed, and placed in 55 mm diameter Petri dishes in the humidity chambers. After 2 days, filter papers were re-weighed to determine the moisture gain at each humidity level. Twenty termites (18 workers and 2 soldiers) were placed in each Petri dish and the dishes were returned to the covered, incubated humidity boxes and maintained in 24 h darkness. Mortality was recorded daily until all termites were dead. After all of the termites in a dish were dead, the remaining filter paper was weighed, oven dried and re-weighed to determine moisture content and consumption. For each termite species, ten experimental units (2 colonies x 5 replicates) were used at each temperature-humidity combination. Assays using *C. formosanus* dealates were conducted at 20, 25, and 30°C and all five RH levels. A filter paper circles (Whatman #4, 42.5mm) was placed in each 55 mm diameter Petri dishes in the humidity chambers at least 2 days before beginning the experiments. On the mornings following swarms, one male and one female reproductive were placed in a Petri dish in one of the humidity chambers. Because few termites were collected some evenings, termites were assigned to treatments on a rotating basis, allowing all treatments to have a similar number of replicates (7 or 8 replicates per treatment). Mortality was recorded daily until all termites were dead.

Data analysis

Data were analyzed separately for each termite species and for *C. formosanus* dealates. For each temperature-RH combination, lethal times (LT_{50} and LT_{90}) were determined using probit analysis for correlated data (Throne *et al.* 1995), executed in Mathematica (Wolfram Research, Inc., Champaign, IL). Non-overlapping confidence intervals were used to determine significant differences among mortality times. To evaluate temperature and humidity effects, survival data were arcsine of the square root transformed and analyzed using repeated measures analysis, with temperature and RH as the between subject effects and time as within subject effect. Analyses were performed using SAS Proc Mixed (SAS Institute Inc. Cary, NC) (Littel *et al.* 1996).

RESULTS

Measurement of filter paper consumption was attempted for 20, 25, and 30° *R. flavipes* treatments. All changes in dry weight were negligible; therefore, filter paper weighs were not recorded for the remaining treatments. Of the filter papers that were weighed, final moisture contents (mean ± sd) were 4 ± 1%, 5 ± 1%, 5 ± 2%, 7 ± 2%, and 12 ± 3% in the 55, 65, 75, 85, and 99% RH treatments, respectively.

In all treatments, *C. formosanus* mortality reached 100% within 52 d. At each temperature, survival times increased with relative humidity (Figs. 1-6, Table 1). Survival was significantly affected by the interaction between temperature and RH over time ($F = 56.76, P < 0.0001$), by temperature over time

Table 1. Lethal times (days) and lower and upper confidence limits for small groups of *C. formosanus* exposed to combinations of six temperatures and five relative humidities.

°C	% RH	LT$_{50}$ (95% CL)	LT$_{90}$ (95% CL)	χ^2	Slope
10	55	6.5 (5.8-7.1)	9.4 (8.6-10.4)	30.4	0.44
	65	7.7 (7.2-8.3)	11.2(10.5-12.0)	24.1	0.37
	75	10.2 (9.1-11.3)	16.2 (14.9-17.9)	37.5	0.21
	85	14.3 (11.9-16.8)	23.5 (20.6-27.7)	108.9	0.14
	99	28.2 (24.5-32.0)	44.7 (40.3-50.8)	132.0	0.08
15	55	4.8 (3.5-6.2)	7.5 (6.1-10.2)	80.6	0.49
	65	6.0 (5.1-6.9)	8.8 (7.8-10.4)	43.1	0.46
	75	5.7 (3.9-7.5)	9.9 (8.0-13.2)	98.5	0.31
	85	9.6 (4.5-14.6)	20.4 (15.3-31.2)	229.5	0.12
	99	32.3 (25.5-39.4)	48.2 (40.8-62.7)	328.2	0.08
20	55	3.0 (2.5-3.4)	4.7 (4.2-5.4)	17.2	0.76
	65	4.5 (3.9-5.0)	6.2 (5.6-7.2)	27.4	0.72
	75	5.5 (5.1-5.9)	7.6 (7.1-8.2)	17.5	0.62
	85	9.4 (8.1-10.8)	13.0 (11.6-15.4)	120.8	0.36
	99	17.1 (14.2-20.0)	26.5 (23.3-31.3)	156.7	0.14
25	55	1.4*	2.1*	6.4	1.70
	65	2.0*	2.9*	20.0	1.50
	75	2.5(1.7-3.4)	3.7 (3.0-5.6)	41.3	1.08
	85	3.7 (3.4-4.1)	5.4 (5.0-5.9)	10.6	0.77
	99	9.4 (8.5-10.4)	11.9 (10.8-13.6)	70.5	0.53
30	55	-	-	-	-
	65	-	-	-	-
	75	-	-	-	-
	85	-	-	-	-
	99	5.7 (4.3-7.0)	7.3 (6.2-11.0)	44.9	0.79
35	55	-	-	-	-
	65	-	-	-	-
	75	-	-	-	-
	85	-	-	-	-
	99	2.8 (2.1-3.6)	3.7 (3.1-5.5)	18.2	1.44

- Probit analysis did not produce LT values because of rapid mortality
* Confidence limits are undefined

($F = 452.07$, $P < 0.0001$), and by RH over time ($F = 572.44$, $P < 0.0001$). In separate repeated measures ANOVAs conducted at each RH, effects of temperature on mortality were always highly significant (all $P < 0.0001$). Likewise, relative humidity effects were significant at all temperatures ($P < 0.0001$). The importance of humidity to *C. formosanus* survival was most dramatically observed at 30° and 35°C. At these temperatures, mortality in the 55-85% RH treatments occurred too rapidly to permit the calculation of LT$_{50}$ and LT$_{90}$ values.

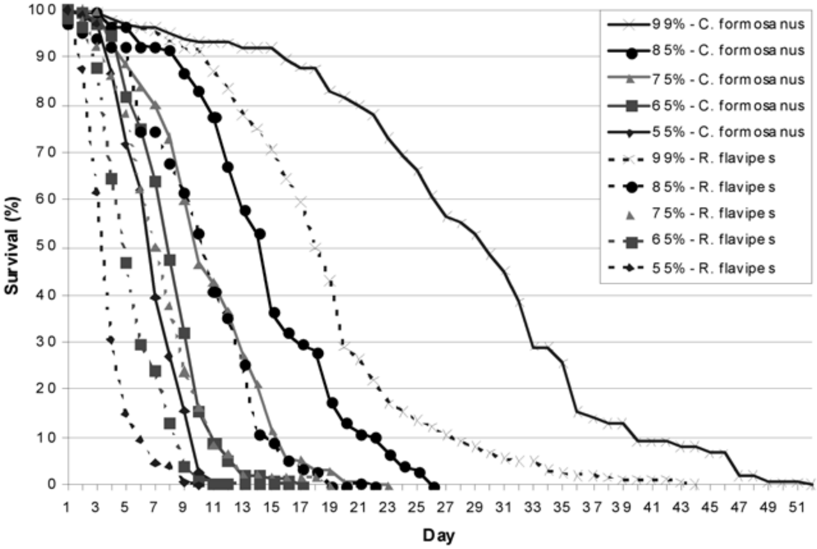

Fig. 1. Survival of small groups (18 workers + 2 soldiers) of *C. formosanus* and *R. flavipes* at 10°C and varying relative humidities.

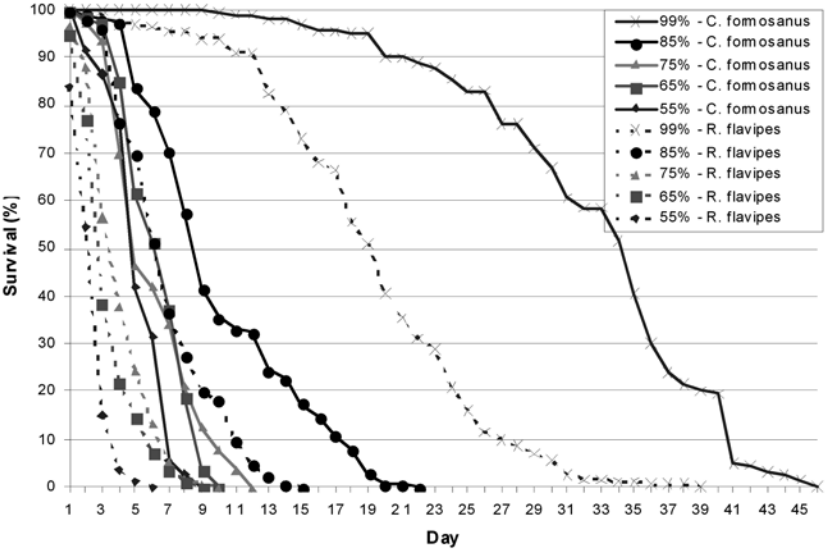

Fig. 2. Survival of small groups (18 workers + 2 soldiers) of *C. formosanus* and *R. flavipes* at 15°C and varying relative humidities.

Table 2. Lethal times (days) and lower and upper confidence limits for small groups of *R. flavipes* exposed to combinations of six temperatures and five relative humidities.

°C	% RH	LT$_{50}$ (95% CL)	LT$_{90}$ (95% CL)	χ^2	Slope
10	55	3.5 (2.1-4.9)	6.5 (5.1-9.0)	79.9	0.44
	65	5.2 (4.3-5.9)	8.2 (7.3-9.5)	39.3	0.41
	75	7.2 (6.5-7.8)	11.3 (10.4-12.3)	23.5	0.31
	85	9.7 (8.1-11.4)	16.2 (14.2-18.9)	65.6	0.20
	99	18.1 (16.3-20.0)	28.9 (26.7-31.7)	80.1	0.12
15	55	2.1 (1.7-2.4)	3.4 (3.0-4.0)	8.2	0.93
	65	3.1 (1.9-4.1)	5.5 (4.4-7.5)	60.8	0.53
	75	3.7 (3.0-4.4)	6.2 (5.4-7.3)	34.9	0.52
	85	6.5 (5.1-8.0)	11.1 (9.5-13.6)	83.2	0.28
	99	18.8 (17.1-20.6)	28.8 (26.7-31.5)	65.3	0.13
20	55	3.1 (2.8-3.4)	4.2 (3.8-4.8)	16.7	1.18
	65	3.7 (3.3-4.0)	5.0 (4.6-5.5)	14.9	0.95
	75	5.0 (3.6-6.5)	7.7 (6.3-10.6)	84.7	0.49
	85	6.8 (6.2-7.3)	8.3 (7.7-9.3)	59.2	0.81
	99	14.4 (12.2-16.8)	20.7 (18.1-25.1)	112.6	0.20
25	55	-	-	-	-
	65	2.2*	2.9*	9.4	1.9
	75	3.0 (2.5-3.4)	3.8 (34.-4.7)	9.2	1.45
	85	3.8 (3.7-3.9)	5.0 (4.8-5.2)	7.9**	1.08
	99	8.7*	16.4*	508.2	0.17
30	55	-	-	-	-
	65	-	-	-	-
	75	-	-	-	-
	85	-	-	-	-
	99	4.1 (3.7-4.5)	5.4 (4.9-6.1)	16.3	0.95
35	55	-	-	-	-
	65	-	-	-	-
	75	-	-	-	-
	85	-	-	-	-
	99	1.8 (1.7-1.9)	3.0 (2.8-3.2)	2.6**	1.07

- Probit analysis did not produce LT values because of rapid mortality

* Confidence limits are undefined

** p > 0.05

Complete mortality of *R. flavipes* was recorded after 44 d (10°C, 99% RH). As with *C. formosanus*, *R. flavipes* survival increased with RH at each of the temperatures evaluated (Figures 1-6, Table 2). Survival of *R. flavipes* was significantly affected by the interaction between temperature and RH over time ($F = 33.98$, $P < 0.0001$), by temperature over time ($F = 280.13$, $P < 0.0001$), and by RH over time ($F = 426.78$, $P < 0.0001$). In one-way repeated measures ANOVA conducted at each RH, effects of temperature on mortality were always highly significant (all $P < 0.0001$) and RH effects were significant when evaluated at each individual temperature ($P < 0.0001$).

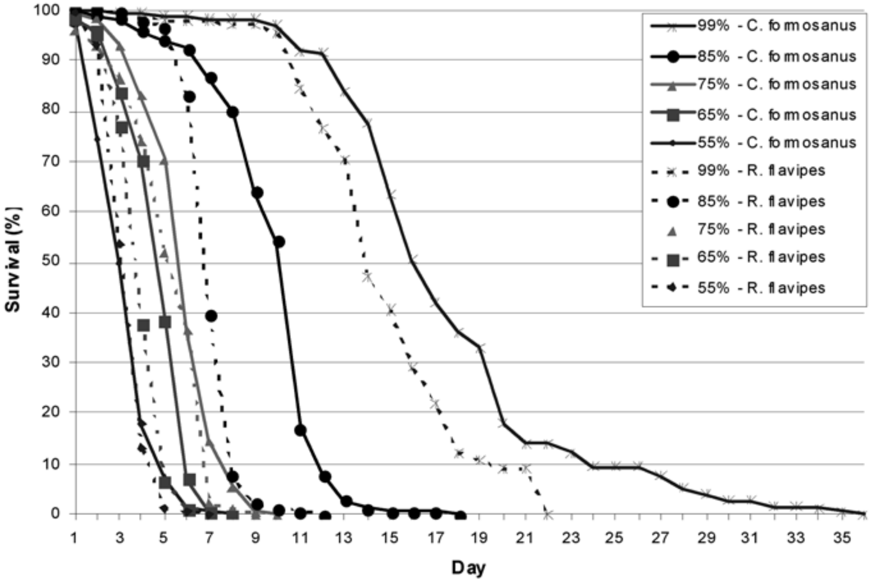

Fig. 3. Survival of small groups (18 workers + 2 soldiers) of *C. formosanus* and *R. flavipes* at 20°C and varying relative humidities.

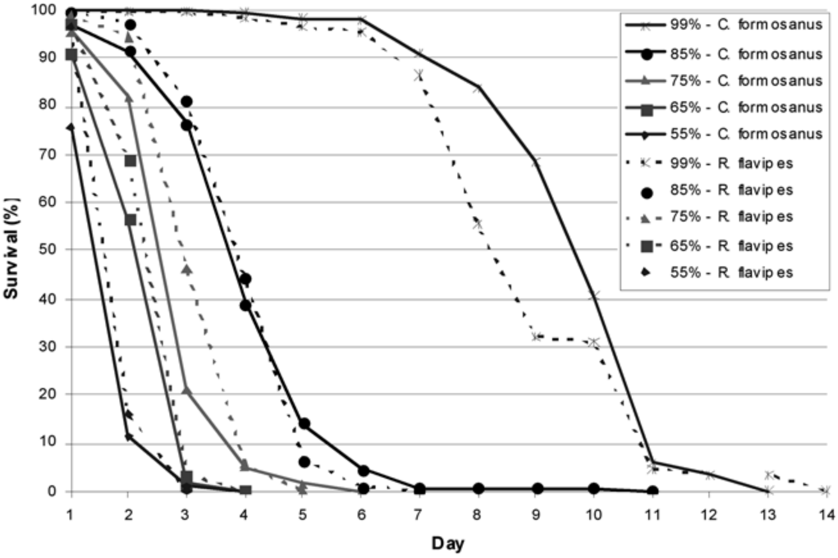

Fig. 4. Survival of small groups (18 workers + 2 soldiers) of *C. formosanus* and *R. flavipes* at 25°C and varying relative humidities.

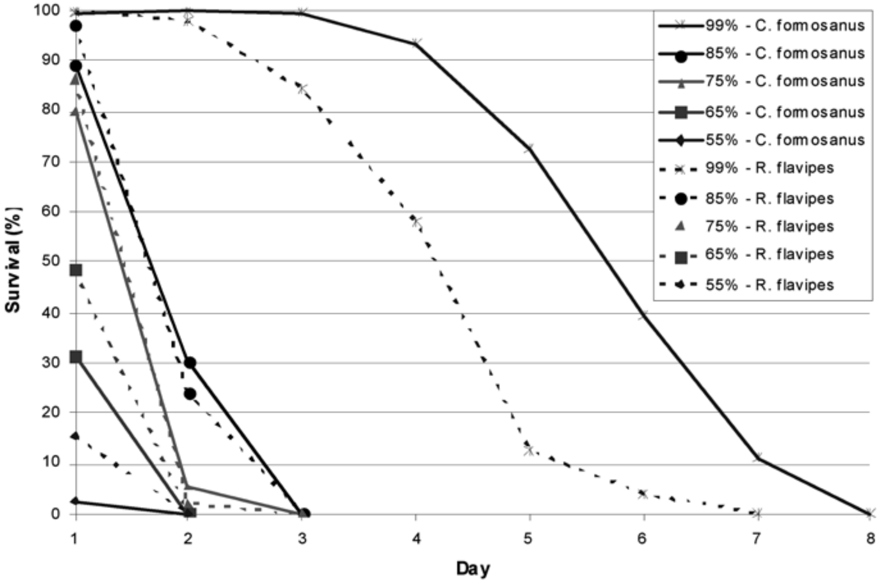

Fig. 5. Survival of small groups (18 workers + 2 soldiers) of *C. formosanus* and *R. flavipes* at 30°C and varying relative humidities.

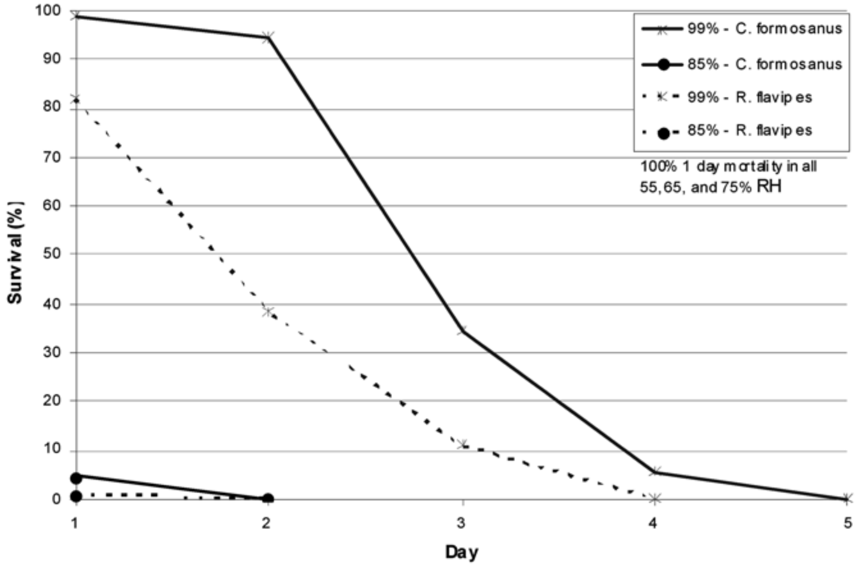

Fig. 6. Survival of small groups (18 workers + 2 soldiers) of *C. formosanus* and *R. flavipes* at 35°C and varying relative humidities.

All *C. formosanus* dealates died within 9d, with high mortality occurring within the first day in most treatments (Figure 7). Survival after 24h exposure was recorded for all RH levels only at the lowest temperature tested (20°C). At 30°C, 2d survival occurred only at 99% RH. Rapid mortality allowed the calculation of LT_{50} and LT_{90} for only six of the treatments (lethal times, followed by lower and upper confidence limits, if available): 20°C/75% RH (LT_{50}= 1.1d (0.0-1.9), LT_{90}=2.7d (1.9-5.5)), 20°C/85% RH (LT_{50}= 1.5d, LT_{90}=4.6d), 20°C/99% RH (LT_{50}= 2.5 d (0.0-4.6), LT_{90}=6.9d (4.8-12.4)), 25°C/85% RH (LT_{50}= 1.8 d (0.8-2.6), LT_{90}=3.5d (2.7-5.5)), 25°C/99% RH (LT_{50}= 3.0 LT_{90}=6.2), 30°C/99% RH (LT_{50}= 3.1 d (2.6-3.6), LT_{90}=4.2d (3.7-5.4)).

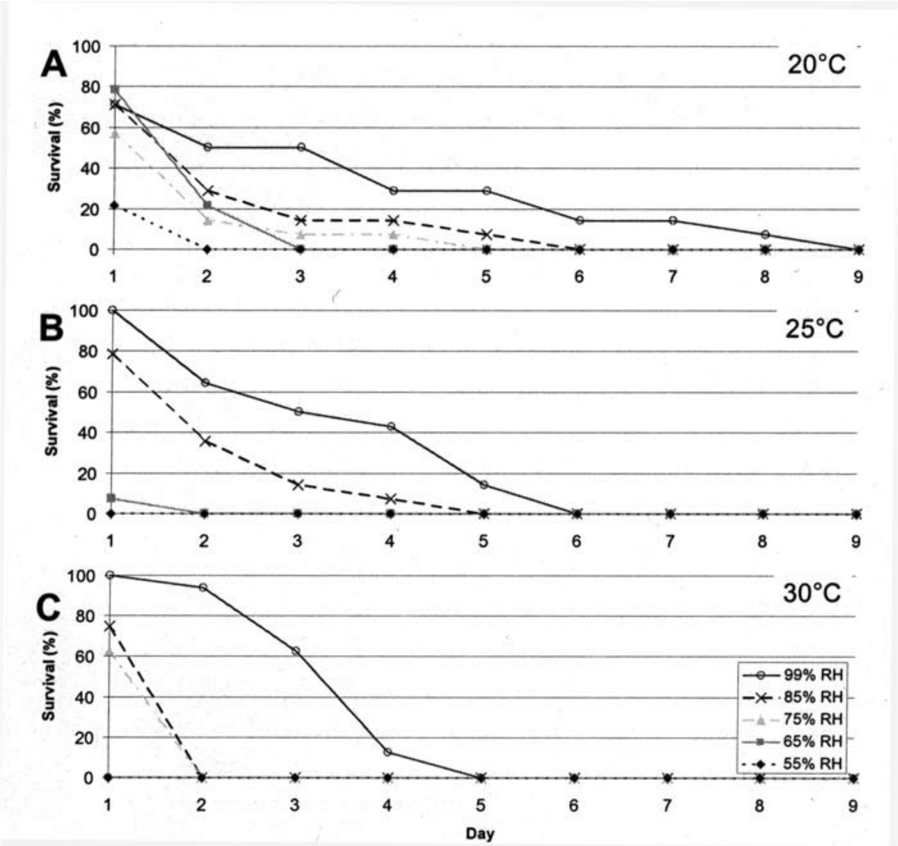

Fig. 7. Survival of *C. formosanus* alates at A) 20°, B) 25°, and C) 30°C.

DISCUSSION

The results presented here concur with those of other studies, finding that, within a certain range of conditions, subterranean termite success increases at low temperature and high RH (Smith and Rust 1993b, Nakayama *et al.* 2004, Kulis *et al.* 2008), but provide data on the most extensive range of temperature and RH combinations tested for both *C. formosanus* and *R. flavipes*. Subterranean termites are extremely susceptible to desiccation (Nakayama *et al.* 2004). At lower temperatures, termites have lower metabolic rates (Smith and Rust 1993b) and lower body water loss (Sponsler and Appel 1990). Reduced susceptibility to desiccation at lower temperatures makes seasonal humidity patterns more important than average humidity. In addition to high humidity increasing the suitability of hotter climates, it is critical for the survival of alates and dealates because they are the stages least protected from fluctuations in environmental conditions.

In the United States, the Formosan subterranean termite has been reported in 11 states (Woodson *et al.* 2001; Scheffrahn and Su 2005; Messenger *et al.* 2002; Hu and Oi 2004; Brown *et al.* 2007; Sun *et al.* 2007). Ongoing surveys indicate the FST distribution is spreading. The physiological limitations affecting the spread of *C. formosanus* are not as well understood as they should be. *R. flavipes* is found throughout much of the eastern United States, but has also recently been reported in Nevada, California, and Oregon (Austin 2005, McKern *et al.* 2006). The Oregon populations, as well as *R. hageni* in that state are believed to be the result of anthropogenic introductions (McKern *et al.* 2006) and their potential western distribution is unknown.

Winter temperature has long been considered the primary environmental factor affecting termites' northern distribution (Kofoid 1934; Abe 1937). For many species, rainfall is another climatic variable closely associated with distribution. However, because subterranean termites are highly susceptible to desiccation, rainfall cannot be used as the sole predictor of available moisture. The amount of moisture available at any given time is a critical factor for survival and reproduction. Precipitation and irrigation provide moisture, while soil texture, groundcover, and relative humidity influence retention and availability of moisture in soil and food sources. Of these factors, seasonal patterns of humidity and temperature may be the most useful in determining potential regional distributions of FST. In addition, FST is able to establish aerial colonies with no ground contact. This is only possible in food sources with sufficient moisture. Data presented here will be valuable in the development of models for potential distribution of *C. formosanus*. Specifically, RH should be considered as a possible limiting factor when temperatures are high and for the establishment of new colonies from nuptial flights. In addition, survival times for *C. formosanus* were at least as high as those of the native species *R. flavipes*. This suggests that RH is probably not a factor that would exclude *C. formosanus* from areas currently occupied by *R. flavipes*.

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