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## The optimum temperature–humidity combination for the feeding activities of Japanese subterranean termites

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**Abstract** Two species of Japanese subterranean termite, *Coptotermes formosanus* Shiraki and *Reticulitermes speratus* (Kolbe), were used in an investigation of the optimum temperature–relative humidity (RH) combination for their feeding activities. Daily wood consumption per worker and survivals were measured, and the protozoan fauna in the hindgut were observed under 15 temperature–RH combinations for 1 week. Five temperatures (20°, 25°, 30°, 35°, and 40°C) and three RH conditions (50%, 70%, and 90% RH) were examined. The activities of the workers were highest at around 30°C for both species, and workers died at 40°C within 5 days and 2 days for *C. formosanus* and *R. speratus*, respectively. The optimum RH condition for feeding activity was 90% RH for *C. formosanus* and 70%–90% RH for *R. speratus*. The optimum temperature–RH combinations for feeding activities were 30°C at 90% RH for *C. formosanus* and 30°C at 70%–90% RH for *R. speratus*.

**Key words** *Coptotermes formosanus* · *Reticulitermes speratus* · Temperature · Relative humidity · Feeding activity

### Introduction

Four kinds of termite, two subterranean species, *Coptotermes formosanus* Shiraki and *Reticulitermes speratus* (Kolbe), and two dry-wood species, *Incisitermes minor* (Hagen) and *Cryptotermes domesticus* (Haviland), cause damage to houses in Japan. Among them, two rhinotermitids, *C. formosanus* and *R. speratus*, are of major

economic importance. The former species is distributed in the southern parts of Japan, whereas the latter is found throughout most of the country.<sup>1,2</sup> The lowest average temperature in January (coldest month) could be responsible for such habitat segregation.<sup>1</sup> Although these termites have been conventionally controlled by the application of insecticidal chemicals up to now, strategies involving decreased use of chemicals or not involving chemicals at all are now being investigated.<sup>3</sup> In Japan, Integrated Termite Management (ITM) focuses on physical barriers, such as stainless steel mesh and gravel barriers, and the positive regulation of the crawl space environment with measures such as humidity control in combination with environmentally acceptable chemical treatments.<sup>4</sup> Therefore, it is important to determine the relationship between the feeding activities of these termites and environmental conditions. Among many environmental factors that could be controlled, humidity seems to be the most promising for controlling termite activity.

The optimum temperature and relative humidity (RH) conditions for the feeding activities of *C. formosanus* and *R. speratus* were reported in previous studies.<sup>5–10</sup> The optimum temperature conditions for the feeding activities of workers of *C. formosanus* and *R. speratus* were reported as 30°–35°C<sup>5,6</sup> and 25°–30°C,<sup>7</sup> respectively, when the termites were fed on filter papers<sup>5,7</sup> and wood blocks.<sup>6</sup> In observations of the wood-attacking activity of *C. formosanus* workers using acoustic emission (AE) monitoring at 12°–40°C, the highest activity was observed at 36°C.<sup>8</sup> On the other hand, the optimum RH for the activity of *C. formosanus* was reported as more than 70% RH,<sup>9</sup> and AE events were maximized at 70% and 80% RH for *R. speratus*.<sup>10</sup> In these studies, the temperature was kept constant at 28°C and 25°C for *C. formosanus* and *R. speratus*, respectively. However, no detailed study of the effect of temperature–RH combinations on the wood-attacking activity of termites has been conducted.

The purpose of the present study was to detect the optimum temperature–RH combination for the feeding activities of two subterranean termites, *C. formosanus* and *R. speratus*, that are economically important in Japan.

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## Materials and methods

### Test insects and wood specimens

Mature workers of *Coptotermes formosanus* Shiraki and *Reticulitermes speratus* (Kolbe) were used as test insects. Workers of *C. formosanus* were obtained from a laboratory colony maintained in a termite culturing room at Wood Research Institute of Kyoto University at  $28^{\circ} \pm 2^{\circ}\text{C}$  and more than 85% RH in the dark. *R. speratus* workers were collected from a field colony on the Uji Campus of Kyoto University in September, 2002.

Oven-dried ( $60^{\circ}\text{C}$  for 3 days) sapwood blocks of Japanese red pine (*Pinus densiflora* Sieb. et Zucc), measuring 10 (R)  $\times$  10 (T)  $\times$  20 mm (L), were used as the test specimens.

A test block was placed at the bottom center of an acrylic cylinder (55 mm in diameter and 60 mm in height) with a plaster bottom, with 50 workers of *C. formosanus* or *R. speratus* (Fig. 1). The assembled test cylinder was put in a plastic container (170  $\times$  230  $\times$  80 mm in height) with a moistened cotton pad, so that the workers could take up water from the plaster bottom. To keep the wood block dry, an acrylic sheet with a 1.0 mm thickness was inserted between the block and the plaster bottom. The plastic container was covered with a piece of aluminum foil with 3 openings to prevent the moistened cotton pads from drying (Fig. 1).

### Measurement of feeding activity

The plastic container was installed in a temperature- and RH-regulated chamber (EYELA KCL-1000, Tokyo Rikakikai, Tokyo) at five temperatures ( $20^{\circ}$ ,  $25^{\circ}$ ,  $30^{\circ}$ ,  $35^{\circ}$ , or  $40^{\circ}\text{C}$ ) in combination with three RH levels (50%, 70%, or 90% RH, a total of 15 conditions) for 1 week. At the same time, the RHs inside the test cylinders were monitored for each condition (Thermo Recorder TR-72S, T and D, Nagano) (Fig. 1).

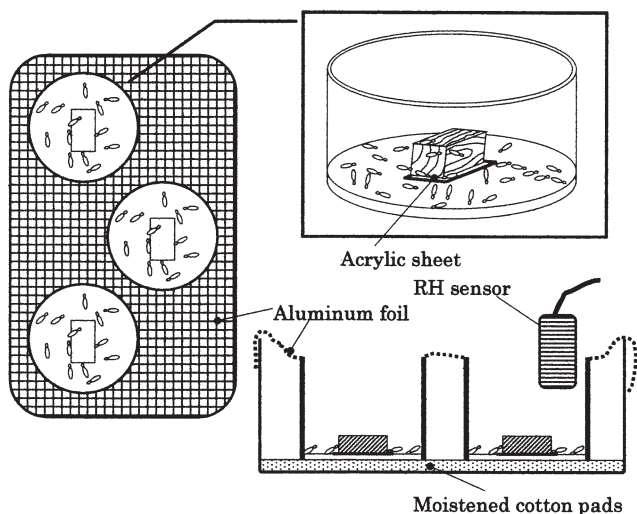


Fig. 1. Test unit

After 1 week, the survival rates of workers were measured, and the blocks were recovered, washed with tap water and oven-dried at  $60^{\circ}\text{C}$  for 3 days so that wood consumption rates could be calculated. Three replicates were employed for each test condition.

In addition, two or three test insects were used to observe protozoan fauna in the hindguts. A worker termite was dissected with fine forceps to take a gut sample out of the body by gentle pulling at the posterior end.<sup>11</sup> Dissected parts were placed in a hole of a slide glass filled with Trager U solution<sup>12</sup> and were observed under a phase-contrast microscope.<sup>11</sup>

## Results and discussion

### Survival rate

The mean survival rates after 1 week of workers of *C. formosanus* and *R. speratus* kept under various temperature–RH conditions are summarized in Table 1. All workers of *C. formosanus* cultured at  $40^{\circ}\text{C}$  with 70%–90% RH died within 5 days. At the  $40^{\circ}\text{C}$  and 50% RH condition, approximately 50% of the workers survived after 1 week. Comparing the mean survival rates and periods to RH conditions, the higher ambient RH levels resulted in lower survival rates and shorter survival periods at  $40^{\circ}\text{C}$ . Two-way analysis of variance (ANOVA) indicated that worker survival rates for *C. formosanus* showed no significant differences among all temperature–RH combinations in the range of  $20^{\circ}$ – $35^{\circ}\text{C}$  ( $P < 0.05$ ).

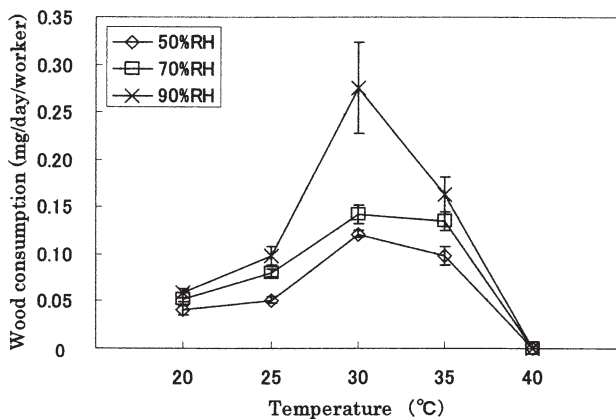
In the case of *R. speratus*, all of the workers cultured under conditions of  $40^{\circ}\text{C}$  and for the conditions of  $35^{\circ}\text{C}$  and 90% RH died within 4 days. As the RH level increased, the survival period shortened under the  $40^{\circ}\text{C}$  temperature condition. In addition, comparing mean survival rates to ambient RHs, the survival rate decreased when the RH level increased for the  $35^{\circ}\text{C}$  condition. Two-way ANOVA analysis indicated that worker survival rates for *R. speratus* in the range of  $20^{\circ}$ – $30^{\circ}\text{C}$  were not significantly different among the different temperature–RH combinations ( $P < 0.05$ ).

It was reported that the mortality of *C. formosanus* was below 5% when cultured for 90 min at  $40^{\circ}\text{C}$ , whereas the lowest temperature that caused 100% mortality was  $42^{\circ}\text{C}$  after 90 min.<sup>13</sup> Using AE monitoring, *C. formosanus* workers stopped feeding behavior after 6 hours when ambient temperature approached  $40^{\circ}\text{C}$ .<sup>8</sup> In the present investigation, the upper tolerable ambient temperature for *C. formosanus* workers was in the range of  $35^{\circ}$ – $40^{\circ}\text{C}$  when tested for 7 days. In the case of dry-wood termites, *I. minor* nymphs have a temperature preference at around  $30^{\circ}\text{C}$ , whereas they died or avoided areas in the range of  $44^{\circ}$ – $47^{\circ}\text{C}$ .<sup>14</sup> Additionally, for *C. domesticus* workers, no AE event was detected at  $45^{\circ}\text{C}$  because all of the insects died within a few min.<sup>15</sup> These results may suggest that Japanese termites have common threshold lines for survival in the range of  $35^{\circ}$ – $45^{\circ}\text{C}$ , regardless of species. Detailed experiments with finer temperature intervals ( $1^{\circ}$ – $2^{\circ}\text{C}$ ) will be needed to decide the exact tolerable temperatures of Japanese termites.

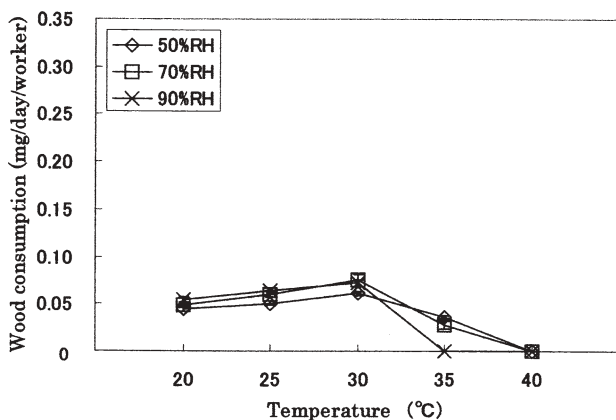
**Table 1.** Mean survival rates of workers of *Coptotermes formosanus* and *Reticulitermes speratus* under various conditions after 1 week

Temperature (°C)	<i>Coptotermes formosanus</i>			<i>Reticulitermes speratus</i>		
	50% RH	70% RH	90% RH	50% RH	70% RH	90% RH
20	97.3 ± 1.2	98.0 ± 3.5	96.7 ± 2.3	94.7 ± 1.2	98.7 ± 2.3	89.3 ± 10.3
25	97.3 ± 1.2	96.0 ± 3.5	98.0 ± 2.0	91.3 ± 1.2	90.0 ± 2.0	90.0 ± 0
30	98.0 ± 0	99.3 ± 0.7	99.3 ± 0.7	84.0 ± 4.0	80.7 ± 1.2	80.7 ± 9.2
35	96.0 ± 2.0	98.0 ± 0	96.7 ± 3.1	50.0 ± 29.6	12.0 ± 12.0	0 ± 0 (4 days)
40	52.7 ± 46.9	0 ± 0 (5 days)	0 ± 0 (1 day)	0 ± 0 (2 days)	0 ± 0 (1 day)	0 ± 0 (5 h)

Values are expressed as means ± SDs. Numbers in parentheses represent the survival period



**Fig. 2.** Effects of temperature–RH combination on the wood consumption of *Coptotermes formosanus* over 1 week. Error bars show standard deviations



**Fig. 3.** Effects of temperature–RH combination on the wood consumption of *Reticulitermes speratus* over 1 week

### Wood consumption

The changes in daily wood consumption per *C. formosanus* and *R. speratus* worker (consumption rate) under various temperature–RH conditions are illustrated in Figs. 2 and 3, respectively. For *C. formosanus* workers, the consumption rates were highest at 30°–35°C (ca. 0.12 mg/day/worker for 50% RH, ca. 0.14 mg/day/worker for 70% RH, and ca. 0.27 mg/day/worker for 90% RH). At 25°–35°C, the higher RH levels resulted in higher consumption rates. In the case of *R. speratus*, no wood consumption was observed at 35°C

and 90% RH nor at any of the conditions at 40°C. Although consumption rates of workers gradually increased with temperature from 20° (ca. 0.05 mg/day/worker) to 30°C (ca. 0.07 mg/day/worker), the rates drastically decreased at temperatures above 30°C.

The effect of ambient temperature conditions on the feeding activities of workers was stronger in *C. formosanus* than in *R. speratus* under all RH conditions. At the lowest temperature of 20°C, the wood consumption rates for both termites were the same (ca. 0.05 mg/day/worker), but when the ambient temperature increased, the rates of the *C. formosanus* workers increased more clearly than those of the *R. speratus* workers (Figs. 2 and 3). These differences may regulate the distribution of these species in Japan: *C. formosanus* is found in the southern parts and *R. speratus* is found throughout most of the country.<sup>1,2</sup>

There are several reports on the optimum temperature conditions for feeding activities of the workers of *C. formosanus* and *R. speratus*.<sup>5–8</sup> When these termites were fed on filter papers, the temperature conditions under which the highest activities were observed were reported to be 30°–35°C for *C. formosanus*<sup>5</sup> and 25°–30°C for *R. speratus*.<sup>7</sup> Additionally, it has been reported the *C. formosanus* workers consumed wood blocks best from 29° to 33°C (100% RH).<sup>6</sup> Observing the wood-attacking activities of *C. formosanus* workers using AE monitoring for temperatures of 12°–40°C, the highest activity was obtained at 36°C.<sup>8</sup> These results coincide well with those of the present investigation, even though further studies should be conducted with finer temperature ranges (1°–2°C) to determine the exact optimum temperature for feeding activities.

Wood consumption rates were significantly different between the highest RH level (90% RH) and the lowest level (50% RH) at 20°–35°C for *C. formosanus* (*t*-test,  $P < 0.01$ ) and at 20°–30°C for *R. speratus* (*t*-test, at least  $P < 0.05$ ) (Figs. 2 and 3). For *R. speratus* workers, the rates were not significantly different between the 70% and 90% RH conditions, but they were significantly different between the 50% and 70% RH conditions (*t*-test,  $P < 0.05$ ) (Fig. 3). When using AE monitoring, it was reported that AE events tended to be maximized at 70% and 80% RH in the fixed-RH test,<sup>10</sup> which is similar to the present results. On the other hand, workers of *C. formosanus* showed higher activities at higher RH levels under all temperature conditions (Fig. 2). For *C. formosanus*, the higher wood-attacking activity was observed at 75% RH and 28°C by AE monitor-

ing.<sup>9</sup> In addition, the highest feeding activity was observed at 70% RH and 27°C for *C. domesticus*.<sup>15</sup> These results suggest that the highest feeding activities of Japanese termites are performed at 70%–80% RH when the activity is measured by AE monitoring. However, higher activities were observed at 90% RH for all temperatures in the present investigation by measuring the wood consumption rates of *C. formosanus* and *R. speratus* (Figs. 2 and 3). It was reported that the number of AE events detected for *C. formosanus* workers maintained at 28°C and 75% RH was 1.5 times higher than those for workers maintained at 85% RH after 4h.<sup>9</sup> AE event rates were constant (ca. 4000/h) over 2–8h (end of experiment) for 85% RH, whereas at 75% RH, the events decreased gradually from approximately 6500/h to approximately 4000/h after 8h.<sup>9</sup> This difference may result in the difference in the optimal RH condition for *C. formosanus* in the AE monitoring and measurement of wood consumption.

#### Observation of protozoan fauna

After 1 week, the protozoan fauna in the hindguts of two to three randomly selected workers of both species were observed using a phase contrast microscope. *C. formosanus* has a relatively simple protozoan fauna consisting of 3 species, *Pseudotriconympha grassii* Koidzumi, *Holomastigotoides hartmanni* Koidzumi, and *Spirotrichonympha leidyi* Koidzumi, while 11 flagellate species live in the hindgut of *R. speratus*.

The results of the observation of the protozoan fauna of the workers exposed to various temperature–RH conditions for 1 week are shown in Table 2. Sound protozoan fauna were observed in workers kept at 20°–35°C for *C. formosanus* and 20°–30°C for *R. speratus* under all RH conditions. Although approximately 50% of the *C. formosanus* workers survived after 1 week at 40°C and 50% RH (Table 1), almost no protozoa were observed in their hindguts, resulting in no wood consumption (Fig. 2). In the case of *R. speratus*, after 1 week the mean survival rates of the workers were 50% and 12% for 35°C–50% RH and 35°C–70% RH (Table 1), respectively, without any protozoa in the hindgut. The wood consumption then decreased to approximately 0.03 mg/day/worker for both conditions (Fig. 3). Because keeping workers at the higher temperature–RH combinations caused the death of all of the insects

within 5 days (Table 1), it was impossible to observe the protozoan fauna under these conditions.

It has been found that protozoan fauna in the hindgut of *R. speratus* workers die at 33°C.<sup>16</sup> In the present investigation, no protozoa were observed in workers kept at 35°C after 1 week (Table 2). On the other hand, protozoan fauna were found in *C. formosanus* workers kept at 35°C after 1 week (Table 2). Although the relationship between protozoan fauna and RH was not clear, the threshold lines of the survival of protozoa were in the range of 35°–40°C and 30°–35°C for *C. formosanus* and *R. speratus*, respectively (Table 2).

The protozoan fauna relate to the feeding activity of their host termite, and the starvation of *C. formosanus* workers caused a decrease in the number of protozoa in the hindgut.<sup>17</sup> Among the three protozoan species, *P. grassii* in the hindguts of starved workers disappeared after 3 days, whereas *H. hartmanni* and *S. leidyi* survived after 2 weeks.<sup>17</sup> Therefore, the protozoa were able to survive for at least several days when their hosts were starved. Taking account of these results, maintaining the temperature at 35°C seems to be responsible for the disappearance of the protozoa in the hindguts of the *R. speratus* workers after 1 week (Table 2). The fact that the workers of *R. speratus* consumed wood at the rate of approximately 0.03 mg/day/worker at 35°C and conditions of 50% and 70% RH may support this assumption (Fig. 3). These results show that the threshold lines of survival of the host termite and protozoa were in the same range, 35°–40°C, for *C. formosanus*, but were in different ranges, 35°–40°C and 30°–35°C, respectively, for *R. speratus*.

#### Conclusions

For two subterranean termites, *C. formosanus* and *R. speratus*, the optimum temperatures for feeding activity and the threshold lines of survival were around 30°C and in the range of 35°–40°C, respectively, regardless of species. The optimum RH conditions for the activities of *C. formosanus* and *R. speratus* were 90% RH, and 70–90% RH, respectively, at the optimum temperatures. These results suggest that the optimum temperature–RH combination for the feeding activity of *C. formosanus* is 30°C and 90% RH.

**Table 2.** Observation of protozoan fauna of workers of *Coptotermes formosanus* and *Reticulitermes speratus* under various conditions after 1 week

Temperature (°C)	<i>Coptotermes formosanus</i>			<i>Reticulitermes speratus</i>		
	50% RH	70% RH	90% RH	50% RH	70% RH	90% RH
20	○	○	○	○	○	○
25	○	○	○	○	○	○
30	○	○	○	○	○	○
35	○	○	○	×	×	–
40	△	–	–	–	–	–

○, normal; △, almost no protozoa; ×, no protozoa; –, not observed



Fifteen combinations using three RH levels (50%, 70%, and 90% RH) and five temperatures (20°, 25°, 30°, 35°, and 40°C) were tested. For *R. speratus*, the optimum combination appeared to be 30°C and 70%–90% RH. In addition, the protozoan fauna in the hindgut of *R. speratus* disappeared at a temperature lower than that at which the host termites died.

For dry-wood termites, fumigation or heat treatments have been reported as the only viable means of eradicating the termites from the entire infested structure.<sup>18</sup> The present results suggest that a heat treatment at more than 40°C may exclude subterranean termites from houses.

As the next step, experiments at fine temperature intervals (1°–2°C) will be conducted for these economically important species to address the practical conditions for termiticidal treatment using heat.

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