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Contents

Difference in Digestibility of Pine Wood by Two Subterranean Termites, *Coptotermes formosanus* Shiraki and *Reticulitermes speratus* Kolbe (Blattodea: Rhinotermitidae)

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Abstract

Forced-feeding of worker termites of *Coptotermes formosanus* Shiraki and *Reticulitermes speratus* Kolbe with specimens of Japanese red pine (*Pinus densiflora* Sieb. and Zucc.) was carried out and chemical properties of the frasses obtained after digestion were compared. Whole profiles indicate superiority of *R. speratus* to *C. formosanus* in digestibility of the polysaccharide portion of the pine wood. Although the amount of wood consumption in the latter termite was 3-4 times higher than the former termite, higher rates of degradation of cellulose and at least xylan were observed in the former termite. Solid-state ^{13}C cross polarization/magic angle spinning NMR and FT-IR spectroscopic analyses, and X-ray diffraction analysis also confirmed the results of chemical composition analyses. High consumption of wood in the *C. formosanus* leading to its most destructive action on wooden structures in Japan can be ascribable to its low efficiency of utilization of polysaccharides in wood.

Key words: *Coptotermes formosanus*, *Reticulitermes speratus*, digestibility, pine wood, forced-feeding

Introduction

Two species of lower termites, *Coptotermes formosanus* Shiraki and *Reticulitermes speratus* Kolbe, gave serious economic loss of wooden made materials in Japan and subtropical countries in participation with symbiotic flagellated protists in their hindgut. However, lower termites participate in preservation of eco-system on earth and contribute partly in its carbon recycling system. In order to get thorough information about the importance of lower termites as recycler of biomass on earth and to minimize attack of termites, more investigation of digestive system of lower termites is necessary. Previously, authors group analyzed digestive systems of these termites (Kanai et al. 1982, Azuma et al., 1984, 1993; Hyodo et al., 1999; Inoue et al., 1997; Yoshimura et al, 1993a, b). Key results given in these reports were utilizability of xylan in *R. speratus* and exclusive importance of cellulose in *C. formosanus* as a diet having relatively high degree of polymerization to maintain the largest flagellated protist, *Pseudotrichonympha grassii* Koidzumi, while the fauna of the other two types of flagellated protists, *Holomastigotoides hartmanni* Koidzumi and *Spirotrichonympha leidyi* Koidzumi could be maintained with low-molecular weight cellulose (degree of polymerization of 17-27) (Yoshimura et al., 1993a). Recent molecular biological analysis further promoted characterization of carbohydrolases from endogenous and microbial origins (representative references, Watanabe et al., 1998; Okuma et al., 2007; Tokuda et al., 2007; Shinzato et al., 2005). However, degradation of wood particles by these lower termites is not fully characterized.

In this report, we investigated differences in digestibility of pine wood by worker termites of two subterranean termites, *C. formosanus and R. speratus*.

Materials and methods

Termites used were workers and soldiers of *C. formosanus*, maintained with pieces of Japanese red pine wood (*Pinus densiflora* Sieb. et Zucc.) in RISH and Shirahama in Wakayama District. Corresponding termites of *R. speratus* were collected from wild colonies inhabited in the same species of wood located in Fukiage Pine Forest in Kagoshima district and Mount Yoshida in Kyoto City. Because of getting similar tendencies, the data obtained in termites from Shirahama and Yoshidayama were cited in this paper.

Forced-feeding experiments were carried out using specimens of the pine wood wetted with tap water placed separately inside acrylic test cylinders (80 mm in diameter and 60 mm in height) whose bottom were sealed with hard dental plaster as described previously (Yoshimura et al, 1993a). Numerical sizes of the specimens were 50×50×3 mm for *C. formosanus* and 50×50×2 mm for *R. speratus*, respectively. For analysis of the effects of soldiers on feeding activity of workers, number of soldier termites was changed from 0 to 30 per 200 workers. In the case of *R. speratus*, experiment was done up to 20 soldiers because of difficulty of collection. Feeding experiments were done under dark at 26 °C for 4 weeks and frasses produced by biting off the specimens were collected. Three replicates were done for each condition. The data were analyzed by a paired *t*-test (Zar, 1999).

The experimental methods for carbohydrate analysis were done according to the combined procedures of Fujii et al. (1996) and Tsubaki et al. (2008). Solid-state ^{13}C cross polarization/magic angle spinning (CP/MAS) NMR spectroscopic and X-ray analyses were done as described previously by Hyodo et al. (1999, 2000).

Results and discussion

1. *Effects of soldiers on biting off Japanese red pine wood specimen by workers*

At first, effects of soldiers on feeding activity of worker termites of *C. formosanus* were analyzed by changing the ratio of worker/soldier numbers from 200/0 to 200/30. The results are shown in Figure 1. A marked difference observed between the two species was in that the feeding activity of the workers of *C. formosanus* was activated by the accompanied soldiers and attained to the maximum at 20 soldiers per 200 workers. In the case of *R. speratus*, however, no such clear dependence was observed up to 30 soldiers per 200 workers. For further experiment we therefore used fixed ratios of worker to soldier termites of 200/20 for *C. formosanus* and 200/0 for *R. speratus*. This may imply that 10 worker termites are enough to keep one soldier in *C. formosanus* in the resent forced-feeding experimental condition. Since soldiers are nutritionally dependent on worker termites in both species, an evadable duty of workers to keep soldiers may prompt them to take more foods than their physiological needs. The results obtained in *R. speratus* indicate that cost of energy for the worker termites to take care of soldiers is not as high as in *C. formosanus*.

2. *Rate of biting off Japanese red pine wood specimen by workers*

According to the experiment described above, we analyzed rate of biting off Japanese red pine wood specimens by the worker termites of *C. formosanus* and *R. speratus* in the fixed worker/soldier ratios of 200/20 and 200/0, respectively, for 28 days (4 weeks). The results of the forced-feeding experiments are shown in Figure 2. The given data indicate significant higher rates of biting off the pine wood in the *C. formosanus* ($P = 0.0006$) and clear proportional increases in both species with 4.0 times higher in the *C. formosanus*. The amount of biting off by one worker termite of *C. formosanus* in a week was calculated to be about 1.90 mg, the value being about 3.6 times higher than did one worker termite of *R. speratus* (0.53 mg).

The present results indicate that *C. formosanus* consumes Japanese pine wood about 3-4 times higher than *R. speratus*. This may be one of the reasons why the *C. formosanus* is the most destructive species for wooden made structures.

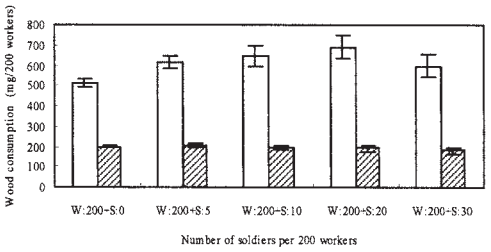

Fig. 1 Effects of number of soldiers on wood consumption by worker termites (●, C. *formosnus*; ○, *R. speratus*) w, number of workers; S, number of soldiers)

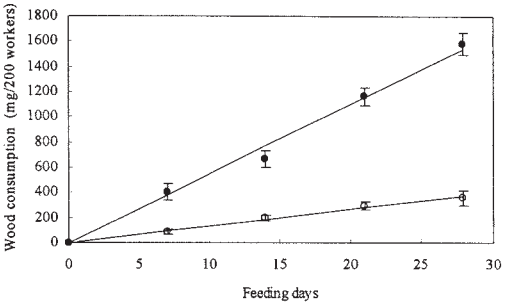

Fig. 2 Wood consumption by worker termites (□, *C. formosnus*; ▨, *R. speratus*;

3. *Chemical properties of frasses produced by workers fed on pine wood*

In order to know and compare about the effects of passing through the gut system of worker termites of *C. formosanus* and *R. speratus* on the bite off components of Japanese red pine wood, chemical analyses of the frasses were thoroughly carried out. The summarized results are listed in Table 1.

The amounts of alcohol-benzene extract and SDS-soluble components in the frasses were higher than the values of the sound pine wood but did not show any time dependent change, indicating low use of the extractives and substantial contribution of microbiota in their hindgut. Lignin content, however, increased after passage through the gut system. As expected from the lignin content, the mounts of polysaccharides (holocellulose, α-cellulose and hemicellulose) decreased in the frasses, more typically observed in the case of *R. speratu*. Indeed the amounts of α-cellulose and hemicellulose in *R. speratus* decreased to 13-35% and 37-61% of the mother wood, respectively, in good accordance of our previous observations that showed high degradability of xylan in this species (Inoue et al., 1997; Azuma et al, 1993). Sugar compositional data which showed decrease in xylose and glucose and remaining of high amounts of arabinose, galactose and mannose (Table 2) further indicate low use of arabinogalactan and galactoglucomannan by these termites. When the effects of degradation on crystalline portion of cellulose was analyzed by X-ray diffraction, degree of crystallinity of the cellulose remained in the frasses decreased gradually throughout the experiment to about 50% in *R. speratus*, in contrast to a small decrease (81-83%) in *C. formosanus*, in good agreement with high degradability of polysaccharides in the former termite (Table 1). The results of no clear time dependent change in chemical compositions of the frasses within 3 weeks indicate low frequency of multiple use of the biting off foods within this experimental period.

Table 1 Chemical composition of the frasses produced by workers fed on pine wood (w%)

Component	Sound pine wood	*C. formosanus* (feeding days)				*R. speratus* (feeding days)			
		7	14	21	28	7	14	21	28
Alcohol-benzene extract	1.7	4.5	3.3	2.6	3.9	3.6	3.8	3.2	3.2
SDS extract	3.3	11.6	9.0	7.8	9.3	9.6	5.8	9.6	9.9
Lignin	24.5	44.9	42.0	40.9	48.2	57.5	56.1	55.7	70.6
Holocellulose	70.5	39.0	45.7	48.7	38.7	29.2	34.3	31.4	16.3
α-Cellulose	41.3.	17.8	22.4	24.7	16.3	11.5	19.0	13.8	5.5
Hemicellulose	29.3	21.2	23.3	23.9	22.3	17.8	15.3	17.6	10.9
Crystalinity of cellulose	69.8	56.6	57.8	57.9	49.6	41.9	38.1	37.2	34.8

Consumption of polysaccharides and remaining of lignin in the frasses were further analyzed by solid-state ^{13}C CP/MAS NMR and FT-IR spectroscopic analyses. The results of ^{13}C CP/MAS NMR spectroscopic analysis shown in Figure 3 (a and b) showed similarity of the spectra in both termites; remarkable decrease in intensity of sugar carbon signals at 61-105 ppm and disappearance

Table 2 Neutral sugar composition of the frasses produced by workers fed on pine wood (w%)

Component	Sound pine wood	*C. formosanus*(feeding days)				*R. speratus* (feeding days)			
		7	14	21	28	7	14	21	28
Arabinose	3	5.2	4.5	4.2	6.2	7.9	8.2	7.0	7.0
Galactose	5.4	10.9	9.6	9.9	9.8	8.4	8.4	7.9	7.8
Glucose	59.6	48.8	53.8	56.6	47.1	41.9	41.4	42.4	40.6
Mannose	14.7	18.3	16.6	16.4	18.8	25.6	26.1	26	26.7
Xylose	17.3	16.9	15.5	13.0	18.0	16.2	15.9	16.6	17.8

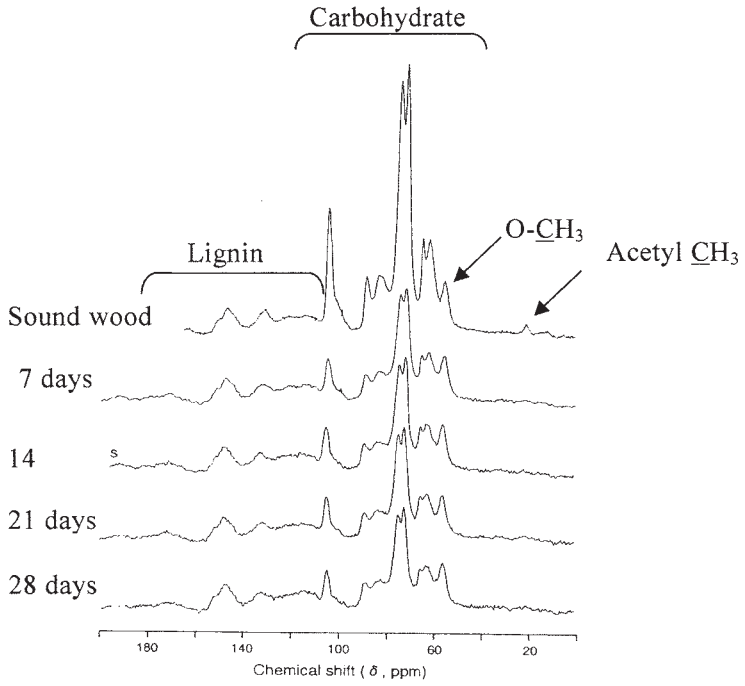

Figure 3 (a) Solid-state ^{13}C CP/MAS NMR spectra of the frasses produced from Japanese pine wood by worker termites of *C. formosanus*

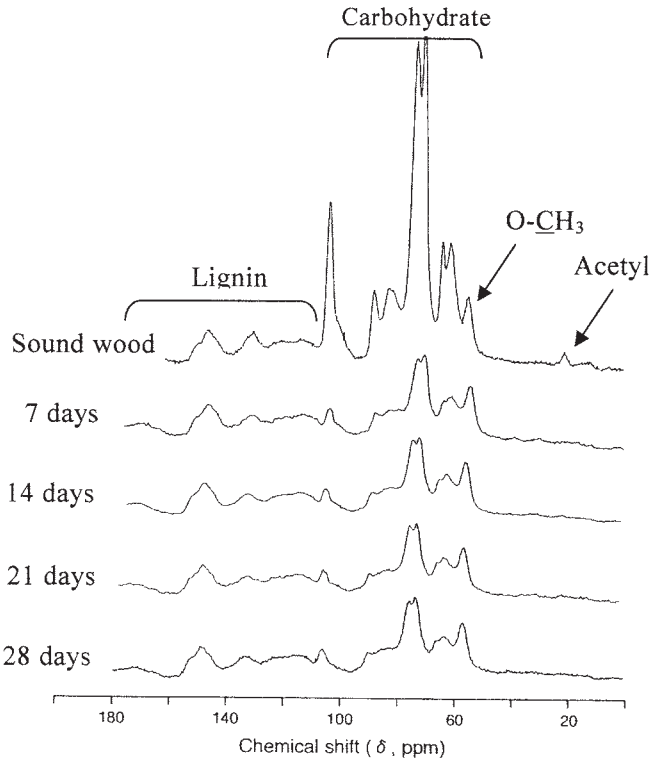

Figure 3 (b) Solid-state ^{13}C CP/MAS NMR spectra of the frasses produced by *R.. speratus*

or weakened signals due to acetyl group at 22 and 174 ppm with remaining signals due to lignin typically appeared at 110-160 ppm (aromatic ring carbons) and 56 ppm (methoxyl methyl carbon). Previously, Hyodo et al. (1999) showed little or no ability of degradation of natural lignin in *C. formosanus*. In this paper we confirmed the previous data and further showed that *R. speratus* belonged to the same category of termites on account of lack of lignin degradation. The strength of the carbon signals due to polysaccharides in *R. speratus* was apparently weaker than those appeared in the case of *C. formosanus* in agreement with the chemical composition data (Tables 1 and 2). Remaining of absorptions due to lignin moiety with corresponding decrease in absorptions due to carbohydrates was also shown by FT-IR spectra at a region of 1400-1500 cm^{-1} in both termites (data not shown). More remarkableness of this trend in the case of *R. speratus* confirmed the superiority of this termite on use of polysaccharides in the Japanese red pine wood in comparison with *C. formosanus*.

Conclusions

The present study dealing with differences in digestibility of Japanese red pine wood by worker termites of two subterranean termites, *C. formosanus Shiraki* and *R. speratus Kolbe*, indicates the superiority of the latter termites on degradability of polysaccharide moiety in the wood. Inferiority of the worker termites of *C. formosanus* to degrade polysaccharides in the wood and their duty to supply foods for soldiers are suggested to be reasons for high consumption of wood, leading to their strongest hazardous species against wooden made structures.

References

Azuma, J., K. Kanai, K. Murashima, K. Okamura and M. Takahashi 1993 Studies on digestive system of termites: III. Digestibility of xylan by termite *Reticulitermes speratus* (Kolbe). *Wood Res.* **79**, 41-51.

Azuma, J., K. Nishimoto, T. Koshijima 1984 Studies on digestive system of termites: II. Properties of cabohydrolases of termites *Coptotermes formosanus* Shiraki. *Wood Res.* **70**, 1-16.

Fujii, Y., J. Azuma, and K. Okamura 1996 Change in chemical composition within an internode of elongating bamboo. *Holzforschung* **50**, 525-530.

Hyodo, F., J. Azuma and T. Abe 1999 Estimation of effect of passage through the gut of a lower termite, *Coptotermes formosanus* Shiraki, on lignin by solid-state CP/MAS ^{13}C NMR. *Holzforshung* **53**, 244-246.

Hyodo, F., T. Inoue, J. Azuma, I. Tayasu, T. Abe 2000 Role of the mutualistic fungus in lignin degradation in the fungus-growing termite *Macrotermes gilvus* (Isoptera; Macrotermitinae). *Soil Biol. Biochem.* **32**, 653-658.

Inoue, T., K. Murashima, J. Azuma, A. Sugimoto, M. Slayter 1997 Cellulose and xylan utilization in the lower termite *Reticulitermes speratus*. *J. Insect Physiol.* **43**, 235-242.

Kanai, K., J. Azuma and K. Nishimoto 1982 Studies on digestive system of termite I. Digestion of carbohydrates by termite *Coptotermes formosanus* Shiraki. *Wood Res.* **68**, 47-57.

Ohkuma, M., K. Saita, T. Inoue, T. Kudo 2007 Comparison of four protein phylogeny of parabasalia symbionts in termite guts. *Mol. Phyl. Evo.* **42**, 847-853.

Sninzato, N., Muramatsu, M., T., Matsui, Y. Watanabe 2005 Molecular phylogenetic diversity of the bacterial community in the gut of the termite *Coptotermes formosanus*. *Biosci. Biotechnol. Biochem.* **69**, 1145-1155, 2005.

Tokuda, G.. H. Watanabe 2007 Hidden cellulases in termites: revision of an old hypothsis. *Biol. Lett.* **3**, 336-339.

Tsubaki, S., H. Iida, M. Sakamoto and J. Azuma 2008 Microwave Heating of Tea Residue Yields Polysaccharides, Polyphenols and Plant Biopolyester. *J. Agric. Food Chem.* **56**, 11293-11299.

Watanabe, H., H. Noda, G. Tokuda, N. Lo 1998 A cellulase gene of termite origin. *Nature* **394**, 330-331.

Yoshimura, T., J. Azuma, K. Tsunoda and M. Takahashi 1993a Cellulose metabolism of the symbiotic protozoa in termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) I. Effect of degree of polymerization of cellulose. *Mokuzai Gakkaishi* **39**, 221-226.

Yoshimura, T., J. Azuma, K. Tsunoda and M. Takahashi 1993b Cellulose metabolism of the symbiotic protozoa in termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) III. Utilization of non-natural celluloses. *Mokuzai Gakkaishi* **39**, 1322-1326.

Zar, J. H. 1999 Biostatistical Analysis, fourth ed., Prentice-Hall, Upper Saddle River, NJ.

From Waste Paper to Food Supplements with the Help of Termites?

by
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Abstract

Groups of *Coptotermes formosanus* and *Reticulitermes speratus* readily fed on a variety of types of paper. However, survival (*R. speratus*, after 8 weeks) was good only on craft pulp and tissue paper, and poor on all others. Eggs and larvae were also only found when craft pulp or tissue paper formed the diet. It is unlikely that termites could be used in a significant way directly for the biodegradation of waste paper nor would waste paper provide a medium for the mass-rearing of termites for the production of food supplements.

Key words: *Coptotermes formosanus, Reticulitermes speratus*; paper consumption, termite survival

Introduction

Japan is the world's 3rd largest producer of paper products. In 2007 the demand for paper and paper board reached 31.7 million tons in Japan. More than 19 million tons were recovered as waste paper during the same year. Large amounts of waste paper products are burnt or used in landfills. Significant volumes of waste paper are also exported from Japan, mainly to China. Increasingly recovered paper is used for the production of a range of recycled paper products and in Japan the use of such materials is encouraged.

Another way of dealing with recovered paper is by biodegradation (Brune 2007; Fox and Noike 2004; Ohkuma 2003). Alternatively, the termites themselves could be used as the agents of bioconversion (Haritos 1992; Haritos *et al.* 1993; Myles 1993; French 1998).

But termites are also of high nutritional value (high protein and lipid content, including essential amino and fatty acids) as detailed analyses have shown (Paoletti *et al.* 2003; Itakura *et al.* 2006). In fact, termites form an important component in the diet of many animals at certain times of the year (termite alates) or throughout the seasons. Humans in several regions of the world supplement their diet with insect, including termites (Defoliart 1999; Paoletti 2003)

Both aspects of termites, decomposers and a source of nutrients, could be combined: Keep and rear termites on cellulose waste products such as paper, harvest the termites and convert them to food supplements for stock (fish, poultry) or to specific products as dietary supplements for humans (Haritos 1992; French 1998; Itakura *et al.* 2006; Severtson 2006; Sogbesan, and Ugwumba 2008a,b).

Despite earlier recommendations of using termites for the decomposition of waste paper (Haritos 1992; Myles 1993), the willingness of termites to feed on different types of paper has only recently been investigated (Severtson 2006) and the question as to whether it represents a medium for rearing these insects is still unanswered.

Experimental procedures and summarized results

Laboratory bioassay

At RISH groups (0.5g) of *Reticulitermes speratus* and *Coptotermes formosanus* were kept for 8 and 4 weeks respectively on different types of paper (4-5 g) in force-feeding trials: pulp, several types of quality print, recycled, tissue, newspaper, glossy pamphlets, and cardboard. All types of paper were readily attacked by both species, most notably corrugated cardboard. Survival after 4 weeks for groups of *C. formosanus* was fairly similar across paper types within a colony. Survival in groups of *R. speratus,* maintained for 8 weeks on the papers, was good only on craft pulp and tissue paper (ca. 50% average for both colonies), lower on corrugated cardboard (35%) and newspaper (24%) and very poor on all other types of paper. Breeding by *Reticulitermes* (eggs, larvae) occurred only on craft pulp and tissue paper, i.e. the least chemically modified products.

Field exposure

Field exposure of paper materials to subterranean termites was conducted at the RISH experimental site in Kagoshima in southern Japan. Although a limited field choice experiment with *C. formosanus* would not allow us to draw conclusions, it appears that craft pulp and tissue paper are among the preferred papers. However, definite conclusions are not possible since only 4 out of 10 replicate sets were contacted by termites, and in only 2 of those did termites contact all satchels with different papers and fed on selected ones. Choice trials with *Coptotermes acinaciformis* in Australia indicated preference for newspaper over glossy-coated paper and bleached office paper was the least preferred (Severtson 2006). The rate of decomposition of newspaper under field conditions by the same species was much lower compared to the laboratory (Severtson 2006).

Conclusions

Haritos (1992) and Haritos *et al.* (1993) did show in short-term experiments that termites are capable of breaking down some of the toxic compounds, particularly polychlorinated biphenyls (PCB's) found in print ink. However, prolonged exposure to those compounds lowers termite survival as our results with newspaper have indicated. Termites did not fair any better on a variety of other paper types, even ones, such as corrugated cardboard, they consumed in significant amounts. Most paper products are clearly not a medium for rearing termites, i.e. not a basis for the production of termite food supplements unless various chemicals added during the manufacture of different papers and deleterious to termites are removed first. However, a number of issues, key among those the processes required and the economics involved, may prohibit the use of waste paper as a medium for mass production of termites. Other substrates, such as the waste of edible mushroom cultures, appear to be suitable for termite breeding (Itakura *et al.* 2008), the first requirement for the production of food supplements from termites.

Acknowledgements

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References

Brune, A. 2007 News and Views: Microbiology: Woodworker's digest. *Nature* **450**, 487-488.

Defoliart, G.R. 1999 Insects as food: why the western attitude is important, *Annual Rev. Entomology,* **44**, 21-50.

Fox, M. and T. Noike 2004 Wet oxidation for the increase in anaerobic biodegradability of newspaper waste. *Bioresource Technology* **91**, 273-281.

French, J.R.J. 1998 Biogenic conversion of waste paper into protein by termites. Summary report, Key Centre for Applied and Nutritional Toxicology, RMIT University, Melbourne.

Haritos, V.S. 1992 The fate for chlorinated xenobiotics in termites. *Thesis, Dept. Applied Biology, RMIT,* Melbourne, 56pp.

Haritos, V.S., J.R.J. French and J.T. Ahokas 1993 The metabolism and comparative elimination of chlorinated biphenyl congeners in termites. *Chemosphere* **26**, 1291-1299.

Itakura, S., J. Okuda, K. Utagawa, H. Tanaka and A. Enoki 2006 Nutritional value of two subterranean termite species, *Coptotermes formosanus* Shiraki and *Reticulitermes speratus* (Kolbe) (Isoptera: Rhinotermitidae). *Jpn. J. Environ. Entomol. Zool.* **17**, 107-115.

Itakura, S., T. Kankawa, H. Nishiguchi, T. Tanaka, H. Tanaka and A. Enoki 2008 The waste of edible mushrooms (*Hypsizigus marmoreus*) affects differentiation and oviposition of the termite *Reticulitermes speratus* (Isoptera: Rhinotermitidae). *Sociobiology* **52**, 67-80

Myles, T.G. 1993 The ecological importance of termites and the potential utilization of termites for the decomposition of lignocellulosic wastes. *Proc. Int. Workshop on Termite – Symbiont Systems,* Kyoto, Japan, 4pp.

Ohkuma, M. 2003 Termite symbiotic systems: efficient bio-recycling of lignocellulose. *Appl. Microbiol. Biotechnol.* **61**, 1-9.

Paoletti, M.G., E. Buscardo, D.J. Vanderjagt, A. Pastuszyn, L. Pizzoferrato, Y.-S. Huang, L.-T. Chuang, R.H. Glew, M. Millison and H. Cerda 2003 Nutrient content of termites (*Syntermes* soldiers) consumed by Makiritare Amerindians of the Alto Orinoco of Venezuela, *Ecology of Food and Nutrition* **42**, 177-191.

Severtson, D. 2006 Bioconversion of waste paper by termites: A landfill of opportunity. *Thesis, Dept. Environmental Biology, Curtin Univsersity of Technology,* Perth,51 pp.

Sogbesan, A.O. and A.A.A. Ugwumba 2008 Nutritional evaluation of termite (*Macrotermes subhyalinus*) meal as animal protein supplements in the diets of *Heterobranchus longifilis* (Valenciennes, 1840) fingerlings. *Turkish J. Fisheries Aquatic Sci.* **8**, 149-157.

Sogbesan, A.O. and A.A.A. Ugwumba 2008 Nutritional value of some non-conventional animal protein feedstuffs used as fishmeal supplement in aquaculture practices in Nigeria. *Turkish J. Fisheries*

Architecture, Thermoregulation and Gas Exchange of Mounds of *Macrotermes carbonarius* in a Tropical Forest of Northeast Thailand: Are Tropical Forests Optimal Habitats for *Macrotermes*?

by
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Abstract

The genus *Macrotermes* termites are well known to maintain the inside conditions optimal (30°C, low CO_2 concentrations) for fungus cultivation in relatively hot environments (i.e. savannas), whereas the situation in relatively cool environments (i.e. tropical forests) is still remain unclear. Here we observed the size frequency of mounds of *M. carbonarius*, and determined mound parameters, inside temperature, and CO_2 concentration for a total of 20 mounds, which covers the major size classes, in the tropical forests of the Sakaerat Environ Research Station, Thailand. The frequency of small mounds was unexpectedly low. For the 20 mounds examined, the mound height usually significantly correlated with the other measures such as mound wall thickness and fungus-comb biomass. The inside temperature was apparently lower than the optimal in small mounds and nearly optimal (28-30°C) in large mounds, while the inside CO_2 concentration was relatively high in small mounds and relatively low (1.0-1.5%, probably suboptimal) in large mounds. The inside conditions in large mounds are equally to or slightly more optimal than those previously reported to *M. bellicosus* in an African gallery forest. These strongly suggest the presence of a trade-off between thermoregulation and gas exchange in the relatively cool environments and that tropical forests are not optimal habitats for mound-building *Macrotermes* termites, especially for the small colonies.

Key words: Heat production, Insulation, Fungus comb, Mound size, Carbon dioxide, Temperature

Introduction

Epigeal nests of termites are a characteristic of the tropical and subtropical regions. Compared to subterranean nests, which are found in from the tropical to temperate regions, epigeal nests are apparently of advantage to gas exchange (Noirot & Darlington 2000). Exchange efficiency of the inside air for the ambient air is sometimes a fatal problem in termite nests, where a mass of population packed within a small volume rapidly consume oxygen and simultaneously emit waste gases such as carbon dioxide (CO_2). Subterranean nests rely for gas exchange mainly on diffusion between the inside air and the intestinal air in the soil, whereas epigeal nests directly connect with moving ambient air, which maintains a high diffusion gradient between the inside air and the ambient air and thus facilitates gas exchange. Instead, epigeal nests are exposed to fluctuations in the ambient temperature. For example, in contrast to constant temperature in the soil, the soil surface temperature in an African shrub savanna reached a maximum of over 50°C, while the minimum was 21°C (Korb & Linsenmair 1998). So far, some epigeal nesters, such as "magnetic termites", have been shown to perform thermoregulation and gas exchange of their nests in sophisticated ways (reviewed in Noirot & Darlington 2000).

The genus *Macrotermes* (Isoptera, Macrotermitinae: fungus-growing termites) usually build earthen

epigeal nests (mounds). These mounds are a conspicuous feature of African savannas as well as African and Southeast Asian tropical forests. Fungus-growing termites cultivate fungi on the gardens (fungus-combs) inside the nests and fully rely on the fungal activity for their living. Since the metabolic activity of the fungi is optimized at the high temperature of 30°C (Thomas 1987) as well as at low CO_2 concentrations (Sands 1969), the thermoregulation and gas exchange is a quite important problem for the termites. Additionally, fungus-combs have generally a much higher biomass than the termites themselves and subsequently release CO_2 and metabolic heat at considerable rates (McComie & Dhanarajan 1990, Darlington *et al.* 1997, Konaté *et al.* 2003, Yamada *et al.* 2005). These facts should have led mound-building *Macrotermes* termites to develop the elaborate systems of thermoregulation and gas exchange of their mounds.

The most striking system may be found in the mounds of *M. jeanneli* in an African savanna. In this relatively hot tropical environment (i.e. savannas), the termites maintain the inside temperature and CO_2 concentrations by venting the inside air through a tall chimney (Darlington *et al.* 1997). It has been shown that the air from the apical hole is constantly around 30°C and contains approximately 0.3% of CO_2 (Darlington *et al.* 1997). This CO_2 concentration, though much higher than those in the ambient air, seem to have no significant effect on the fungus activity (respiration rate, Darlington *et al.* 1997). Since the air discharged from the chimney should reflect the inside conditions, *M. jeanneli* have achieved fully optimal inside conditions for fungus cultivation.

A series of studies have been carried out for *M. bellicosus* by Korb and Linsenmair (reviewed in Korb 2003). In an Africa shrub savanna, *M. bellicosus* build the "cathedral" mounds, where the inside temperature is maintained at 30°C (Korb & Linsenmair 1998) and the CO_2 concentrations of the air passages (equivalent to Korb's air channels) in the mounds at 0.2–1.0% (Korb & Linsenmair 1999). In this relatively hot environment, the inside conditions are shown to be maintained optimal for fungus cultivation by efficient gas exchange through the thin wall and complex surface structure (Korb & Linsenmair 1999). On the other hand, *M. bellicosus* mounds can be found in the neighboring gallery forest, while the mound density is comparatively low (Korb & Linsenmair 1998). By contrast with the "cathedral" mounds in the savanna, the mound architecture in this relatively cool environment seems to be adapted to counteract the loss of heat by insulating the inside and is the dome-shaped structures with thick walls, which have a lower surface complexity (Korb & Linsenmair 1998). Nevertheless, the inside conditions are suboptimal; and the inside temperature is 2°C lower than 30°C (Korb & Linsenmair 1998) as well as the CO_2 concentration (of the air passages in the mounds) has increased up to 1.0–1.5% (Korb & Linsenmair 1999). Accordingly, Korb and Linsenmair have suggested that the suboptimal conditions both in the inside temperature and CO_2 concentration are a result of a trade-off between thermoregulation and gas exchange in the relatively cool environments (Korb & Linsenmair 1999).

In tropical forests of SE Asia, the two *Macrotermes* species, *M. gilvus* and *M. carbonarius* are known to build the thick-walled mounds without complex surface structures (McComie & Dhanarajan 1993, Inoue *et al.* 1997, 2001). As described above, the mound architecture appears to be an adaption to the relatively cool environments, but there are very few studies on the thermoregulation and gas exchange. In order to better understand the cool-environmental adaptation of mound-building *Macrotermes*, we investigated the mound architecture of *M. carbonarius* and the inside conditions in a wide range of mound size in a tropical forest of NE Thailand.

Study sites and methods

Observation sites were chosen in the Sakaerat Biosphere Reserve, Nakhon Ratchasima Province, Thailand (14°26' to 14°32'N; 101°50' to 101°57'E), which is centered in the Sakaerat Environmental Research Station (SERS). The core area of the biosphere is 57 km ^{2} and consists of natural, primary forest, some areas of natural regeneration and the SERS headquarters. The forest area is mainly classified to dry evergreen and dry dipterocarp tropical forests. Mean rainfall is 1027 mm, with mean maximum and minimum temperatures of 31.2 and 21.1°C (SERS Records form 1999 to 2008). Dry season generally starts in November and continues until February, with the monthly rainfall of less than 30 mm and with the relatively low mean monthly maximum and minimum temperatures.

The size distribution of live mounds of *Macrotermes carbonarius* (Hagen) was obtained by

observing the forest edges up to 10 m from the road within the SERS in February 2007 (dry season).

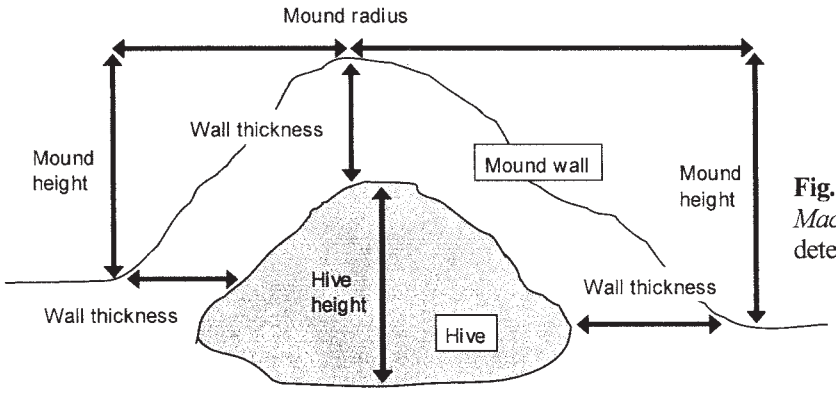

Fig. 1. Mound parameters of *Macrotermes carbonarius* determined in the present study.

In other forest sites, a total of 20 mounds, representing the major size classes (Fig. 2), were selected for further measurements in October 2008 (rainy season). For each mound, passing through the top of the mound, a line was drawn on the surface to divide the mound into two equal parts, and the mound heights were measured from the two opposite basal points of the line. On the mid-points of the two slopes of the mound along the line, two holes were excavated down to the center of the mound by using a steal pipe with the diameter of 2.5 cm. The probe of the digital thermometer CT-430WP (Custom Co. Ltd., Japan) was immediately inserted into one hole, and the hole as well as the other hole was sealed with clay soil. After the temperature of the mound center (i.e. nursery zone) reached a constant value, a 10-cm CO_2 detector tube (No. 2L, Gastec, Japan) was inserted into the other hole through the clay soil cover. One minute later, a 100-ml air was extracted directly through the gas detector tube with the gas sampler GV-100S (Gastec, Japan). The length of the color-changed zone in the gas detector tube indicated the CO_2 concentration in the sampled air after 2 minutes. When the CO_2 concentration was over the maximum limit (3%) of the CO_2 detector, a 50-ml air was re-sampled. Preliminarily, we made the gas measurements for several times in 10-20 minutes for some mounds, but the values were almost constant for each mound. The ambient temperature was also recorded at the beginning of the measurements. Along the line drawn on the surface, the mound was opened to make the vertical cross section down to the bottom of the hive, and the mound parameters shown in Fig. 1 were determined. In addition, all of the fungus-combs were collected, and the wet weights were determined in the field laboratory.

Results

During the observation in the forest edges within 10 m from the road, we found a total of 60 live mounds of *M. carbonarius*. The mound height (the mean of two opposite heights) ranged from 14 to 135cm (Fig. 2). Compared to the histogram of *M. bellicosus* mounds in mound height classes (Collins 1981), the frequency of small mounds was apparently very low in *M. carbonarius*.

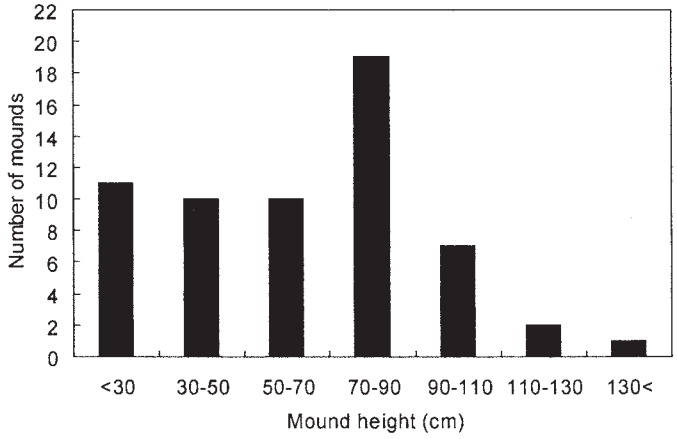

Fig. 2. A histogram of *Macrotermes carbonarius* mounds in mound height classes. The census was done in the forest edges within 10 m from the road in the SERS.

We measured mound parameters (height, radius, wall thickness, and hive height), the inside and ambient temperatures, CO_2 concentrations at the points of 10 cm from the mound surface (inside CO_2 concentrations), and fungus-comb biomass for a total of 20 mounds of *M. carbonarius*. The mound height and radius as well as the horizontal wall thickness were represented by the mean of two measures in each mound. The mound height ranged from 22 to 97 cm, and this range includes
49 of the 60 mounds described above. Collins (1981) reported that for *M. bellicosus* mounds, the mound height well represents the age and colony size; thus, the other parameters were shown in relation to the mound height in Fig. 3. The architecture of *M. carbonarius* mounds was conical- to dome-shaped structures (height to radius ratio: 0.51–1.35) with thick walls (9–61 cm both in vertical and horizontal). There were not clear air passages (*cf.* Noirot & Darlington 2000, Korb 2003), while we found air passage-like cavities and/or fungus-comb chambers excavated within the thick mound walls especially in large mounds (Fig. 4).

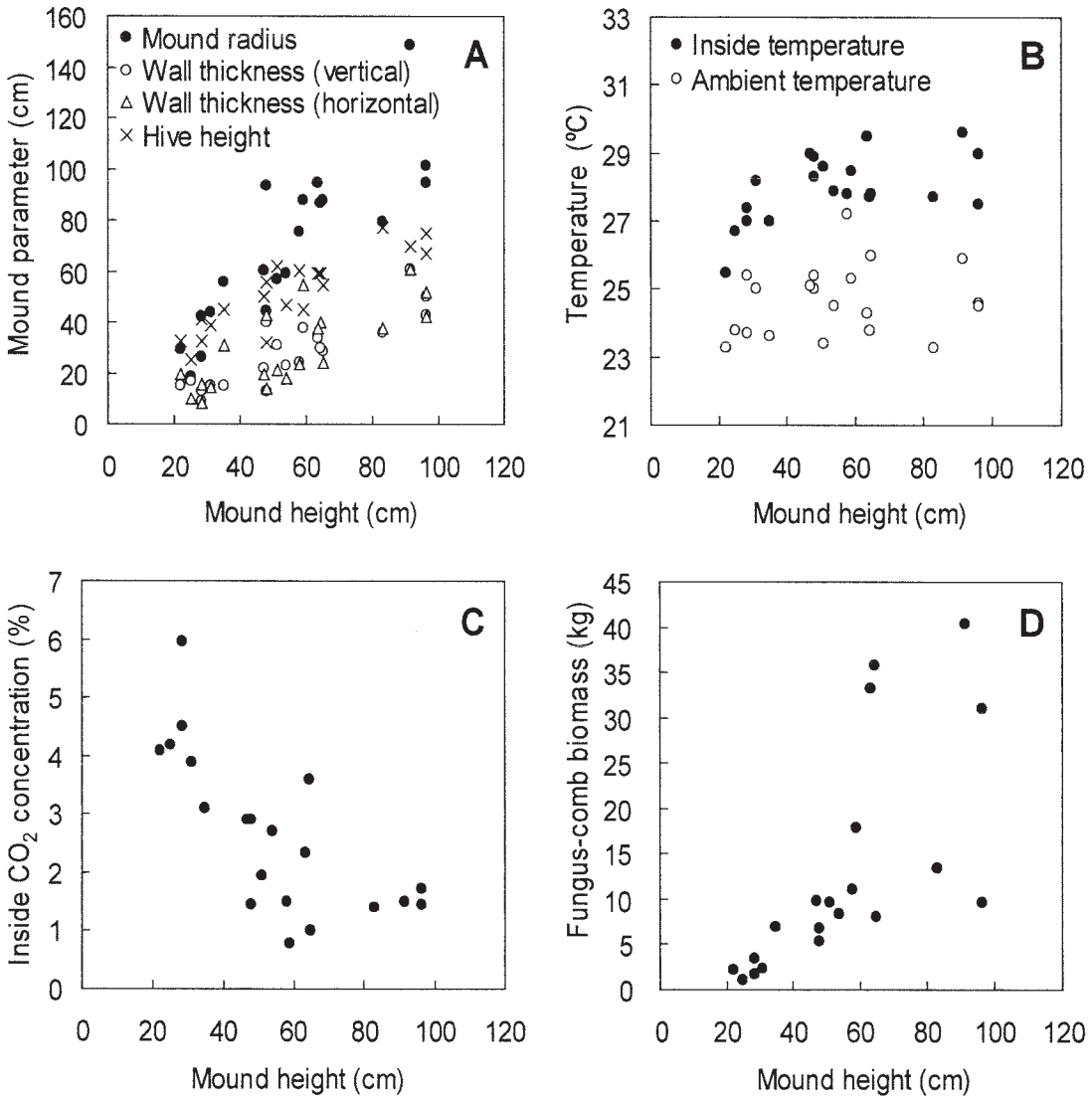

Fig. 3. (A) Mound parameters, (B) temperatures, (C) inside CO_2 concentration and (D) fungus-comb biomass in relation to the mound height of *Macrotermes carbonarius*. The mound height, radius and wall thickness (horizontal) are the mean of two measures for each mound.

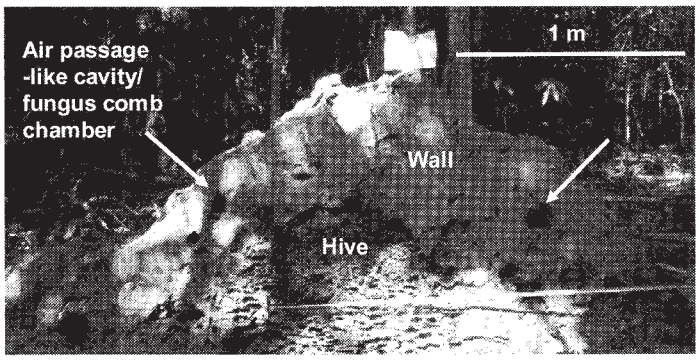

Fig. 4. A cross-section of a mound of *Macrotermes carbonarius*.

Using the observed mound parameters, the outer and inner surface areas of the mounds were estimated with the formula for an ideal cone, which is the best available approximation for mound structure. In relation to these observed and estimated parameters, ambient temperate and fungus-comb biomass, we analyzed the mound inside conditions, the inside temperature and CO_2 concentration (Table 1). The inside temperature increased significantly with the other parameters except for the ambient temperature (Table 1), while the correlations were not strong ($r < 0.7$, $r^2 < 0.5$). The inside CO_2 concentrations were significantly decreased with the other parameters except for the ambient temperature and fungus-comb biomass, and strongly correlated with the mound height and wall thickness. Most of the mound parameters and fungus-comb biomass were significantly and usually strongly correlated among each other.

In order to separate the influence of the different parameters on the inside temperature and CO_2 concentration, a multiple regression analysis was performed. The results revealed that the fungus-comb biomass and ambient temperature explained 43% of the variability in the inside temperature, while only the fungus-comb biomass was significant (Table 2). The causal relationship between ambient and inside temperature has been shown (Korb & Linsenmair 1998), whereas our observations were done at the similar ambient temperatures (Fig. 3B) and, as expected, the ambient temperature was not a significant explanation factor for the inside temperature (Table 2). Likewise, the wall thickness and ambient temperature explained 54% of the variability in the inside CO_2 concentration, and the former was significant. Although a seasonality of the inside CO_2 concentration is not taken into consideration, there has been no significant and apparent difference in the inside CO_2 concentration of the mounds of *M. bellicosus* in the gallery forest between rainy and dry seasons (Korb & Linsenmair 1988). Mastsumoto (1977) measured the CO_2 concentration of the mound center (i.e. nursery zone) of *M. carbonarius*, showing the high concentrations in large mounds (for example, 5.0% of CO_2, the height: 1.3m). This, however, does not contradict with the results, simply because there will be a CO_2 gradient within the mound. The present CO_2 concentrations are well expected to be comparable to those reported for *M. bellicosus* by Korb & Linsenmair (1999); they have measured the air in the air passages (air channels), of which the place inside the mounds is apparently very similar to the inside place where we took the air samples (*cf.* Korb & Linsenmair 1999, Korb 2003).

Table 1. Bivariate correlation analysis between mound parameters, ambient and inside temperature, inside CO_2 concentration, and fungus-comb biomass. Shown are for each combination the Pearson's correlation coefficients (r) in the upper cells and the p-values in the lower cells. *Italic* face: $p < 0.05$, **Bold** face: $r > 0.7$; number of mounds = 20. M height: mound height, M surface: mound outer surface area, M thickness: wall thickness (vertical), H height: hive height, M inner surface: mound inner surface area, A temp: ambient temperature, I temp: inside temperature, CO_2: inside CO_2 concentration, Fungus-comb: fungus-comb biomass.

		1	2	3	4	5	6	7	8	9
1	M height	-	***0.850***	***0.868***	***0.882***	***0.796***	0.203	*0.538*	***-0.730***	*0.692*
2	M surface	***<0.001***	-	***0.934***	*0.667*	***0.933***	0.331	*0.604*	*-0.645*	***0.780***
3	M thickness	***<0.001***	***<0.001***	-	*0.647*	***0.804***	0.160	*0.583*	***-0.746***	*0.692*
4	H height	***<0.001***	*0.001*	*0.002*	-	*0.669*	0.143	*0.449*	*-0.588*	*0.594*
5	M inner surface	***<0.001***	***<0.001***	***<0.001***	*0.001*	-	*0.470*	*0.622*	*-0.612*	***0.730***
6	A temp	0.391	0.154	0.500	0.548	*0.036*	-	0.397	-0.307	0.094
7	I temp	*0.014*	*0.005*	*0.007*	*0.047*	*0.003*	0.083	-	*-0.536*	*0.609*
8	CO_2	***<0.001***	*0.002*	***<0.001***	*0.006*	*0.004*	0.188	*0.015*	-	-0.404
9	Fungus-comb	*<0.001*	***<0.001***	*<0.001*	*0.006*	***<0.001***	0.692	*0.004*	0.077	-

Table 2. Results of the multiple regression analysis of inside CO_2 concentration and temperature in relation to the mound parameters (mound height, mound outer and inner surface, vertical wall thickness, hive height), ambient temperature and fungus-comb biomass. The combinations shown are selected on the basis of the AIC values (= number of samples × $\log_e(1-r^2)$ × 2 × number of variables) by testing all the combinations (= 120) with deletion of multicollinear variables. **Bold** face: significant variable, r_s: adjusted r, Fungus-comb: fungus-comb biomass, A temp: ambient temperature, M thickness: wall thickness (vertical).

Inside temperature (AIC = -9.341)					Inside CO_2 concentration (AIC = -16.935)				
Variable	r_s^2	*df*	*f*	*p*	Variable	r_s^2	*df*	*f*	*p*
	0.4264	2; 17	8.062	0.003		0.5441	2; 17	12.337	<0.001
Fungus-comb				0.004	**M thickness**				<0.001
A temp				0.066	A temp.				0.236

Discussion

In the relatively hot tropical environments, the mounds of *Macrotermes* species such as *M. jeanneli* and *M. bellicosus* have achieved the optimal inside conditions for fungus cultivation by using the elaborate architecture-based ventilation systems on the basis of higher ambient temperatures (Darlington *et al.* 1997, Noirot & Darlington 2000, Korb 2003). Although *M. bellicosus* also inhabit relatively the cool environment by building the apparently insulated dome-shaped mounds with thick walls, the termites are suggested to be suffering from a trade-off between thermoregulation and ventilation due to the low ambient temperature (Korb 2003).

In the present tropical forest of NE Thailand, *M. carbonarius* had made the dome- or conical-shaped massive mounds as is the case of *M. bellicosus* in the gallery forest (Korb 2003), but these mounds lacked clear air passages. Despite the presence of more or less clear air passages in African *Macrotermes* mounds including *M. bellicosus* mounds in the gallery forest (Noirot & Darlington 2000, Korb 2003), the absence from the mounds of *M. gilvus* has been also reported form a tropical forest of NE Thailand (Inoue *et al.* 1997). The inside conditions of large mounds of *M. carbonarius* (Fig. 3B, 3C) were usually maintained being equally to or slightly more optimally than those of *M. bellicosus* in the gallery forest (ca. 28°C and 1.0–1.5% of CO_2; Korb & Linsenmair 1998, 1999).The concordance of *M. carbonarius* with the forest *M. bellicosus* strongly supports the common architecture of the mounds (dome-shape, thick walls) is an adaptation to such cool environments and the existence of a trade-off between thermoregulation and ventilation. Furthermore, since air passages are generally related with the presence of the thin part of mound walls (Noirot & Darlington 2000, Korb 2003), the architecture of *M. carbonarius* mounds may have higher insulation and be an adaptation to cooler environments. Supporting this, *M. bellicosus* mounds have been found only in relatively hot sites (open stands) of the gallery forests (Korb & Linsenmair 1998).

The inside temperature of small *M. bellicosus* mounds has been below 30°C even in the savanna (Korb & Linsenmair 1998). In line with this, small colonies of *M. bellicosus* build dome-shaped mounds with thick walls (Korb 2003). On this basis, Korb (2003) supposed that this insulation does not compromise with gas exchange due to the low gas exchange requirements. However, it does not seem to be the case at least for *M. carbonarius* in the present forest. Compared to large mounds, our results showed that the inside conditions of small mounds were apparently far from optimal not only for the temperature, but also for the inside CO_2 concentration (Fig. 3B, 3C). It is implied that these small mounds are in a compromise at lower levels, which probably means a high mortality of such small colonies. This could give an explanation to the apparently low frequency of small mounds of *M. carbonarius* in the forest (Fig. 2) compared to that of *M. bellicosus* in an African savanna (Collins 1981).

The small mounds of *M. carbonarius* appear not to be insulated enough to maintain the inside temperature being optimal. Higher insulation means less heat loss by heat transfer between inside and outside through the walls. Theoretically, insulation linearly increases with the increasing thickness of walls and with the decreasing inside surface areas under a constant difference in the temperature between inside and outside.

Assuming that an ideal colony of *M. carbonarius* develops with keeping the same ratio of the mound height to the other mound dimensions, the inside surface area will be a quadric function of the height, and the wall thickness will be a linear function of the height; the insulation linearly decreases with the increasing height. Meanwhile, the volume of the mound inside (more or less an approximation of fungus-comb biomass and thus heat production) will be a cubic function of the height. Here, since heat production (a cubic function of the height) is expected to more sharply increase than heat loss (a quadric function of the height), the colony will get to lose relatively less heat by heat transfer via walls along the development. In fact, the importance of fungus-comb biomass was indicated by the multiple regression analysis, which showed that the fungus-comb biomass is the single significant explanation factor for the inside temperature (Table 2). These suggest that tropical forests are unexpectedly cool environments for small colonies living in small mounds. Some small colonies may build unproportionally thick walls to heighten the insulation, but the present study indicates that the limited resources had better be used for the colony development, which means the increasing biomass of fungus-combs (i.e. heat production).

A paradox can be found in the decreasing CO_2 concentrations in the larger mounds (Fig. 3C). As mentioned above, the ratio of the inside surface area to the inside volume (an approximation of CO_2 production) will decrease with the increasing height; the CO_2 concentration should increase with the increasing height, if gas exchange occurs only though the inside wall surface (Noirot &Darlington 2000, Korb 2003). In the case of the mounds of *M. bellicosus* in the gallery forest, gas exchange seems to occur in the central and peripheral turrets with comparably thin walls (Korb & Linsenmair 2000). The *M. carbonarius* mounds, however, did not have such conspicuous structures. A possible explanation could be found in air passage-like cavities and/or fungus-comb chambers excavated within the thick mound walls. In spite of no hard data, such wall-inner structures seemed to be more frequently and abundantly found in larger mounds. It is speculated that gas exchange occurs in the wall-inner structures through the comparably short distance from the outside, resulting in the lower CO_2 concentrations in the larger mounds, while at the same time these structures are expected not to compromise the insulation (see above). The multiple regression analysis at least partly supports the hypothesis, showing that the wall thickness is the single significant explanation factor for the CO_2 concentration and simultaneously is not a significant explanation factor for the inside temperature (Table 2). Nevertheless, even the large mounds of *M. carbonarius* in the forest are still in a compromise between thermoregulation and gas exchange at higher, but not fully optimal levels, although the increased heat in the large mounds is probably allowing gas exchange in such a relatively high rate.

Conclusions

In the forest of NE Thailand, the termites of *M. carbonarius* make dome- or conical-shaped mounds with thick walls. The inside conditions, the inside temperature and CO_2 concentration, are both suboptimal or nearly optimal in large mounds, but not in small mounds probably due to the low heat production from fungus-combs. The presence of a trade-off between thermoregulation and gas exchange is strongly suggested in relatively cool environments (i.e. tropical forests). Although African tropical forests have been suggested to have allowed the evolution of fungus-growing termites (and probably the genus *Macrotermes*, Aanen & Eggleton 2005), tropical forests are not fully optimal habitats for mound-building *Macrotermes* termites, especially for the small colonies.

Acknowledgments

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References

Aanen, D. K. and P. Eggleton 2005 Fungus-growing termites originated in African rain forest. *Current Biology* **15**, 851–855.

Collins, N. M. 1981 Populations, age structure and survivorship of colonies of *Macrotermes bellicosus*

(Isoptera, Macrotermitinae) *Journal of Animal Ecology* **50**, 293–311.
Darlington, J. P. E. C, P. R. Zimmerman, J. Greensberg and C. Westberg 1997 Production of metabolic gases by nests of the termite *Macrotermes jeanneli* in Kenya. *Journal of Tropical Ecology* **13**, 491–510.
Inoue, T., N. Kirtibutr and T. Abe 2001 Underground passage system of *Macrotermes carbonarius* (Isoptera, Termitidae) in a dry evergreen forest of northeast Thailand. *Insectes Sociaux* **48**, 372–377.
Inoue, T., P. Vijarnsorn, and T. Abe 1997Mound structure of the fungus-growing termite *Macrotermes gilvus* in Thailand. *Journal of Tropical Ecology* **13**, 115–124.
Konaté, S., X. Le Roux, B. Verdier and M. Lepage 2003 Effect of underground fungus-growing termites on carbon dioxide emission at the point- and landscape-scales in an African savanna. *Functional Ecology* **17**, 305–314.
Korb, J. 2003 Thermoregulation and ventilation of termite mounds. *Naturwissenschaften* **90**, 212–219.
Korb, J. and K. E. Linsenmair 1998 The effects of temperature on the architecture and distribution of *Macrotermes bellicosus* (Isoptera, Macrotermitinae) mounds in different habitats of a West African Guinea savanna. *Insectes Sociaux* **45**, 51–65.
Korb, J. and K. E. Linsenmair 1999. The architecture of termite mounds: a result of a trade-off between thermoregulation and gas exchange? *Behavioral Ecology* **10**, 312–316.
Korb, J. and K. E. Linsenmair 2000. Ventilation of termite mounds: new results require a new model. *Behavioral Ecology* **11**, 486–494.
Matsumoto, T. 1977. Respiration of fungus combs and CO_2 concentration in the center of mounds of some termites. *In*: Proceedings of the Eighth International Congress of the IUSSI, Wageningen, the Netherlands, September. Wageningen. J. de Wilde J, ed. Centre for Agricultural Publishing and Documentation, Wageningen, pp. 104–106
McComie, L. D. and G. Dhanarajan 1990 Respiratory rate and energy utilization by *Macrotermes carbonarius* Hagen (Isoptera, Termitidae, Macrotermitinae) in Penang, Malaysia. *Insect Science and its Applications* **11**, 197–204.
McComie, L. D. and G. Dhanarajan 1993 The physical and chemical composition of mounds of *Macrotermes carbonarius* (Hagen) (Termitidae, Macrotermitinae), in Penang, Malaysia. *Journal of Insect Science* **44,** 427–433.
Noirot, C. and J. P. E. C. Darlington 2000 Termites nests: architecture, regulation and defence. *In*: Termites: Evolution, Society, Symbioses, Ecology. T. Abe, D.E. Bignell & M. Higashi, eds. Kluwer Academic, Dordrecht, pp. 121–139.
Sands, W. A. 1969 The association of termites and fungi. *In*: Biology of Termites I. K. Krishna & F.M. Weesner, eds. Academic Press, New York, pp. 495–524.
Thomas, R. J. 1987 Factors effecting the distribution and activity of fungi in the nests of Macrotermitinae (Isoptera). *Soil Biology and Biochemistry* **19**, 343–349.
Yamada, A., T. Inoue, D. Wiwatwitaya, M. Ohkuma, T. Kudo, T. Abe & A. Sugimoto 2005.Carbon mineralization by termites in tropical forests, with emphasis on fungus combs. *Ecological Research* **20,** 453–460.

Morphometric Study of *Hospitalitermes* and *Lacessititermes* Based on Soldier Caste Characters

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Abstract

Morphometric study of the related termite genera *Hospitalitermes* and *Lacessititermes* (Isoptera; Termitidae, Nasutitermitinae) have been carried out. Multivariate analyses (Principal Component Analysis and Canonical Variate Analysis) were used to assess the fidelity of the existing allocation of museum and field-collected specimens to these two genera and to species level within each genus. The two genera can easily be differentiated using the mandible characters of the worker caste. In *Lacessititermes* there is a distinct notch on the lower dorsal edge of the molar plate while *Hospitalitermes* does not have this character. Multivariate analysis confirmed a clear distinction between the genera based on the soldier caste and without reference to worker characteristics. From examination of specimens and from the result of multivariate analysis it is proposed that *Hospitalitermes hospitalis* forma *hospitaloides* and *H. medioflavus* are synonyms of *H. hospitalis; H. madras* is a synonym of *H. jepsoni; H. schmidti* is synonym of *H. rufus; H. irianensis* and *H. moluccanus* are synonyms of *H. papuanus* and *H. umbrinus* forma *sharpi* is a synonym of *H. umbrinus.* Of the 16 species of *Lacessititermes* currently recognised, I have added two species previously allocated to *Hospitalitermes* i.e. *H. butelli* and *H. nemorosus. L. filicornis* has been overturned as being invalid and synonymised to *L. laborator.*

Key words: morphometric study, multivariate analyses (PCA and CVA), *Hospitalitermes, Lacessititermes*

Introduction

Processionary termites (genus *Hospitalitermes* and *Lacessititermes* of the subfamily Nasutitermitinae, family Termitidae) are recognised as being of exceptional biological and sociobiological interest, and are a notable feature of lowland forests in South East Asia, including Borneo and parts of the Indonesian archipelago. While the majority of termite species are cryptic and forage through tunnels and galleries in soil or wood, and under covered runways over surfaces (Wood, 1978), *Hospitalitermes* and *Lacessititermes* forage above ground in exposed processionary columns, searching for food on tree-trunks and in the high canopy of tropical rain forests (Jones and Gathorne-Hardy, 1995; Miura and Matsumoto, 1998).

The systematics of these two genera are very poorly known. At an alpha taxonomic level there are many names, and species are often notionally separated by small differences in size and head shape, and by poorly defined colour characters. Since the original descriptions were made without the use of standard colour charts, the colour characters are difficult to use with live specimens. Furthermore colour is often impossible to use with preserved specimen because the colour can fade over time in preservative, and notably so in alcohol, which is used in all museum collections.

There are 36 species of *Hospitalitermes* and 16 species of *Lacessititermes* currently described. The status of many species needs to be clarified due to misidentification and misplacement within the genus. Tho (1992) stated that at least two species described under *Hospitalitermes* may in fact be species of *Lacessititermes*. It is recognized that a revision of the genera is therefore urgently required (Jones and Brendell, 1998), both for purely taxonomic reasons and also to assist in biodiversity assessment and conservation management. What is required is to clearly define each species, and to confirm which genus each species belongs to. New statistical methods have revolutionized taxonomic work, even without resort to DNA sequencing, and it is therefore appropriate to review all current taxonomic designations with these methods, as some descriptions are a century old.

Materials and methods

Origin of materials

From 36 species of *Hospitalitermes* currently described, I have only been able to examine 22 (Table 1). The following type (holotype or paratype) specimens of *Hospitalitermes* species are represented in this study: *asahinai, ataramensis, bicolor, birmanicus, blairi, butteli, diurnus, ferrugineus, hospitalis, hospitalis* f. *hospitaloides, irianensis, jepsoni, madrasi, medioflavus, moluccanus, monoceros, nemorosus, papuanus, rufus, schmidti, umbrinus* and *umbrinus* f. *sharpi*. I have attempted to obtain the type specimens for the other species, but failed to get responses to loan requests from the relevant institutions. Light and Wilson (1936) said that the type specimens of two of Oshima's species (*H. flavoantennaris* and *H. luzonensis*) were destroyed when the alcohol in the specimen tubes evaporated.

For *Lacessititermes*, I examined 12 species out of the 16 species currently described (Table 1). The species represented in this study are: *albipes, atrior, batavus, breviarticulatus, cuphus, filicornis, holmgreni, laborator, lacessitiformis, lacessitus, piliferus* and *sordidus*.

Table 1 List of species *Hospitalitermes* and *Lacessititermes* included in Ordination analyses

No.	Species name	Abbreviation	Number of specimens examined
1	*Hospitalitermes asahinai*	Hasah	6
2	*Hospitalitermes ataramensis*	Hatar	2
3	*Hospitalitermes bicolor*	Hbico	10
4	*Hospitalitermes birmanicus*	Hbirm	6
5	*Hospitalitermes blairi*	Hblai	1
6	*Hospitalitermes butteli*	Hbutt	2
7	*Hospitalitermes diurnus*	Hdiur	11
8	*Hospitalitermes ferrugineus*	Hferr	9
9	*Hospitalitermes hospitalis*	Hhosp	10
10	*Hospitalitermes hospitalis* f. *hospitaloides*	Hhhpt	10
11	*Hospitalitermes irianensis*	Hiria	1
12	***Hospitalitermes jepsoni***	Hjeps	7
13	*Hospitalitermes madrasi*	Hmadr	4
14	*Hospitalitermes medioflavus*	Hmed	10
15	*Hospitalitermes moluccanus*	Hmolu	6
16	*Hospitalitermes monoceros*	Hmono	11
17	*Hospitalitermes nemorosus*	Hnemo	2
18	*Hospitalitermes papuanus*	Hpapu	5
19	*Hospitalitermes rufus*	Hrufu	10
20	*Hospitalitermes schmidti*	Hschm	6
21	*Hospitalitermes umbrinus*	Humbr	10
22	*Hospitalitermes umbrinus* f. *sharpi*	Husha	10
23	*Lacessititermes albipes*	Lalbi	5
24	*Lacessititermes atrior*	Latri	6
25	*Lacessititermes batavus*	Lbata	1
26	*Lacessititermes breviarticulatus*	Lbrev	6
27	*Lacessititermes cuphus*	Lcuph	1
28	*Lacessititermes filicornis*	Lfili	5
29	*Lacessititermes holmgreni*	Lholm	1
30	*Lacessititermes laborator*	Llabo	6
31	*Lacessititermes lacessitiformis*	Llacf	6
32	*Lacessititermes lacessitus*	Llact	6
33	*Lacessititermes piliferus*	Lpili	14
34	*Lacessititermes sordidus*	Lsord	6

Those species for which type material could not be examined were excluded from the ordination analyses because the published descriptions were not adequate and did not give all the sizes and characters used in the analyses.

149 *Hospitalitermes* and 63 *Lacessititermes* soldier caste type specimens were examined. Specimens for this study were obtained from the following institutions: Cambridge Museum of Zoology, the Natural History Museum (BMNH), and American Museum of National History (AMNH).

Characters

22 characters were used for the morphometric studies presented here (Table 2). Fig. 1 shows how the characters were measured. Measurements for morphometric studies were carried out on a Leica MZ8 microscope with a VTO232 Video Text Overlay. The equipment had been calibrated prior to the measurements.

Table 2 List of characters used in morphometric studies.

No.	Characters (in mm)	Abbreviation
1	Length of head to tip of nasus (**a1**)	LoHd-Ns
2	Length of head to base of mandibles (**a2**)	LoHd-Md
3	Length of nasus (**a3**)	LoNs
4	Height of head excluding postmentum (**a4**)	HoHd
5	Maximum width of head capsule (**b1**)	WoHd
6	Width of head at point of constriction (**b2**)	WoHd-C
7	Nasus width at base of mandibles (**b3**)	WoNs
8	Length of hind femur (**c1**)	LoHiFm
9	Length of hind tibia (**c2**)	LoHiTb
10	Length of 2nd antennal segment (**d1**)	LoAS-2
11	Length of 3rd antennal segment (**d2**)	LoAS-3
12	Length of 4th antennal segment (**d3**)	LoAS-4
13	Pronotum length (**e1**)	LoPr
14	Pronotum width (**e2**)	WoPr
15	Head index I (**b1/a2**)	HdI-1
16	Head index II (**a4/a2**)	HdI-2
17	Head index III (**a4/b1**)	HdI-3
18	Nasus-head index I (**a3/a1**)	NsHdI-1
19	Nasus-head index II (**b3/b1**)	NsHdI-2
20	Ratio of antennal segment III/II (**d2/d1**)	RAS-3/2
21	Ratio of antennal segment IV/III (**d3/d2**)	RAS-4/3
22	Pronotum index (**e1/e2**)	PrI

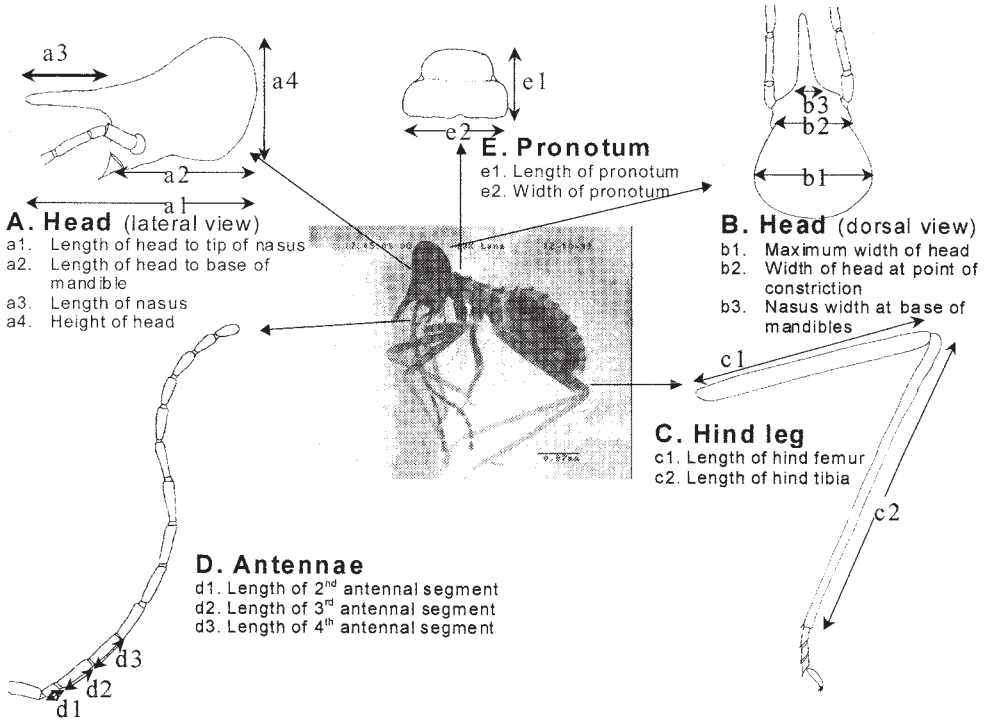

Figure 1 Soldier of *Hospitalitermes hospitalis* showing the measurements used as characters for morphometric studies.

Morphometric studies

The morphometric studies were carried out using principal component analysis (PCA) to establish the patterns of variation between the species, and canonical variate analysis (CVA) to estimate the value of the species-level clusters. Both analyses were performed using CANOCO software version 4.5 (ter Braak and Smilauer, 2002). The PCA and CVA were based on the numbers of type specimens per species (Table 1). All specimens were measured, except those that were damaged.

For multivariate analyses in CANOCO, data from *Hospitalitermes* and *Lacessititermes* was prepared in spreadsheet files, namely HL_char (containing the measurements of 22 characters of *Hospitalitermes* and *Lacessititermes* with the name of each specimen in the first column); HL_spec (containing a matrix of species of both *Hospitalitermes* and *Lacessititermes* with each specimen belonging to a species having a value of 1 for this variable and 0 values for other variables); Ht_char (containing the measurements of 22 characters of *Hospitalitermes* with the name of each specimens in the first column); Ht_spec (containing a matrix of species of *Hospitalitermes* with each specimen belonging to a species having a value of 1 for that variable and 0 values for all other variables); Lt_char (containing measurements of 22 characters of *Lacessititermes* with the name of each specimens in the first column); Lt_spec (containing a matrix of species of *Lacessititermes* with each specimen belonging to a species having a value of 1 for that variable and 0 values for all other variables).

Results and discussion

Principal component analysis (PCA)

The two genera are clearly separated in the PCA (Figure 2). The Principal component analysis yielded good separation of *Hospitalitermes* and *Lacessititermes*, except for *H. butteli* and *H. nemorosus* (shown by red circles). In subsequent analyses *H. butteli* and *H. nemorosus* are treated as *Lacessititermes*. The following

characters are most influential in making the *Lacessititermes* cluster together; Nasus Head Index I, Nasus Head Index II, Width of Nasus, Length of Nasus, Head Index I, Head Index II (all of these size characters are contributing into the shape of head) and Ratio Antennal Segment IV/III.

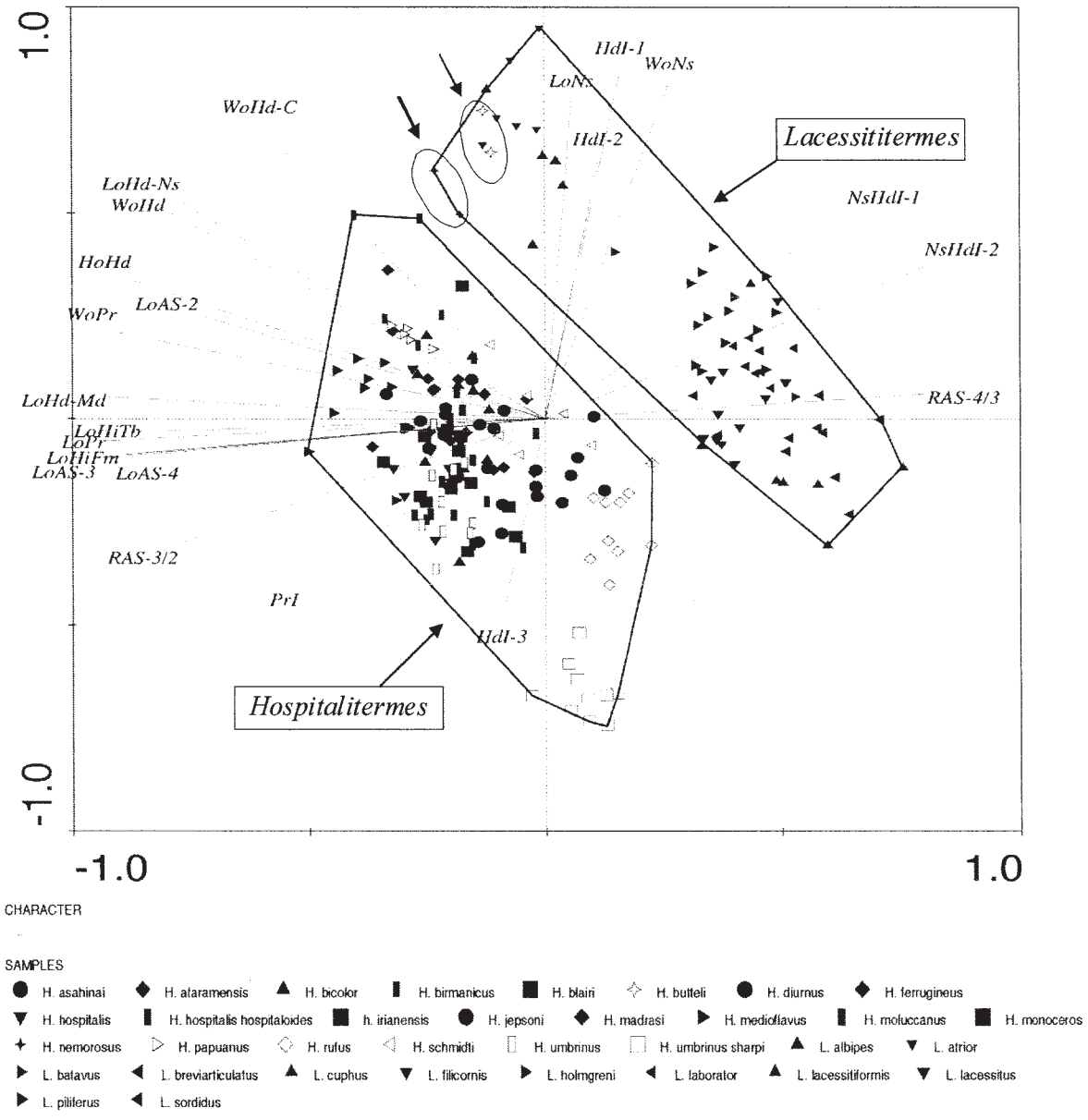

Figure 2 Principal component analysis of type specimens of *Hospitalitermes* and *Lacessititermes* based on soldier caste characters. The envelopes enclose all specimens of each genus, except *H. butteli* and *H. nemorosus* (arrowed, see text).

Canonical variate analysis (CVA)

As shown in Figure 2 and 3, principal component analysis and canonical variate analysis of type specimens of *Hospitalitermes* and *Lacessititermes* yield complete separation between the two genera.

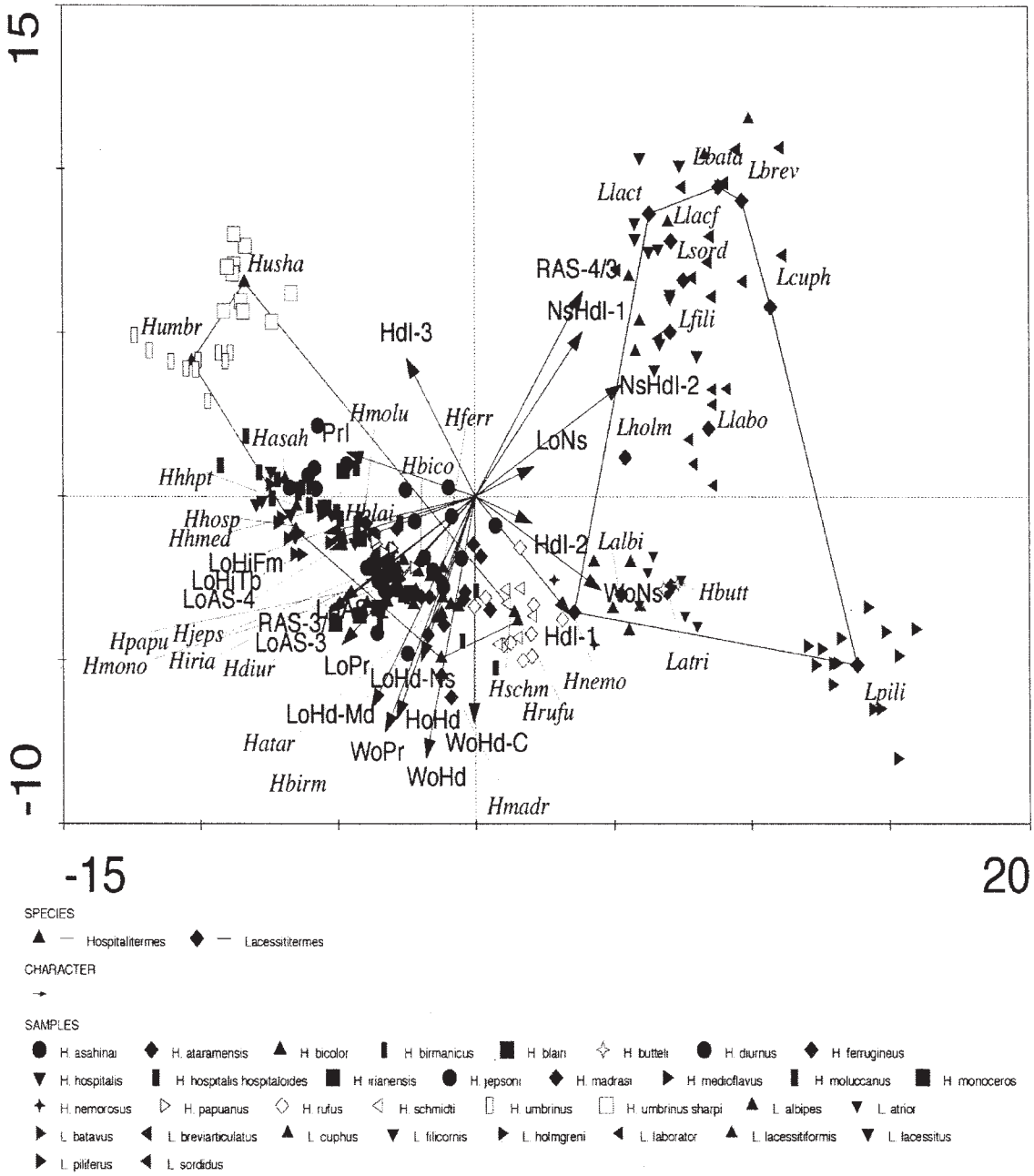

Figure 3 Canonical variate analysis of type specimens of *Hospitalitermes* and *Lacessititermes* based on soldier caste characters. The envelopes enclose the centroids of each species of *Hospitalitermes* and *Lacessititermes*. *H. butteli* and *H. nemorosus* fall within the *Lacessititermes* envelope (see text)

From Figure 3, we can see that the same sets of characters as in the principal component analysis also separate the *Lacessititermes* clusters from the *Hospitalitermes* clusters in the CVA. From the result of these two analyses it is clear that the nasus, head shape and antennal segments IV and III are important characters in differentiating the two genera. This result also supports the morphological characters currently used to separate the two genera: *Lacessititermes* have

a nasus which is cylindrical to conical and broader at the base, while *Hospitalitermes* have a nasus which is short, cylindrical, not much broader at the base and never markedly conical (see Figure 4).

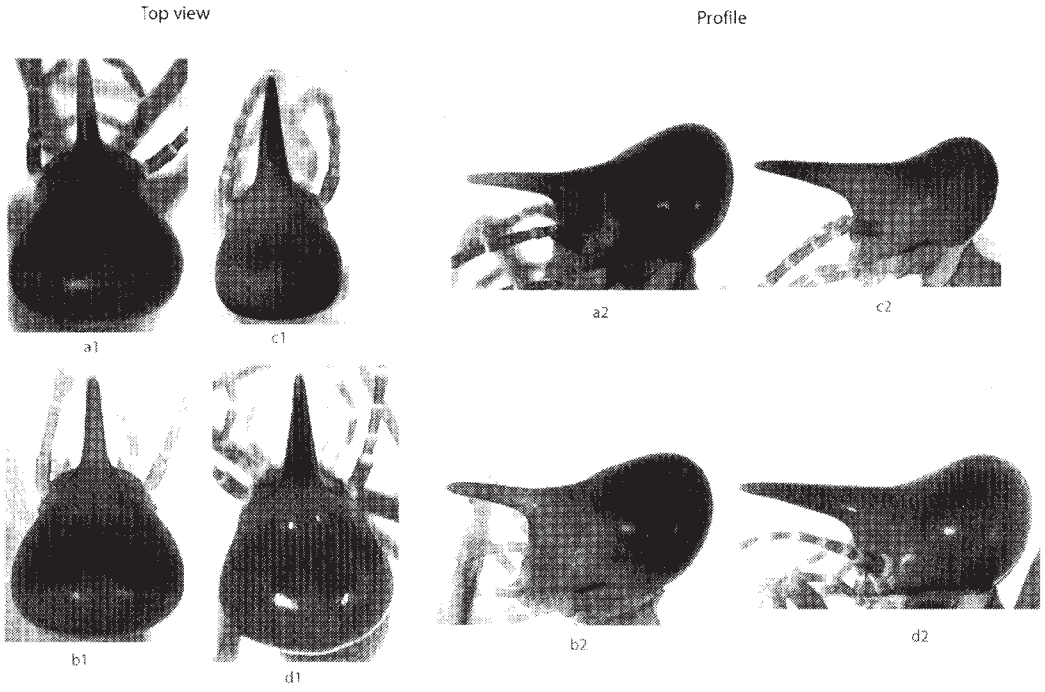

Figure 4 Head shape comparison between *Hospitalitermes* and *Lacessititermes* (a1, a2=*H. hospitalis*, b1, b2= *H. bicolour;* c1, c2 = *L. lacessitus,* d1, d2= *L. atrior*).

In the result shown in Figure 3 we can see that the specimens of *H. butteli* and the specimens of *H. nemorosus* are placed within the *Lacessititermes* cluster. This suggests that *H. butteli* and *H. nemorosus* should be reclassified as *Lacessititermes.* This is supported by the fact that the worker castes of both species have a notch in the right mandible. Tho (1992) has already speculated that *H. butteli* and *H. luzonensis* may be species of *Lacessititermes.* The current CVA results support his opinion that *H. butteli* is within *Lacessititermes.* In addition, although *H. luzonensis* was not included in the analysis (because specimens could not be obtained) the figures in the original description clearly show the worker mandible as having a notch, and thus also being within *Lacessititermes.* The other *Hospitalitermes* species that may be *Lacessititermes* is *H. flavoantennaris* Oshima. It was not possible to inspect the specimens, but from the description and figure available it is clear that the head shape and the presence of the worker right mandible with a notch provide enough evidence to exclude *H. flavoantennaris* from the list of species of *Hospitalitermes.*

In the analysis of *Hospitalitermes, H. rufus, H. umbrinus* and *H. umbrinus sharpi* are very different from other species of *Hospitalitermes.* When compared with other species of *Hospitalitermes, H. rufus* and *H. umbrinus sharpi* are small with average Lo-Hd-Ns (Length of head to tip of nasus) 1.577 ± 0.053 mm and 1.582 ± 0.048mm respectively. All other species have an average of more than 1.7 mm. *H. umbrinus* and *H. umbrinus sharpi* have a larger mean PrI (Pronotum index: pronotum length/pronotum width) when compared with other *Hospitalitermes* species. For *Hospitalitermes,* key characters for separation are size of specimen (LoHd-Ns, i.e. Length of head to tip of nasus), Nasus-head index (NsHdI-1, i.e. Length of nasus/Length of head to tip of nasus) and Pronotum index (PrI).

In *Lacessititermes, L. piliferus is* very different to other species of *Lacessititermes* because it has a small Nasus-head index I (NsHdI-1, Length of nasus/Length of head to tip of nasus) (0.386 ± 0.012 mm) while other species have an average of more than 0.4 mm. *H. butteli, H. nemorosus* (the two species of *Hospitalitermes*

that in fact are species of *Lacessititermes), L. atrior* and *L. albipes* are on average bigger than other *Lacessititermes* species.

Hospitalitermes umbrinus and *H. umbrinus sharpi* are clearly separated one from the other (see Figure 3). However, I proposed these two species to be synonymised. After further field collections and observation, there are specimens which have body sizes between those hitherto considered characteristic of these two species. These lead to the conclusion that the small size of *H. umbrinus sharpi* is solely because they were collected from a newly founded colony.

These morphometric analyses were able to separate species of the two genera. However within genera the analyses were only able to separate some species at either end of the size range (i.e. large and small). Clusters of species with intermediate sizes were not separated clearly. This suggests that we cannot rely on dimensions alone in trying to separate species within a genus.

Conclusions

1. *Hospitalitermes* and *Lacessititermes* are clearly separable on soldier characters using modern multivariate analyses, and this separation is confirmed by the presence or absence of the worker mandible notch.
2. *Hospitalitermes butteli* and *H. nemorosus* are *Lacessititermes* not *Hospitalitermes.*
3. Within *Hospitalitermes,* the most important characters for separating species are Ratio antennal segment IV/III, Head index III, Ratio antennal segment III/II and Head index II.
4. Within *Lacessititermes,* the most important characters for separating species are Pronotum index, Nasus head index I, Head index III, Nasus head index II and Ratio antennal segment IV/III.

References

Jones, D. T. and M. J. D. Brendell 1998 The termite (Insecta: Isoptera) fauna of Pasoh Forest Reserve, Malaysia. *Raffles Bulletin of Zoology*, **46**, 79-91.

Jones, D. T. and F. Gathorne-Hardy 1995 Foraging activity of the processional termite *Hospitalitermes hospitalis* (Termitidae: Nasutitermitinae) in the rain forest of Brunei, north-west Borneo. *Insectes Sociaux*, **42**, 359-369.

Light, S. F. and F. J. Wilson 1936 The Nasute termites of the Philippines. *The Philippine Journal of Science*, **60** (4), 461-520.

Miura, T. and T. Matsumoto 1998 Foraging organization of the open-air processional lichen-feeding termite *Hospitalitermes* (Isoptera, Termitidae) in Borneo. *Insectes Sociaux*, **45** (1), 17-32.

Tho, Y. P 1992 Termites of Peninsular Malaysia. (Kirton, L.G. Ed.). Malayan Forest Records, No. 36: 224 pp. Forest Research Institute Malaysia, Kepong.

Ter Braak, C. J. F. and P. Smilauer 2002 *Canoco Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (version 4.5).* Ithaca, NY: Microcomputer Power, 500 pp.

Wood, T. G 1978 Food and feeding habits of termites. In Production Ecology of Ants and Termites (ed M.V. Brian), pp. 55-80. Cambridge University Press, Cambridge.

Determining the Sample Size on Termite Sex Ratio Studies

by
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Abstract

The most challenging part of a termite sex ratio study is gathering the data. Many different sample sizes have been used for termite sex ratio studies in the literature and most samples sizes were less than 50. This paper scrutinizes the relationships between different sample sizes (varied from 25 to 1600 observations). It shows that a sample size of less than 50 is too small to expose the true sex ratio of a population, and 100 termites are required to provide a respectable ±10% error in a termite sex ratio census. The results reported here help improve precision in estimates of termite colony sex ratios and propose a standard sample size for such studies.

Key words: Isoptera, sex ratio, sample size, sexual dimorphism, *Reticulitermes flavipes*

Introduction

Sex allocation and sex-determination mechanisms in termites are intriguing because they display a remarkably complex and diversified caste system. Investigations of sex ratios are essential parts of the study of termite mating systems and caste development. Progress has been made toward understanding sex allocation in termites, such as *Amitermes* (Noirot 1955), *Coptotermes* (Dean and Gold 2004, Henderson 1996, Huang and Chen 1983, Roisin and Lenz 2002), *Macrotermes* (Darlington 1986), *Nasutitermes* (McMahan et al. 1983, Thorne 1983) and *Reticulitermes* (Hayashi et al. 2007, Herfs 1951, Matsuura 2006, Zimet and Stuart 1982). However, a variety of sample sizes have been utilized in the literature; most studies report using sample sizes of 25 to 50 (Dean and Gold 2004, Howard and Haverty 1980, Jones et al. 1988, Pawson and Gold 1996, Zimet and Stuart 1982), which have provided contradictory results (Table 1).

According to mark-release-recapture estimates of *Reticulitermes* spp. and *Coptotermes* spp., colony foraging populations range from 127 to 6.8 $\times 10^6$ termites per colony and workers account for 95-98% of the whole population (Forschler and Townsend 1996, Grace 1990, Howard and Haverty 1980, Su et al. 1993). But what size sample is required for illuminating the sex ratio of such large populations? The answer to this sample size question is influenced by several factors, including population size, the desired level of precision, the level of confidence for risk, and the degree of variability in the attributes being measured (Miaoulis and Michener 1976). This paper describes an attempt, using worker castes members from *Reticulitermes flavipes* (Kollar), to verify the sample size appropriate to meet several levels of precision for termite sex ratio studies. Our study assummed a worker population of >300,000 and applied two strategies to determine appropriate sample size; first was a sex ratio census of a field collected population, and the second applied mathematical formulas to calculate sample size.

Materials and methods

Four distinct populations of *Reticulitermes flavipes* were collected for this study; three from Athens in Clarke County and one from Sapelo Island in MacIntosh County, Gerorgia, USA. Termites were fixed in 100% ethanol. Sixty-four groups of 25 workers from each collection were randomly chosen and viewed under a dissecting microscope. Sex was determined by the arrangement of sternal plates as described by Zimet and Stuart (1982). The sternal plate character was verified as a correct indication of sex in worker subterranean termites by dissecting 10-20 workers of each sex to confirm the presence of ovaries or testies. The seventh sternite of males is similar in size to the other sternites and is not convex (Fig.1a). Females are characterized by an enlarged, elongated and distally convex seventh sternite (Fig.1b). The sex ratio of sample sizes of 25, 50, 100, 200, 400 and 800 were compared with the sample size of 1600 within ±5%, ±10% or ±20% error.

Results and discussion

Using a census of a large population

According to the results, 24.87±8% of the 25 sample size replicates were within ±5% error. The sample size of 100 provided 34.26±6% of the replicates consistent with the sex ratio of the sample of 1600. While the sample size of 25 had 44.68±7% of the replicates within ±10% error, 84% of the 100 sample size replicates were consistent with the sample size of 1600. And within ±20% error, 76.27±11% of replicates of sample size of 25, and 100% replicates of sample size of 100 were consistent with the sample size of 1600 (Fig. 2).

With a sample size of 25, the sex ration estimate varied as much as 9 fold the population estimate (Fig. 3) and a sample size of 50 varied as much as 3 times. Either within ±5% or ±10%, or even ±20% error, a sample size of 25 or 50 is too small to express a colony's sex ratio. The sample size of 100 is enough to express a population's sex ratio within ±20% error with 100% confidence, but we usually allow sampling error within ±5% or ±10%. Though the sample size of 400 is more accurate within ±10% error, it is often impractical to obtain such numbers from a field collection.

Using a formula to calculate a sample size

Cochran (1963) developed the equation below to yield a representative sample for proportions.

$$N_0 = \frac{Z^2 pq}{e^2}$$

Where n_0 is the sample size, the value for Z is the upper $\alpha/2$ point of the normal distribution, Z=1.96, p is the estimated proportion of an attribute that is present in the population, and q is 1-p (assume its maximum value when p=0.5), e is the desired level of precision.

Example. How large a sample is needed to obtain and estimate the true sex proportion within e=0.1?

$$N_0 = \frac{1.96^2(0.5)(0.5)}{0.1^2} = 96.04$$

Thus, a sample size of 96 or 97 would be sufficient to meet the criteria despite the actual population sex proportion (p). According to our experiment results, a sample size of 100 has a ±16% error. This can be explained by two important factors. First, we assumed a 100% confidence level using a census, while the formula provides a 95% confidence level. Second, the mean value of 1600 specimens we compared has an error of ±3% for being the true value in a large population. Although the rest of ±2.5% error of the sample size of 100 could not be explained, using a census was consistent with the formula calculation because they vary less than 10%. We suggest a sample size of 100 be adopted in sampling for termite sex ratios.

Is a ratio the best way to express the numbers of males and females in a sample?

Although sex ratio is easily understood as an expression of the numbers of males and females in a population, the error caused by the ratio transformation of the data can be double the number expressed as a percentage. That's because in a ratio, both numerator and denominator have proportional errors. The sex ratio can therefore double the error compared to the percentage. If a ratio is chosen as the method to express the number of sexes, one must augment the sample size beyond our recommendation of 100.

Determination of a neutral or biased distribution of sex within the worker caste

In the strictest condition, only 50:50 is neutral and any increase in either sex is biased. We suggest the range that determines if a colony is neutral or biased depends on the sample size (i.e. error). For example, because with a sample size of 100 we are willing to accept ±10% error, a sex ratio between 40:60 or 60:40 is reasonable error in reporting a sample to have a neutral, 50:50, distribution. Related to ratio, a sex ratio of 0.67-1.5 is neutral and <0.67 and >1.5 are biased for a sample size of 100. However, neutral and biased are man-made definitions and should be clearly described and understood by researchers examining proportions of sexes in termite societies.

References

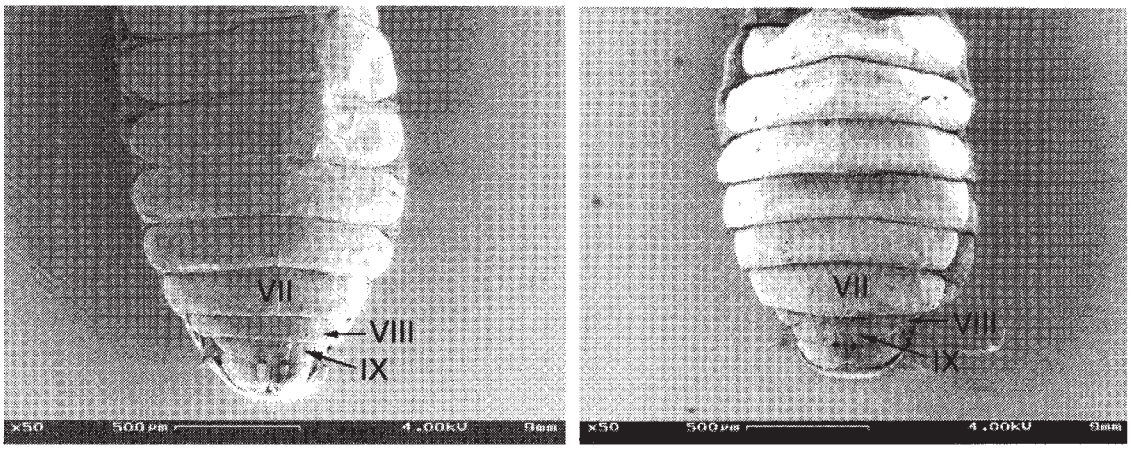
Cochran, W. G. 1963 *Sampling techniques*. New York: John Willey & Sons. Inc.

Costa-Leonardo, A. M., A. Arab, and F. E. Casarin 2004 Neotenic formation in laboratory colonies of the termite

Coptotermes gestroi after orphaning. *Journal of Insect Science* **4**, 1-6.
Darlington, J. 1986 Seasonality in mature nests of the termite *Macrotermes michaelseni* in Kenya. *Insectes Soc.* **33**(2), 168-189.
Dean, S. R. and R. E. Gold 2004 Sex ratios and development of the reproductive system in castes of *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae). *Ann. Entomol. Soc. Am.* **97**(1), 147-152.
Forschler, B. T. and M. L. Townsend 1996 Mark-release-recapture estimates of *Reticulitermes* spp. (Isoptera: Rhinotermitidae) colony foraging populations from Georgia, USA. *Environ. Entomol.* **25**(5), 952-962.
Grace, J. K. 1990 Mark-recapture studies with *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). *Sociobiology* **16**(3), 297-303.
Hayashi, Y., N. Lo, H. Miyata and O. Kitade 2007 Sex-linked genetic influence on caste determination in a termite. *Science* **318**, 985-987.
Henderson, G. 1996 Alate production, flight phenology, and sex-ratio in *Coptotermes formosanus* Shiraki, an introduced subterranean termite in New Orleans, Louisiana. *Sociobiology* **28**(3), 319-326.
Henderson, G. and K. S. Rao1993 Sexual dimorphism in soldiers of Formosan Subterranean Termite (Isoptera: Rhinotermitidae). *Sociobiology* **21**(3), 341-345.
Herfs, A. 1951 Der Schwarmflug von *Reticulitermes lucifugus* Rossi. *Z Angew Entomol.* **33**, 69-77.
Howard, R. W. and M. I. Haverty 1980 Reproductives in mature colonies of *Reticulitermes flavipes*: sex-ratio and association with soldiers. *Environ. Entomol.* **9**(4), 458-460.
Huang, L.-W. and L.-L. Chen 1983 The inceptive swarming of reproductive *Coptotermes formosanus* in laboratory reared colonies. *Acta Entomol. Sin.* **26**(4), 463-464 (In Chinese).
Husseneder, C., J. E. Powell, J. K. Grace, E. L. Vargo and K. Matsuura 2008 Worker size in the Formosan subterranean termite in relation to colony breeding structure as inferred from molecular markers. *Environ. Entomol.* **37**(2), 400-408.
Jones, S.C., J. P. La Fage and R. W. Howard 1988 Isopteran sex ratios: phylogenetic trends. *Sociobiology* **14**(1), 89-156.
Matsuura, K. 2006 A novel hypothesis for the origin of the sexual division of labor in termites: which sex should be soldiers? *Evol. Ecol.* **20**(6), 565-574.
McMahan, E., P. Sen-Sarma, and S. Kumar 1983 Biometric, polyethism, and sex ratio studies of *Nasutitermes dunensis* Chatterjee and Thakur (Isoptera: Termitidae). *Ann. Entomol.* **1**, 15-25.
Miaoulis, G. and R. D. Michener 1976 *An Introduction to Sampling*. Dubuque, Iowa: Kendall/Hunt Publishing Company.
Muller, H. and J. Korb 2008 Male or female soldiers? An evaluation of several factors which may influence soldier sex ratio in lower termites. *Insectes Soc.* **55**(3), 213-219.
Noirot, C. 1955 Recherches sur le polymorphisme des termites supérieurs (Termitidae). *Ann. Sci. Nat. Zool. (11e sér.)* **17**, 399-595.
Pawson, B. M. and R. E. Gold 1996 Caste differentiation and reproductive dynamics of three subterranean termites in the genus *Reticulitermes* (Isoptera: Rhinotermitidae). *Sociobiology* **28**(3), 241-251.
Roisin, Y. and M. Lenz 1999 Caste developmental pathways in colonies of *Coptotermes lacteus* (Froggatt) headed by primary reproductives (Isoptera: Rhinotermitidae). *Insectes Soc.* **46**(3), 273-280.
Roisin, Y. and M. Lenz 2002 Origin of male-biased sex allocation in orphaned colonies of the termite, *Coptotermes lacteus*. *Behav. Ecol. Sociobiol.* **51**(5), 472-479.
Su, N. Y., P. M. Ban and R. H. Scheffrahn 1993 Foraging populations and territories of the eastern subterranean termite (Isoptera: Rhinotermitidae) in southeastern Florida. *Environ. Entomol.* **22**(5), 1113-1117.
Thorne, B. 1983 Alate production and sex ratio in colonies of the neotropical termite *Nasutitermes corniger* (Isoptera: Termitidae). *Oecologia* **58**(1), 103-109.
Zimet, M. and A. M Stuart 1982 Sexual dimorphism in the immature stages of the termite, *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). *Sociobiology* **7**(1), 1-7.

Table 1. Examples of sample size from the termite sex ratio literature.

Species	Caste	Sample size	Sex ratio (♂/♀)	Reference
C. formosanus	Workers	20-25	0.22-3.40	(Husseneder et al. 2008)
C. formosanus	Soldiers	209	3	(Henderson and Rao 1993)
C. gestroi	Neotenics	1-28	0:28-8:0	(Costa-Leonardo et al. 2004)
C. lacteus	Larvae	9-103	1-3	(Roisin and Lenz 1999, 2002)
C. lacteus	Workers	100-106	0.49-1.13	(Roisin and Lenz 2002)
C. domesticus and *C. secundus*	False workers and soldiers	≤51	1	(Muller and Korb 2008)
M. darwiniensis	Alates	20-200	1	(Jones et al. 1988)
R. flavipes	Workers	32-100	1.13-1.56	(Zimet and Stuart 1982)
R. flavipes	Workers, soldiers, nymphs, and alates	24-52	1.03-1.27	(Dean and Gold 2004)
R. flavipes	Soldiers	1	1.13-1.56	(Zimet and Stuart 1982)
R. flavipes	Soldiers	200	1	(Matsuura 2006)
R. speratus	Workers	100-463	1:1-1:0	(Hayashi et al. 2007)



a b

Fig. 1 Electron micrographs of the ventral abdomen of *R. flavipes* workers illustrating the distinct arrangement of the sternites VIII and IX. (a) male (b) female

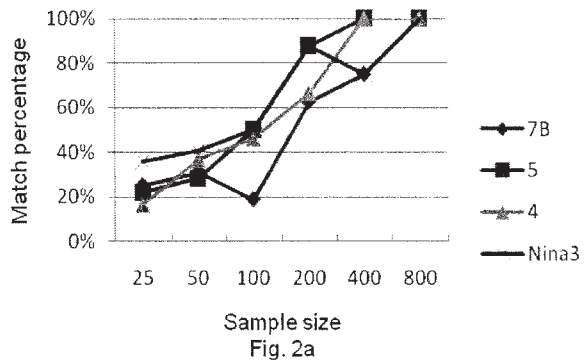


Fig. 2a

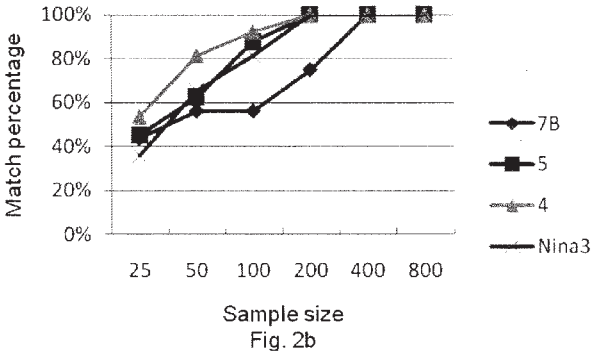


Fig. 2b

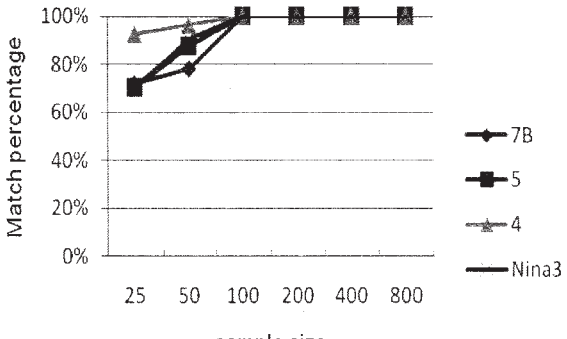


Fig. 2c

Fig 2. Match of females' percentage compared with 1600 observations within ±5% (a), ±10% (b) and ±20% (c) error between different sample sizes (Four colonies: 7B, 5, 4, and Nina 3).

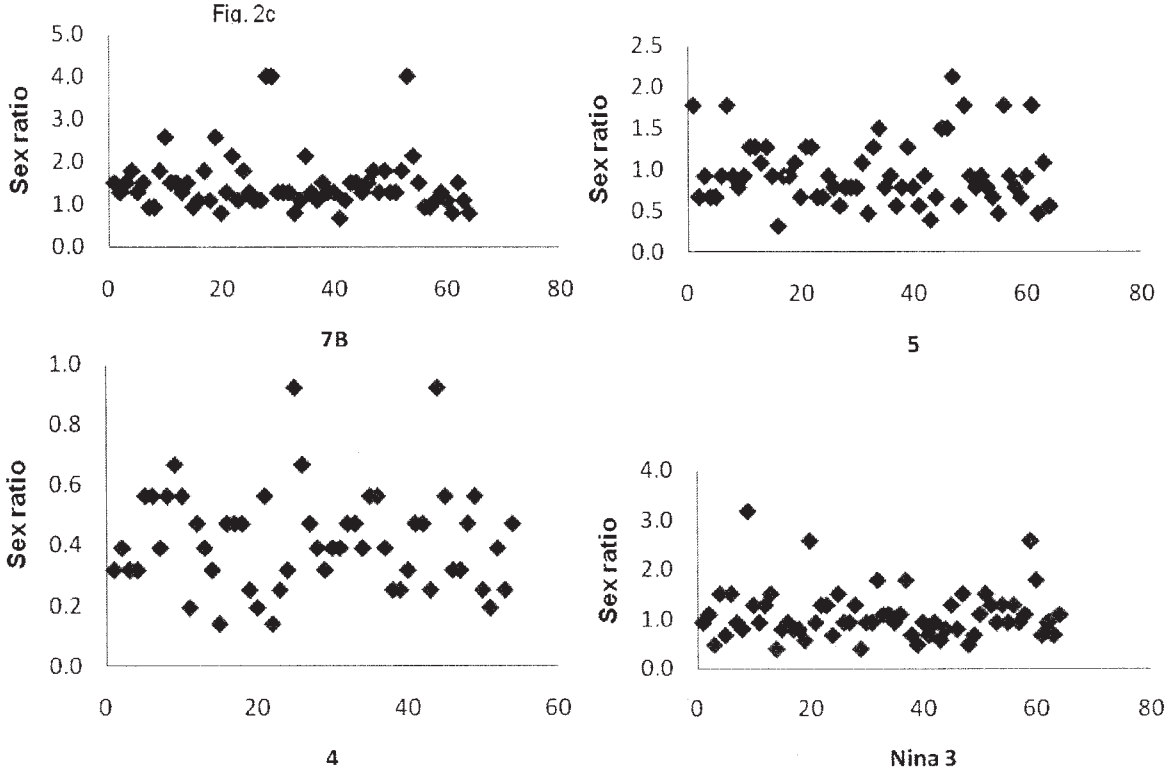


Fig 3. The sex ratio's distribution for the sample size of 25 (Four colonies: 7B, 5, 4, and Nina 3).

Temporal Change in the Species Richness of Termites on *Acacia* Hybrid Plantation

by
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Abstract

Changes in species richness of termites with the lapse of year after plantation were studied in *Acacia* hybrid plantation forests and a primary forest in Borneo, Malaysia. The objective was to clarify the influence of *Acacia* plantation after forest clearance on the recovery of species assemblage of termites. There was little difference in the species richness among *Acacia* hybrid forests irrespective of their years after plantation, but a remarkable difference was found between the *Acacia* hybrid forests and the primary forest. This difference was related to the difference in the abundance of the termitid soil feeders between the plantation forests and the primary forest.

Key words: species richness, disturbance gradient, *Acacia* hybrid plantation, Sabah

Introduction

Termites are widely distributed in tropical and subtropical regions, especially in tropics and play a crucial role in forest ecosystems as a decomposition agent. Termite diversity varies according to inhabiting environments. Indeed, there have been many studies of the effects of environmental factors on termite assemblages, such as the difference of vegetation, latitudinal changes, disturbance level and so on (Bandeira 2003; Davies 1997; Donovan *et al.* 2007; Eggleton *et al.* 1995, 1999, 2002; Gathorne-Hardy *et al.* 2001; Inoue *et al.* 2006; Jones 2000, etc.). It has been reported that termite diversity tends to be higher in the tropical rain forests than in disturbed areas (Eggleton *et al.* 1995, Jones 2000).

In this study, to clarify the influence of plantation after forest clearance on the recovery of species assemblage of termites, we examined changes in species richness with the lapse of years after plantation.

We thank KM Hybrid Plantation SDN. BHD. and Sarawak Forestry Corporation for their kind support and cooperation. This study is supported by KAKENHI no. 20405031.

Materials and methods

1. Research sites

The study was conducted in *Acacia* hybrid (*Acacia auriculiformis* - *mangium*) plantation forests of KM Hybrid Plantation SDN. BHD. in Keningau, Sabah, Malaysia in 2006-2008. We selected 7 sites of the *Acacia* hybrid forest; they were 4 young forests of 2,3,4 and 6 years after plantation (abbr. as YF2, YF3, YF4 and YF6, respectively) and 3 *Acacia* abandoned forests of 20 years (disturbed and undisturbed) and 30 years (abbr. as AF20, AFD20 and AF30, respectively). We made the survey also in a clear land (abbr. as CL). For comparison, a sampling was conducted in a primary forest (a lowland dipterocarp forest) in Lambir Hills National Park, Sarawak, Malaysia (abbr. as PF).

2. Method

The 100m belt transect protocol was used to assess the species richness and abundance of termites. This protocol is a standardized sampling protocol for evaluating faunal and functional diversity, as described by Jones and Eggleton (2000). Sampling was carried out along a 100m belt transect of 2m width. A transect was divided into forty sections each 5m long and 1m wide, and sampled sequentially. To standardize the sampling

effort, each section was searched for thirty minutes by one people collecting termites from dead woods, mounds, soil and arboreal nests. One transect (100 x 2m) was run at each site. The number of species and the number of encounters for each species were summarized. The number of encounters was assumed as the index of relative abundance of termites.

3. Identification and feeding group classification

The collected termites were sorted and identified. Termites were then classified into three feeding groups in regard to their decomposing abilities as follows:

Wood-feeder; termites feeding on cellulose materials.

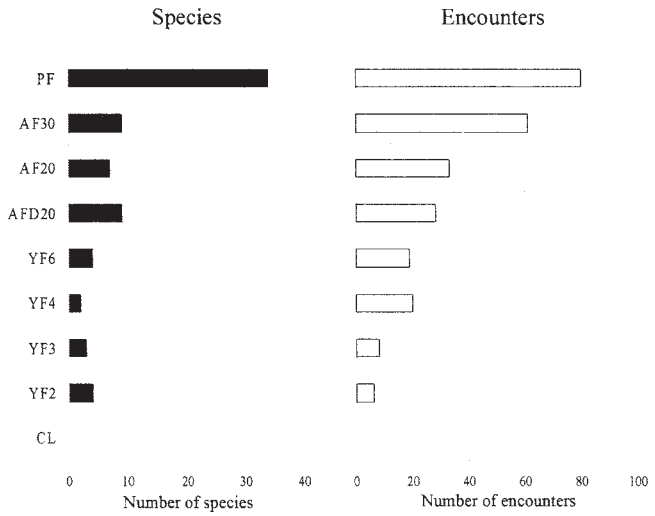
Fungus-feeder: termites feeding on woods and litters that grow fungi and decomposing cellulose and lignin.

Soil-feeder: termites distributed in the soil and humus and feeding on some products of plant degradation or microbial populations in the soil. (Their ability to decompose cellulose and lignin is low.)

Results and discussion

1. Species richness and abundance

Total 16 species were collected from *Acacia* hybrid forests (Table 1), 7 of them were collected from young forests. The rhinotermitid species, especially *Schedorhinotermes* were dominant in the *Acacia* hybrid forests. *M. gilvus* was found in all four young forests. There was none in the clear land. On the other hand, 33 species were collected from the primary forest, which was far more abundant than those from the *Acacia* hybrid forests.



The number of species and encounters are shown in Fig. 1. The number of species was equally low in 7 *Acacia* hybrid forests (YF2 to AF30), but it was outstandingly high in the primary forest (PF). The abundance (=number of encounters) increased with the increase of the forest years.

Fig. 1. The number of species and encounters of termites in 9 transects.

2. Feeding groups and taxonomic diversity

Feeding group composition and taxonomic composition of termites in 9 sites are shown in Fig. 2 and Fig. 3, respectively. Both feeding group composition and taxonomic composition were nearly constant among the same disturbance levels, that is, among young forests and abandoned forests.

The species number of wood feeders of abandoned forests was relatively higher than that of young forests and was markedly high in the primary forest (Fig. 2). The number of fungus feeders was low over all 9 sites. The number of soil feeders was also low in the *Acacia* forests but remarkably high in the primary forest. The taxonomic composition of termites (Fig. 3) shows that Termitinae (many soil feeders belong to this subfamily) dominant in the primary forest. In the young forests (YF2-YF6), the proportion of rhinotermitid species (which is all wood feeders) was rather higher than other two feeding groups and no nasutitermitin species was found (Fig. 3). Thus the differences of species richness among the feeding groups and the taxonomic groups were remarkable.

Table 1. List of termite species found in 10 transects. FG; feeding group, W=wood feeder, F=fungus feeder, S=soil feeder.

	Family		FG	CL	YF2	YF3	YF4	YF6	AF20	AFD20	AF30	PF
1	Kalotermitidae	*Glyptotermes sepilokensis*	W							1		
2		*Glyptotermes* sp.1	W									2
3	Rhinotermitidae	*Heterotermes tenuior*	W			4			2	1	11	5
4		*Coptotermes sepangensis*	W		1			1		3		
5		*Schedorhinotermes javanicus*	W		3		14				16	
6		*Schedorhinotermes tarakanensis*	W									8
7		*Shedorhinotermes javanicus*	W			3		10	17	13		
8		*Shedorhinotermes sarawakensis*	W						1			
9		*Parrhinotermes minor*	W									4
10		*Termitogeton minor*	W									1
11	Macrotermitinae	*Odontotermes denticulatus*	F									1
12		*Odontotermes sarawakensis*	F									2
13		*Odontotermes* sp.	F							1		
14		*Macrotermes gilvus*	F		1	1	5	7			2	
15		*Macrotermes mallacensis*	F									3
16	Nasutitermitinae	*Nasutitermes havilandi*	W						7	1	6	
17		*Nasutitermes longinasus*	W									6
18		*Bulbitermes borneensis*	W									1
19		*Bulbitermes constrictus*	W							4	1	
20		*Havilanditermes atripennis*	W									1
21		*Longipeditermes longipes*	W									1
22		*Malaysiotermes* sp.1	S									1
23		*Oriensubulitermes* sp.1	S									2
24	Termitinae	*Prohamitermes mirabilis*	S									8
25		*Microcerotermes sabahensis*	W									3
26		*Microcerotermes serrula*	W						3		11	6
27		*Homallotermes foraminifer*	W									1
28		*Dicuspiditermes nemerosus*	S									3
29		*Dicuspiditermes santschii*	S									6
30		*Pericapritermes dolichocephalus*	W		1							
31		*Pericapritermes mohri*	S									2
32		*Pericapritermes semarangi*	S									2
33		*Procapritermes prosetiger*	S					1	1	2	5	
34		*Procapritermes sandakanensis*	S						2			
35		*Procapritermes silvaticus*	S									1
36		*Procapritermes* sp.1	S									1
37		*Procapritermes* sp.2	S									1
38		*Procapritermes* sp.3	S									1
39		*Procapritermes* sp.4	S									1
40		*Procapritermes* sp.5	S									1
41		*Procapritermes* sp.6	S									1
42		*Termes propinquus*	S							2		
43		*Termes rostratus*	S									1
44		Termitidae sp.1	S									1
45		Termitidae sp.2	S									1
46		Termitidae sp.3	S									1
47		Termitidae sp.4	S								6	
		Species		0	4	3	2	4	7	9	8	33
		Encounters		0	6	8	19	19	33	28	58	80

Conclusion

It was found that the species richness of termites was very low in all of the *Acacia* plantation forests (YF and AF) irrespective of the lapse of years after plantation, making a marked contrast to the high richness in the primary forest. The termite communities in plantations must be recovered by migration of some species from neighboring forests. We found that the rate of recovery differed according to feeding groups. The termitid soil feeder was the most dominant group in the primary forest and may be expected to play a role for the recovery of the species richness. However the species richness of the soil feeder remained very low in plantation forests for many years (more than 20-30 years), probably because of their low ability of immigration to such new, disturbed areas. On the other hand, the number of rhinotermitid wood feeders was higher than termitid soil feeders in all plantation forests. Then it may be considered that termite communities start to recover from disturbed states mainly by migration of rhinotermitid wood feeders from neighboring areas. But it should be

noted that the number of rhinotermitid species in the neighboring areas was not so high as to make a significant contribution to the recovery processes.

The present study seems to provide an evidence that species assemblages of termites in a natural forest are damaged by serious disturbances imposed upon the forest such as thorough forest clearance, and that even by the subsequent plantation the communities would not significantly recover for the long time.

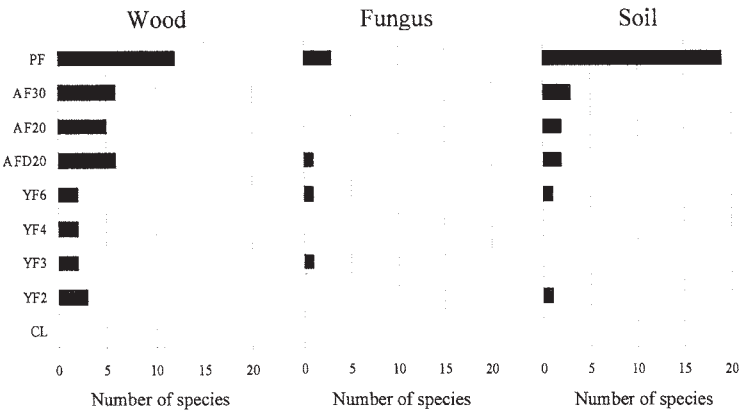


Fig. 2. The number of species of each feeding group in 9 transects.

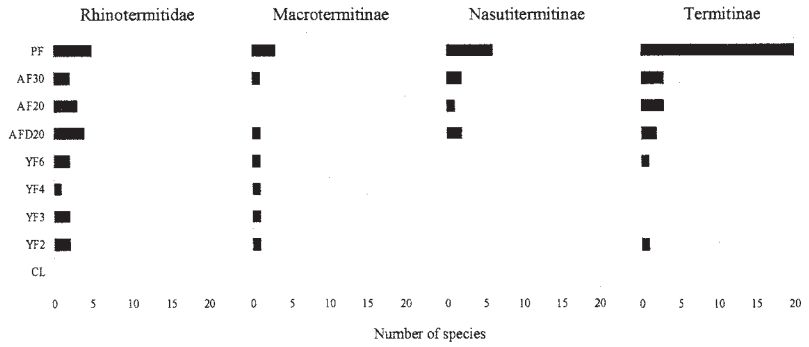


Fig. 3. The number of species of 4 family ranks in 9 transects.

References

Davies, R. G. 1997 Termite species richness in fire-prone and fire-protected dry deciduous dipterocarp forest in DoiSuthep-Pui National Park, northern Thailand, *J. Trop. Ecol.* **13**, 153–160.

Donovan, S. E., G. J .K. Griffiths, R. Homathevi and L. Winder 2007 The spatial pattern of soil-dwelling termites in primary and logged forest in Sabah, Malaysia. *Ecol. Ent.* **32**, 1-10.

Eggleton, P., D. E. Bignell, S. Hauser, L. dibog, L. Norgrove and B. Madong 2002 Termite diversity across an anthropogenic disturbance gradient in the humid forest zone of West Africa. *Agr. Ecosyst. Environ.* **90**, 189-202.

Eggleton, P., D. E. Bignell, W. A. Sands, B. Waite, T. G. Wood and J. H. Lawton 1995 The species richness of termites (Isoptera) under differing levels of forest disturbance in the Mbalmayo Forest Reserve, southern Cameroon. *J. Trop. Ecol.* **11**, 85–98.

Eggleton, P., R. Homathevi, D. T. Jones, J. A. MacDonald, D. Jeeva, D. E. Bignell, R. G. Davies and M. Maryati 1999 Termite assemblages, forest disturbance and greenhouse gas fluxes in Sabah. East Malaysia. *Phil. Trans. R. Soc. Lond. B.* **354**, 1791-1802.

Gathorne-Hardy, F., Syaukani and P. Eggleton 2001 The effects of altitude and rainfall on the composition of the termites (Isoptera) of the Leuser Ecosystem (Sumatra, Indonesia). *J. Trop. Ecol.* **17**, 379–393.

Inoue, T., Y. Takematsu, A. Yamada, Y. Hongoh, T. Johjima, S. Moriya, Y. Somnuwat, C. Vongkaluang, M. Ohkuma and T. Kudo 2006 Diversity and abundance of termites along an altitudinal gradient in Khao Kitchagoot National Park, Thailand. *J Trop Ecol.* **22**, 1-4.

Jones, D. T. 2000 Termite assemblages in two distinct montane forest types at 1000m elevation in the Maliau Basin, Sabah. *J. Trop. Ecol.* **16**, 271–286.

Jones, D. T. and P. Eggleton 2000 Sampling termite assemblages in tropical forests: testing a rapid biodiversity assessment protocol. *J. Appl. Ecol.* **37**, 191–203.

Studies on the Population of Subterranean Termite, *Macrotermes gilvus* Hagen (Blattodea: Termitidae) from Natural Forest

by
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Abstract

Termites play a very important role in ecosystem, especially in nutrient cycle through its functions as decomposer of organic material in natural forest. The studies have ever been conducted to look at the population and foraging of *Macrotermes gilvus* Hagen in natural forest ecosystem. The research was focused to observe various variables including major caste, minor caste, worker caste, nympha, and reproductive caste and average of population based on nest size of *Macrotermes gilvus* Hagen. Research was conducted in Yanlappa Sanctuary, Bogor West Java, Indonesia. Termites were surveyed by collecting termite individual *Macrotermes gilvus* Hagen at different size of nest, small (0 - 0.99 m), midle (1 - 1.99 m), and large (≥ 2 m). Mounds were fully excavated, termites collected by means of vacuuming, and colony size estimated by sub-sampling. The proportion of termites in the mound (above and underground sections) amounts to more than 70% of the colony; the rest being present in the surrounding soil (excavated beyond mound peri-meter). Results indicated that mean of the termite's colony *Macrotermes gilvus* Hagen for large nest 183,825 ind; middle nest 46,267; and small nest 20,223 ind. Mean of soil subterranean termite ratio major soldier caste *Macrotermes gilvus* Hagen 1.61% for large nest, 8.69% for middle nest, and 6.42% for small nest. Mean of minor soldier caste 0.20% for large nest, 1.96% for middle nest, and 1.29% for small nest. Mean of worker caste 15.93% for large nest, 6.85% for middle nest, and 50.42% for small nest. Mean of nymph caste 82.26% for large nest, 82.51% for middle nest, and 41.87% for small nest.

Key words: termite colonies, caste composition, *Macrotermes gilvus* Hagen, natural forest.

Introduction

Termites are pivotal in nutrient cycling and hence an important ecosystem component that requires analysis (e.g., Pomeroy, 1978; Lamotte & Bourlière, 1983; Nkunika, 1986; Meyer *et al.*, 1999). The rationale for carrying out this research is dichotomously described: intracolonially and ecologically. The former implies trophallaxis – exchange of nutrients between individuals on contact (La Fage & Nutting, 1978), either stomodeally (mouth-to-mouth) or proctodeally (from the rectum). Secondly, termites have been shown to fix nitrogen (Curtis & Waller, 1998). If the nitrogen fixation rate per individual termite is known, caste numbers and proportions provided by the present study can be used to accurately derive overall nitrogen fixation, as rates of fixation vary among species and castes via microbes and fungi (e.g., Matsumoto & Abe, 1979; Collins, 1983). Furthermore, termites are important in the capture and release of essential ions and soil nutrients and in the degradation of complex carbohydrates (cellulose) to simple carbon compounds. When plants, in turn, take up these compounds, the available nitrogen facilitates growth (Hesse, 1955). Nitrogen fixation is necessary, as mobile nitrogen is easily leached from the root zone into deeper soil horizons (Tainton, 1988). It is therefore vital to obtain baseline data and other fundamental information about this species, so that the necessary projections can be made.

The objective of the research was to study soil's termite population *Macrotermes gilvus* Hagen in Indonesian Natural Forest and to evaluate the need of food of the species as well as factors affecting it. The research was focused to observe various variables including caste population nest and abiotic parameter average based on nest size of *Macrotermes gilvus* Hagen.

Materials and methods

Research was conducted between March - April 2007 in Yanlappa Sanctuary, Bogor, West Java,

Indonesia. Insect samplings were conducted by using colony classification (Meyer 2001) i.e small nest (0 – 0.99 m), middle nest (1 – 1.99 m) and large nest (≥ 2 m).

Three small (height: 0–0.99 m), three medium (1.00–1.99 m), and three large (≥ 2.00 m) mounds of *M. gilvus* Hagen were fully excavated. Excavations were done in the Yanlappa Sanctuary regions during daylight hours when no dispersal or foraging was evident. A circular trench (as deep as termites occurred, often a metre down) was dug beyond the mound perimeter so as to include the pediment. This took 1–2 days depending on mound size. Excavation was performed by gradually exposing sections of the mound, while digging proceeded. Vacuum samples that consisted of termites mixed with soil were placed in water so that the termites could be separated by flotation (Collins, 1981).

Estimation of colony sizes was done using a helminthological method (Clark *et al.*, 1971; Clark & Turton, 1973). Data processing and analysis were conducted using SAS (SAS Institute, 1989 a, b). In order to normalize the data, counts were transformed using the natural logarithm (Steel and Torrie, 1980).

Result and discussion

Yanlappa Sanctuary is about 32 Ha and located between 6°40' S and 106°45' E., with rainfall was about 2,399 mm/year. Soil is recorded grey alluvial association and primer sediment was clay and sand with waved phisiography. Vegetation of the area was dominated *Dipterocarpus hasseltii, Hopea sangal* Korth, *Schima walichii* Korth, *Altingia excelsa* Noronhae, *Litsea* spp, *Lagerstroma* spp and *Ficus septica.*

In the study area there were 43 points of termite colonies found consisted of 15 spots of large nests, 23 spots of middle nests, and 5 spots of small nests. Population *Macrotermes gilvus* Hagen in natural forest large nest was 183,825 individuals, middle nest is 46,267 individuals, and small nest is 20,223 individuals.

Average of major soldier of *Macrotermes gilvus* Hagen in natural forest was found to be higher in large nest 2,964 (1.61 %), middle nest 4,021 (8.69 %), and small nest 1,298 (6.42 %). Average of minor soldier of *Macrotermes gilvus* Hagen in natural forest was found to be higher in large nest 1,057 (0.20 %), middle nest 572.67 (1.96 %), and small nest 261 (1.29 %). Average worker *Macrotermes gilvus* Hagen in natural forest was found to be higher in large nest 29,277 (15.93 %), middle nest 31,670 (6.85 %), and small nest 10,196 (50.42%). Average of nymph was recorded to be higher in large nest 51,223 (82.26 %), middle nest 76,545 (82.51 %), and small nest 468 (41.87 %). Mean number of abundace in different castes and developmental stages of the colony can be seen in Table 1

A mature mound of *Macrotermes ukuzii* Fuller in Swaziland and *Macrotermes carbonarius* (Hagen) in Malaysia roughly 48,000 and 30,000 neuters, respectively, were calculated to occur (Darlington, 1984).

Mean of abiotic parameter in the colony of soil termite *Macrotermes gilvus* Hagen in natural forest based on the characteristic of nest, for small nest, mound high 0.70 ± 0.18 m, excavated diameter 1.44 ± 0.69 m, excavated depth 0.62 ± 10 m, wide nest 2.75 ± 2.12 m^2, volume nest 3.33 ± 2.67 m^3. For middle nest, mound high 1.67 ± 0.15 m, excavated diameter 2.24 ± 0.36 m, excavated depth 0.87 ± 0.13 m, wide nest 4.33 ± 1.65 m^2, volume nest 5.79 ± 3.44 m^3. For large nest, mound high 2.03 ± 0.06 m, excavated diameter 2.50 ± 0.52 m, excavated depth 1.07 ± 0.12 m, wide nest 4.24 ± 1.59 m^2, volume nest 11.88 ± 3.42 m^3. Mean number of abiotic parameter can be seen in Table 2.

The proportion of caste changes during the colony lifetime, most often varying seasonally and with sexual brood production. The underlying mechanisms of such fluctuations are not fully understood. They pointed out that a higher proportion of soldiers in a colony may enhance survival of the colony, but decrease the rate of colony growth, because soldier must be fed by other members of the colony. Therefore, for any species an optimal proportion of soldiers may have evolved which maximizes the energy expended in producing alates while maintaining adequate defence of colony.

The mounds are constructed of soil particles (like coarse sand, fine sand, silt, clay and organic carbon), extracta and saliva, these in varying proportions (Wilson, 1971). The *M. gilvus* Hagen uses re-packed, orally transported soil particles, cemented with saliva into walls. It is thought that mound structure of termites is determined by three important factors: the species, the soil composition and the microclimatic conditions (like rain and temperature).

Table 1. Nest populations of *Macrotermes gilvus* Hagen showing abundance in different castes and developmental stages of the colony, i.e. small, medium and large mound sizes. (calculated by using ANOVA)

Nest Type	**Sample**	**Major Soldier**	**Minor Soldier**	**Worker**	**Nymph**	**Total Individuals**
Small Nest	I	1,503 (6.77%)	364 (1.64%)	11,663 (52.52%)	8,675 (39.07%)	22,205
	II	1,216 (6.05%)	228 (1.13%)	10,172 (50.63%)	8,475 (42.18%)	20,091
	III	1,174 (6.39%)	191 (1.04%)	8,753 (47.64%)	8,255 (44.93%)	18,373
	Average	1,298 (6.42%)	261 (1.29%)	10,196 (50.42%)	468 (41.87%)	20,223
	SD	179	91	179	210	1919
Middle Nest	I	4,348 (8.96%)	1,567 (3.23%)	39,780 (8.20%)	38,641 (79.62%)	83,336
	II	3,919 (8.56%)	606 (1.32%)	28,370 (5.85%)	38,441 (83.93%)	71,336
	III	3,797 (8.54%)	545 (1.23%)	26,860 (6.04%)	37,437 (84.19%)	183,756
	Average	4021 (8.69%)	572.67 (1.96%)	31670 (6.85%)	76545 (82.51%)	46,267
	SD	289	573	7064	645	61734
Large Nest	I	3,135 (1.68%)	1,260 (0.07%)	30,916 (16.56%)	152,554 (81.70%)	187865
	II	2,935 (1.68%)	1,060 (0.06%)	29,616 (16.92%)	142,393 (81.34%)	176004
	III	2,823 (1.49%)	850 (0.45%)	27,298 (14.39%)	158,723 (83.67%)	189694
	Average	2964 (1.61%)	1057 (0.20%)	29277 (15.93%)	51223 (82.26%)	183.825
	SD	158	205	1833	8246	7432

Table 2. Abiotic parameter from nest populations of *Macrotermes gilvus* Hagen showing abundance in different castes and developmental stages of the colony, i.e. small, medium and large mound sizes. (calculated by using ANOVA)

Parameter	Sample	Small Nest	Middle Nest	Large Nest
Mound High (m)	1	0.50	1.50	2.00
	2	0.85	1.70	2.00
	3	0.75	1.80	2.10
	Average	0.70	1.67	2.03
	SD	0.18	0.15	0.06
Excavated Diameter (m)	1	0.73	1.83	2.20
	2	2.10	2.50	2.20
	3	1.50	2.40	3.10
	Average	1.44	2.24	2.50
	SD	0.69	0.36	0.52
Excavated Depth (m)	1	0.50	0.75	1.00
	2	0.65	0.85	1.00
	3	0.70	1.00	1.20
	Average	0.62	0.87	1.07
	SD	0.10	0.13	0.12
Wide Nest (m^2)	1	0.36	5.45	3.32
	2	4.41	2.43	6.07
	3	3.47	5.11	3.32
	Average	2.75	4.33	4.24
	SD	2.12	1.65	1.59
Volume Nest (m^3)	1	0.29	9.60	7.93
	2	5.33	4.87	13.75
	3	4.36	2.91	13.96
	Average	3.33	5.79	11.88
	SD	2.67	3.44	3.42

Conclusions

1. *Macrotermes gilvus* Hagen colonies in Indonesia more is bigger than population *Macrotermes carbonarius* Hagen in Malaysia and *Macrotermes ukuzii* in Swaziland.
2. Density colony termite in Yanlappa Sanctuary habitat of large nest is 183,825 individuals, middle nest is 46,267 individuals, and small nest is 20,223 individuals.
3. Factors affecting soil termite population *Macrotermes gilvus* Hagen are micro climate, macro climate, pH, predators, soil type, and resource food.

References

Clark, C. J., A. M. Tucker and J.A. Turton 1971 Sampling technique for estimating roundworm burdens of sheep and cattle. *Exp. Parasitol.* **30**, 181–186.

Clark, C. J. and J. A. Turton 1973 Estimating roundworm burdens and group sizes in anthelmintic trials with sheep and cattle. *Exp. Parasitol.* **34**, 69–75.

Collins, N. M. 1981 Populations, age structure and survivorship of colonies of *Macrotermes bellicosus* (Isoptera: Macrotermitinae). *J. Anim. Ecol.* **50**, 293–311.

Collins, N. M. 1983 The utilization of nitrogen resources by termites (Isoptera). In: *Nitrogen as an Ecological Factor* (J.A. Lee, S.McNeill and I.H. Rorison, Eds.), Blackwell Scientific Publications, Oxford, London, Edinburgh, Melbourne, pp. 381–412.

Darlington, J. P. E. C. 1984 A method for sampling the populations of large termite nests. *Ann. appl. Biol.* **104**, 427–436.

Lamotte, M. and F. Bourlière 1983 Energy flow and nutrient cycling in tropical savannas. In: *Ecosystems of the World 13, Tropical Savannas* (F. Bourlière, Ed.), Elsevier, Amsterdam, Oxford, New York, pp. 583–603.

Meyer, V. W., L. E. O. Braack, H. C. Biggs and C. Ebersohn 1999 Distribution and density of termite mounds in the northern Kruger National Park, with specific reference to those constructed by *Macrotermes* Holmgren (Isoptera: Termitidae). *Afr. Ent.* **7**, 123– 130.

Nkunika, P. O. Y. 1986 An ecological survey of the termites (Isoptera) of Lochinvar National Park, Zambia. *J. ent. Soc. sth Afr.* **49**, 45–53

Pomeroy, D. E. 1978 The abundance of large termite mounds in Uganda in relation to their environment. *J. appl. Ecol.* **15**, 51–63

Sands, W. A. 1998 *The Identification of Worker Castes of Termite Genera from Soils of Africa and the Middle East.* CAB, Oxon and New York, 500 pp.

SAS Institute, Inc., 1989a. *SAS/STAT User's Guide: Version 6,* 4th Edition, Volume 1. SAS Institute, Cary, 943 pp.

Wilson, E. O. 1971 *'The Insect Societies'*, Harvard University Press.

Chemical Defensive Secretions of the Subterranean Termite Soldiers of *Coptotermes curvignathus* Holmgren (Blattodea: Rhinotermitidae)

by
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Abstract

Termite soldiers of *Coptotermes curvignathus* eject a viscous white and sticky defensive secretions from the frontal gland. These soldier defensive secretions used to overcome their enemy. Defensive secretions are produced in frontal gland with 2.5 mm long and 0.8 mm diameter. This frontal gland is almost entirely of termite's abdomen. In this research, soldier defensive secretions (SDS) from *Coptotermes curvignathus* Holmgren was isolated and extracted in ethyl acetate and ethanol solution then analysis with GCMS to detection the compound. The result showed that extract SDS has pH value 4-5.5, and the viscosity value were 0.00002-0.0005 poise and temperature value were 27-27.5°C. Gas chromatography mass spectra (GCMS) analysis resulted 8 volatile compounds, mainly aldehyde compounds, including tetradecanal, pentadecanal undecanal and octadecanal. These defensive secretions had an activity as antifungal and antibacterial agents.

Key words: soldier defensive secretions, *Coptotermes curvignathus,* GCMS, tetradecanal, pentadecanal

Introduction

A major cost of social life is the increased exposure to pathogens. This cost of sociality is expected to be particularly high for social insects, which live in crowded, persistent, warm and resource-rich nests providing ideal conditions for the development of microorganisms. Hence, social insects have evolved a variety of behaviour and physiological defence mechanisms, including antibiotic-producing symbionts and allogrooming (Roseangus *et al.* 1998), antibiotic secretions (Roseangus *et al.* 2000), removal of wastes and corpses (Roseangus & Traniello 2001a) and immune defences (Roseangus & Traniello 2001b. Another potential mechanism of defence may be to add plant compounds with antimicrobial properties to the nest. Soldier termites of the subfamilies Coptotermitinae employ a chemical secretions in colony defense, applying a lipophilic mixture of electrophilic compounds which act as contact insecticides (Pasteels & Bordereau 1998). So far, chemical defence of termites soldier hasn't been investigated. This research aimed to investigation the compound of soldier defensives secretions from *Coptotermes curvignathus* Holmgren.

Materials and methods

Isolation and extraction soldier defensive secretions

Colonies of subterranean termites *C. curvignathus* Holmgren were obtained from dead Pine tree in Yanlappa Jasinga, Bogor, West Java and rearing on Laboratory of Forest Biology IPB (Bogor Agricultural University) for one year (*Laboratory reared termites colony)*. Soldiers were removed from the colony and the defensive secretions were isolated and extracted according to Chuah *et al* (Chuah *et al.* 1990) methods. The soldier defensive secretions (SDS) then dilution with ethyl acetate and ethanol solution. Each solution was mix with defensive secretions from 4000 termites soldier.

Analysis the bioactive compound

The SDS extract were identified by gas chromatography mass spectra (GCMS) analysis. The GC was Shimadzu QP 5050 model equipped with GC 17a , column DB5 MS (30 m length x 0.25 mm diameter). The GC settings were as follows : initial column temperature set at 40°C for 5 min; temperature programmed from 40°C to 330°C with a rate 4 °C/min. Mass spectra resolution was 1000, with interval 0.5 sec; ionisasi energy 0.90 kv and retention time 1.6-56.0. Identified the compound were obtained according to Clement *et al.* (2001) and compared with data on NIST 62 (*National Institute Standard and Tehnology)*, WILEY 229 and PESTICD.LIB

Results and discussion

Characterization of soldier defensive secretions

Soldier defensive secretions were characterized on colour, viscosity, pH and temperature. Characteri- zation of SDS were shown in Table 1.

Table 1. Characterization of soldier defensive secretions from *C. curvignathus* Holmgren

Solution	pH	Temperature (°C)	Viscosity (poise)	Color
Ethyl Acetate	4	27	0,0005	White
Ethanol	5	27,5	0,00002	White

Chemical constituents of soldier defensive secretions

The chemical composition of soldier defensive secretions and the relative amount of each component were determined by GCMS analysis. Chromatogram of extract SDS in ethanol solution was shown in Figure 1 and the chemical compound was shown in Table 2. The chemical compound were consisted aldehyde group, namely Tetradecanal, Pentadecanal, Undecanal, and Octadecanal and 2-methyl pentane from alcane group.

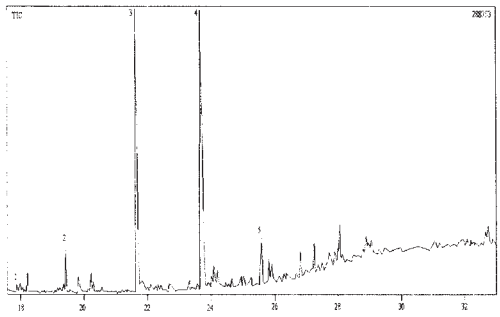

Figure 1. Chromatogram of soldier defensive secretions *C. curvignathus* Holmgren in ethanol extract. Peak Number is based on Number at Table 2.

Table 2. Chemical Compound and Retention Time from Ethanol extract of soldier defensive secretions *C. curvignathus* Holmgren

Peak No.	Retention Time	LRIexp	LRIref	Chemical Compound	Group
1.	1.419	-	673 [(a)]	2-methyl-Pentane	Alcane
2.	19.420	1027	-	Undecanal	Aldehyde
3.	21.654	1096	1107 [(b)]	Tetradecanal	Aldehyde
4.	23.706	1134	-	Pentadecanal	Aldehyde
5.	25.573	1178	-	Octadecanal	Aldehyde

Remarks : LRI experiment from GCMS, DB-5 Column
LRI reference (a) Larrayoz *et al.* (2001), DB-5 Column
LRI reference (b) Mondello *et al.* (2002), DB-5 Column

Chromatogram of extract SDS in ethyl acetate solution was shown in Figure 2 and the chemical compound was shown in Table 3. The chemical compound were consisted aldehyde group, namely Tetradecanal, Pentadecanal, and Undecanal ; Ethyl propionate and n-butyl acetate from Ester group and 1-butanol from alcohol group.

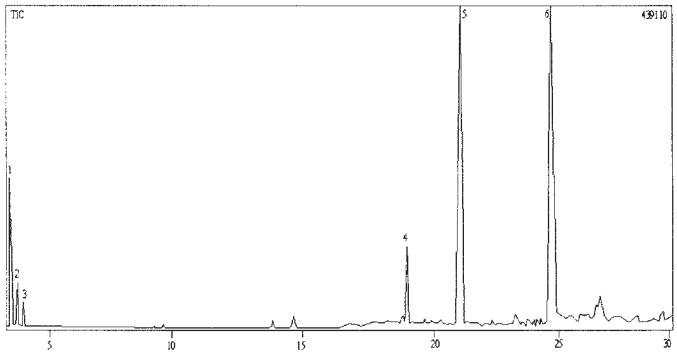

Figure 2. Chromatogram of soldier defensive secretions *C. curvignathus* Holmgren in Ethyl acetate extract. Peak Number is based on Number on Table 3.

Table 3. Chemical Compound and Retention Time from Ethyl acetate extract of soldier defensive secretions *C. curvignathus* Holmgren

Peak No.	Retention Time	LRIexp	LRIref	Chemical Compound	Group
1.	1.954	0	696 [(a)]	Ethyl propionate	Ester
2.	2.067	0	-	1-butanol	Alcohol
3.	2.925	0	795 [(a)]	n-butyl acetate	Ester
4.	19.422	1025	-	Undecanal	Aldehyde
5.	21.669	1096	1107 [(b)]	Tetradecanal	Aldehyde
6.	23.717	1137	-	Pentadecanal	Aldehyde

Remarks : LRI experiment from GC-MS, DB-5 Column
LRI reference (a) Boscaini *et al.* (2003), DB-5 Column
LRI reference (b) Mahattanatawee *et al.* (2004), DB-5 Column

Extract SDS in ethyl acetate solution has a bioactivity as antifungal. The research results of Diba (2008) shown that SDS in ethyl acetate solution has inhibited the growth of *Rhizoctonia solani* fungi. Meanwhile extract SDS in ethanol solution has a bioactivity as antibacterial. The extract has inibited the growth of *Staphylococcus aureus* with average inhibition zone was 30.52 mm (Diba 2006). Defensive secretions is a chemical weapon in termites. This defensive secretions mechanisms as chemical weapon was variety, such as antibiotic function on *Pseudacanthotermes spiniger* (Lamberty *et al.* 2001), antifungal on *Zootermopsis angusticolis* (Roseangus *et al.* 2003) and immune defense on *Nasutitermes costalis* (Roseangus & Traniello 2001b).

Conclusion

Extract soldier defensive secretions from *C. curvignathus* has white colour, pH value 4-5.5, viscosity value were 0.00002-0.0005 poise and temperature value were 27-27.5°C. Gas chromatography mass spectra (GCMS) analysis resulted 8 volatile compounds, mainly aldehyde compounds, including tetradecanal, pentadecanal, undecanal and octadecanal. These defensive secretions had an activity as antifungal and antibacterial agents.

Reference

Boscaini, E, S. V. Ruth, F. Biasioli, F. Gasperi, T. D. Mark 2003 Gas Chromatography –Olfactometry (GC-O) and Proton Transfer Reaction – Mass Spectrometry (PTR-MS) Analysis of the Flavor Profile of Grana Padano, Parmigiano Reggiano and Grana Trentino Cheese. International Conference on Proton Transfer Reaction Mass Spectrometry and Its Application. 18 – 23 January 2003, Austria.

Chuah C. H , S H Goh, Y P Tho 1990 Chemical Defense Secretions of Some Species of Malaysian Rhinotermitide (Isoptera: Rhinotermitidae). *Journal of Chemical Ecology* **16,** 685 – 692.

Clement, J. L., A. G Bagnerer, P. Uva, L. Wilfert, A. Quintana, J. Reinhard and S. Dronnet 2001 Biosystematics of *Reticulitermes* termites in Europe. *Morphological, Chemical and Molecular Data. Insectes Soc.* **48**, 202 – 215.

Diba, F. 2006 Study the Anatomy, Physiology and Bioactivity of Soldier Defensive Secretions of *Coptotermes curvignathus* Holmgren (Isoptera:Rhinotermitidae). Disertation. IPB. Bogor Indonesia.

Diba, F. 2008 Utilization of Soldier Defensive Secretions from Subterranean Termites *Coptotermes curvignathus* Holmgren (Isoptera:Rhinotermitidae) to Inhibition Damping-Off on Pine Seed (*Pinus merkusii* Jungh et de Vriese). Procedings TRG 5 Bali Indonesia.

Lamberty M, A. Caille, C. Landon, S. Tassin-Moindrot, C. Hetru, P. Bulet and F. Vovelle 2001 Solution Structures of The Antifungal Heliomicin and a Selected Variant With Both Antibacterial and Antifungal Activities. *Biochemistry* **40**, 1995 – 2003.

Larrayoz P, M. Addis, R. Gauch, J.O. Bosset. 2001 Comparison of Dynamic Headspace and Simultaneous Distillation Extraction Techniques Used for the Analysis of the Volatile Components in Three European PDO ewes milk cheeses. International Dairy Journal 11, p 911 – 926.

Mahattanatatawee K, R. Rouseff, M. F. Valim dan M. Naim 2004 Identification and Aroma Impact of Norisoprenoids in Orange Juice. Journal of Agricultural and Food Chemistry. 18, 2176 – 2180.

Mondello L, G Zappia, A. Cotroneo, I. Bonaccorsi, J. U. Chowdhurry, M. Yusuf, G Dugo. 2002 Studies on the Essential Oil-Bearing Plants of Bangladesh. Part VIII. Composition of Some *Ocimum* oils *O. basilicum* L. Var. *Purpurascens; O. sanctum* L. Green; *O. sanctum* L. Purple; *O. americanum* L. Citral type; *O. americanum* L. Camphor type. Flavour and Fragrance Journal Vol. 17 Issues 5, p 335 – 340.

Pasteels, J. M and C. Bordereau 1998 Releaser Pheromones in Termites. In: R.K. Van Der Meer, M.D Breed, K.E Espelie, M.L. Winston (eds). Pheromone Communication in Social Insects – Ants, Wasps, Bees and Termites. Pp 193 – 215. Westview Press, Boulder, Colorado.

Roseangus, R. B.; M. L. Lefebvre; J. F.A . Traniello 2000 Inhibition of Fungal Spore Germination by *Nasutitermes:*Evidence for a Possible Antiseptic Role of Soldier Defensive Secretions. *Journal of Chemical Ecology* **26**, 21 - 39.

Roseangus R. B., A. B. Maxmen, L. E. Coates and J. F. A. Traniello 1998 Disease Resistance: a Benefit of Sociality in the Dampwood Termite *Zootermopsis angusticolis* (Isoptera:Termopsidae). *Behav. Ecol. Sociobiol*, **44**, 125-134.

Roseangus R. B and J. F. A. Traniello 2001a Disease Susceptibility and the Adaptive Nature of Colony Demography in the Dampwood Termite *Zootermopsis angusticollis. Behav Ecol Sociobiol* **50**, 546 – 556.

Roseangus R. B., J. E. Moustakas, D. V Calleri and J. F. A. Traniello 2003 Nesting Ecology and Cuticular Microbial Loads in Dampwood (*Zootermopsis angusticulis)* and drywood termites (*Incisitermes minor, I schwarzi, Cryptotermes cavifrons). Journal of Insect Science* **31**, 1 – 6.

Roseangus R. B and J. F. A. Traniello 2001b The Development of Immunity in a Social Insect: Evidence for the Group Facilitation of Disease Resistance. *PNAS* **99**, 6838 – 6842.

Dipteran Parasitism of Subterranean Termite Soldiers, *Macrotermes gilvus* (Hagen) and *Macrotermes carbonarius* (Hagen) (Termitidae: Macrotermitinae)

by
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Abstract

In this study, we report a new species of *Misotermes* (Diptera: Phoridae) found infecting the soldiers of *Macrotermes gilvus* (Hagen), and the Oriental Bengaliinae blow fly, *Verticia fasciventris* Malloch, 1927 (Diptera: Calliphoridae) as an internal parasitoid of the soldiers of *Macrotermes carbonarius* (Hagen). The infected soldiers of both species showed enlarged head capsule and a pair of deformed mandibles. There was a single larva per host. The larvae developed in the head of the soldiers and fed on the entire head content. Interestingly, larval puparation process of both species varied. In *M. gilvus*, perforation of host's abdominal wall was made in an attempt to obtain a dry microenvironment for puparation inside the host body. The action is usually fatal to the host. Only the major soldiers of *M. gilvus* were attacked by the parasitoid. On the contrary, the mature larva exited the *M. carbonarius* soldier host between the abdominal cerci and buried itself into the substratum for puparation. The host would remain alive for 2-3 days after larval emergence. Both soldier castes were victims of the parasitism. It is unknown at this stage why the parasitism is only restricted to the soldier caste.

Key words: Diptera, parasitism, *Macrotermes*, *Misotermes*, *Verticia fasciventris*, soldier termite.

Introduction

The genus *Macrotermes* has two distinct morphological soldier castes, notably the major and minor soldiers. The soldiers play protective roles, in which, accompanying foragers in food searching, sealing holes to prevent predator's intrusion (Jmhasly and Leuthold 1999) and involved in the integrated actions of alates flight initiation (Michell 2007). Polyethism in major and minor soldiers is common in Macrotermitinae (Coaton and Sheasby 1972; Badertscher et al. 1983). The tasks above pose termite soldiers a risk of predating raid and also being parasitized. *M. gilvus* soldiers have a reddish brown head capsule and is distinguishable from other *Macrotermes* by its labial hyaline tip that is broadly conical at the anterior region. In contrast, *M. carbonarius* soldiers have a heavily chitinized black head capsule that possess tri-lobed hyaline tip.

Parasitism had received great attention from parasitologist since 1980s (Feener and Brown 1997). Order Hymenoptera, Diptera, Coleoptera, Lepidoptera and Neuroptera are known to be the common parasitoids. In Diptera, approximately 20% of its species are parasitoids. Adaptive and evolutionary strategies have enabled Dipteran to be successful parasitoids. In the US, extensive studies on parasitism were made on *Pseudacteon spp.* (Diptera: Phoridae) as a biological control agent against the fire ant, *Solenopsis spp.* (Estrada et al. 2006; Orr et al. 1995).

Dipteran parasitism in termite is not unique. Parasitism has been documented for several termite genera, eg. *Odontotermes* (Disney and Darlington 2000), *Nasutitermes* (Disney 1986) and *Macrotermes* (Tsang et al. 2008). This reflects the diversity of termite parasitization. However, to date, documentation of mechanism of dipteran parasitism of termite remains fragmentary. Thus, information is required to provide direct evidence on host-parasite interaction. Here, we presented the first dipteran parasitism of *M. gilvus* and *M. carbonarius* by phorid fly *and* blow fly, respectively. We also discussed the morphological changes of infected soldiers and larval parasitoid emergence in the present study.

Materials and methods

Infected soldiers of *M. gilvus* and *M. carbonarius* were collected from Universiti Sains Malaysia, Minden Campus, Penang, Malaysia (2°59'N and 102°18'E) in July 2008. Sampling sites focused on mounds that were previously used for experimental purposes, especially those that had been opened. Dissected or disturbed mounds were found to have higher prevalence of parasitism. Excavations of mounds were exercised with caution to minimize injuries to the termites. Parasitised soldiers found in the colonies were collected and placed into plastic storage boxes (320 mm x 250 mm x 130 mm) with moistened vermiculite, transported to the laboratory and kept at a constant temperature (28 ± 1°C) and relative humidity of ~ 90%. The infected soldiers were kept along with fungus-comb, major and minor workers to ensure that the infected soldiers were not starved.

Host and larval behaviour of pre- and post-emergence were video-recorded. When adult flies emerged, they were preserved in 70% alcohol. Flies were identified up to species level by R.H.L. Disney (Cambridge University, UK) and H. Kurahashi (International Department of Dipterology, Tokyo, Japan). Random samples of infected soldiers (n = 10) were taken and preserved in 70% alcohol prior to morphometric analysis. Various parts of termite based on Okot-Kotber (1981), were observed under stereo microscope Olympus SZ61 with IC Imaging Standard V2.1 and were measured by using Analysis® Image Processing Software. Two-dimensional scatter plots of infected soldiers to normal soldiers' measurements (unpubl. data) were generated by using Sigmaplot 8.02 SPSS software.

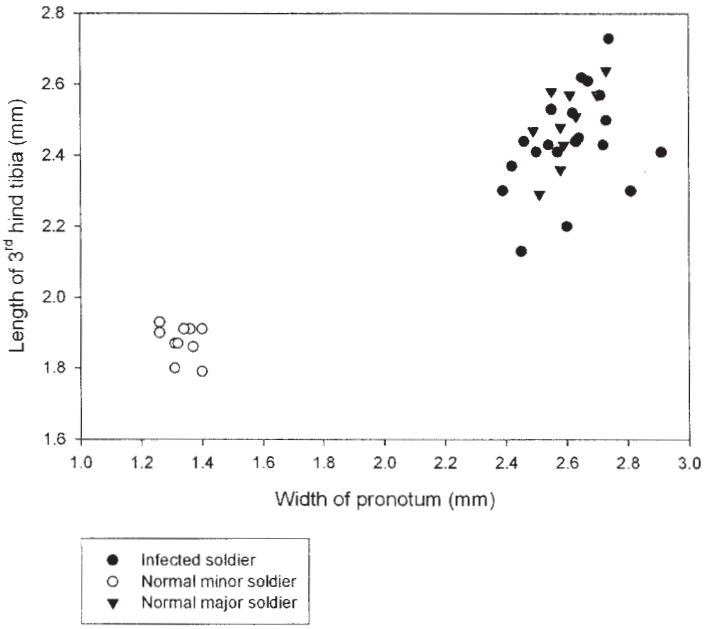
Results and discussion

Fly species and infected soldiers

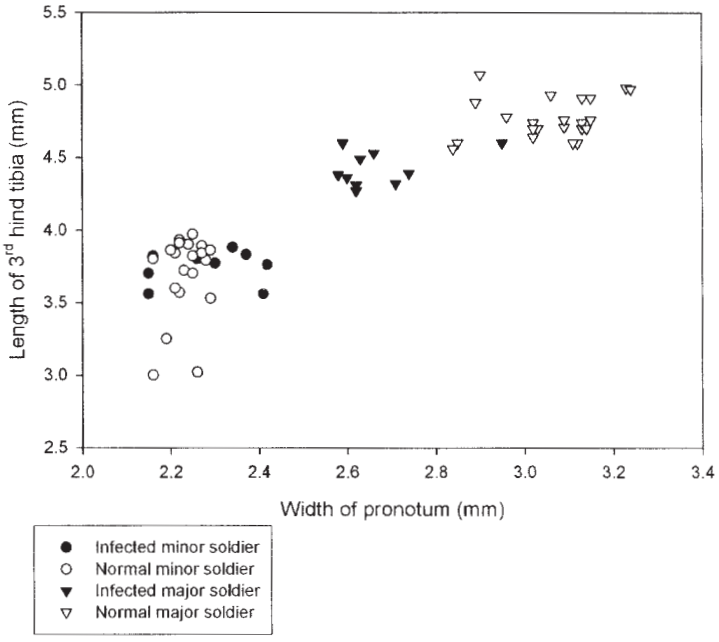
The parasitoid that we discovered that infected *M. gilvus* soldier was identified as *Misotermes sp.* nov (Diptera: Phoridae). It is a new species to science and the third species of the genus *Misotermes*, after the discoveries of *M. exenterans* in Java (Schmitz 1938) and *M. vicinus* in Thailand (Borgmeier 1967). The cluster of infected soldier overlapped with the cluster of uninfected major soldier (Fig.1a). This indicated that only major soldier was infected. The infected soldier's head capsule appeared abnormally large with a pair of remarkably short mandible. They usually shied away when subjected to an external stimulus.

On the other hand, *V. fasciventris* (Diptera: Calliphoridae) was found as an endoparasite to the soldiers of *M. carbonarius*. The measurements fall into two clusters of uninfected soldier, respectively (Fig. 1b), which demonstrated that both minor and major soldiers were victims of the parasitism. The findings were parallel to the report by Tsang et al. (2008) that the same species was found to parasitize both minor and major soldiers of *M. subhyalinus*. The head capsule of infected soldiers was enlarged. The mandibles were subtly shorter than those in healthy soldiers (Fig. 2A). They were highly aggressive when stimulated; in contrast to the *M. gilvus* in the present study and the *M. subhyalinus* (Tsang et al. 2008).

There was a single larva per host, residing in the head capsule of soldier throughout developmental period. Larval parasitoid could be observed in the head capsule of infected soldier during pre-emergence (Fig. 2B). We were unable to elucidate the mechanism of parasitism in the course of our experiment due to limited data and difficulties in rearing the fly parasitoid. The adult female of *Verticia spp.* has a rather short, non-telescopic and non-piercing ovipositor, but equipped with strong bristles (Tsang et al. 2008). The above features indicated that possible involvement of extensive physical contact on premature soldier caste (less sclerotization). This is supported by the presence of premature infected soldier (poor sclerotization) during the sampling. Askew (1971) have suggested several routes of entering host in parasitic dipteran.

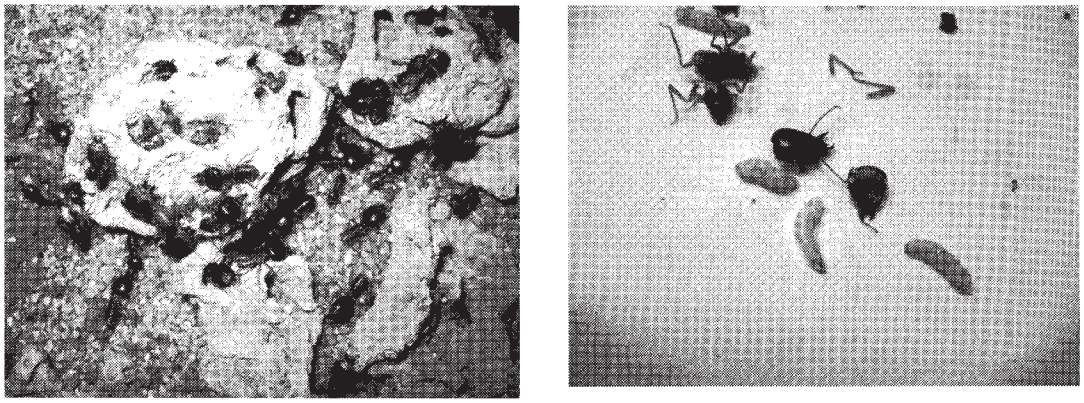


(a)



(b)

Fig. 1. a) *M. gilvus*; **b)** *M. carbonarius*. Distribution of pronotal width and 3rd hind tibial length of infected soldier and uninfected soldiers.



(A) (B)

Fig. 2. *M. carbonarius*. (A) Infected soldiers (mixed caste) and (B) Presence of larval parasitoid in the head

Emergence of parasitoids from hosts

Emergence of larvae from hosts usually occur under laboratory condition after ≈ 7 days. Before larval emergence, infected soldiers with swollen abdominal part became inactive and all movements were comparatively slow.

Both dipteran species demonstrated different approaches in larval puparation. In *M. gilvus*, the larva applied sideways pressure to perforate the host's abdominal wall by using spiracles, allowing the host body fluid to ooze out. The process took approximately 10 minutes. Loss of body fluid of host creates dry microenvironment and enable larva to pupate inside the host body. Generally, the host died of dehydration. High humidity condition, approximately 90% was favored for pupal parasitoid of *Misotermes spp.* development and took ca. 14 days.

In *M. carbonarius*, the mature larva left the host from the head through body and exit between the abdominal cerci by using mouth-hooks. The emerged larva moved away from the host and buried itself into vermiculite for puparation. No aggressive behaviour by other termite individuals was showed against the larva. The host would remain alive 2-3 days under laboratory condition after larval departure. The development of puparium took about 12 days.

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References

Askew, R. R. 1971 Protelean parasitic Diptera. In: *Parasitic insects*. Heinemann Education Books Limited, London. pp. 185-210.

Badertsher, S., C. Gerber and R. H. Leuthold 1983 Polyethism in food supply and processing in termite colonies of *Macrotermes subhyalinus* (Isoptera). *Behav. Ecol. Sociobiol.* **12**, 115-119.

Borgmeier, T. 1967 Studies on Indo-Australian Phorid flies, based mainly on material of the Museum of Comparative Zoology and the United States National Museum (Diptera, Phoridae). *Stud. Ent.* **9**: 129-328.

Coaton, W. G. H. and J. L. Sheasby 1972 Preliminary report on a survey of the termites (Isoptera) of South West Africa. *Cimbebasia Mem.* **2**, 1-129.

Disney, R. H. L. 1986 Two remarkable new species of scuttle fly (Diptera: Phoridae) that parasitize termites (Isoptera) in Sulawesi. *Syst. Ent.* **11**, 413-422.

Disney, R. H. L. and J. P. E. C. Darlington 2000 Alates termites (Isoptera: Termitidae) parasitized by a scuttle fly (Diptera: Phoridae). *Sociobiology* **35**, 63-78.

Estrada, C., R. J. W. Patrock, P. J. Folgarait and L. E. Gilbert 2006 Host specificity of four *Pseudacteon spp.* (Diptera: Phoridae), parasitoids of fire ants in Argentina (Hymenoptera: Formicidae). *Fla. Entomol.* **89**, 462-468.

Feener, D. H. Jr. and B. V. Brown 1997 Diptera as parasitoids. *Annu. Rev. Entomol.* **42**, 73-97.

Jmhasly, P. and R. H. Leuthold 1999 The system of underground passages in *Macrotermes subhyalinus* and comparison of laboratory bioassays to field evidence of intraspecific encounters in *M. subhyalinus* and *M. bellicosus* (Isoptera, Termitidae). *Insectes soc.* **46**, 332-340.

Kalshoven, L. G. E. 1938 Weiteres über das Benehmen der *Misotermes* abdominal larven und der Myiagenen. *Entomol. Meded. Nederlandisch-Indië* **16**, 395-397.

Mitchell, J. D. 2007 Swarming and pairing in the fungus-growing termite, *Macrotermes natalensis* (Havilandi) (Isoptera: Macrotermitinae). *Afr. Entomol.* **15**, 153-160.

Okot-Kotber, B. M. 1981. Instars and polymorphism of castes in *Macrotermes michaelseni* (Isoptera, Macrotermitinae). *Insect. Soc.* **28**: 233-246.

Orr, M. R., S. H. Seike, W. W. Benson and L. E. Gilbert 1995 Flies suppress fire ants. *Nature*. **373**, 292-293.

Schmitz, H. 1938 *Misotermes exenterans* n. g. n. sp., eine parasitische fliege aus der Familie der Phoriden, weiche die Entstehung myiagener Solclaten bei *Macrotermes gilvus* Hagen in Java verursacht. Treubia. **16**: 369-397.

Tsang, W. S., T. Pape and D. K. Toole 2008 The first blow fly parasitoid takes a head start in its termite host (Diptera: Calliphoridae, Bengaliinae; Isoptera: Macrotermitidae). *Syst. Biodivers.* **6**, 25-30.

Intra- and Interspecific Agonistic Behaviour of *Microcerotermes crassus* Snyder (Blattodea: Termitidae)

by

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Abstract

Intra- and interspecific (with *Coptotermes gestroi*, *Globitermes sulphureus* and *Odontotermes* sp.) agonistic behaviours of *Microcerotermes crassus* collected from different locations were observed in a laboratory assay. All termites were tested in pairwise tests (soldiers vs. soldiers, soldiers vs. workers, and workers vs. workers). No intraspecific agonism was found in *M. crassus* colonies, while all interspecific pairings resulted in combat. Survivorship of *M. crassus* in intraspecific study was mostly recorded at 100%. No or relatively low numbers of *M. crassus* survived in the interspecific study. Different behaviours exhibited by *M. crassus* towards the opponent species are discussed.

Key words: termites, intraspecies, interspecies, aggression, survivorship

Introduction

Subterranean termites are an important group of urban insects pests in tropical countries (Lee 2002, Lee *et al.* 2007). Lee *et al.* (2007) reported that in the suburbia and rural settlements in Malaysia and Singapore, there are 7 genera of subterranean termites (*Coptotermes, Macrotermes, Microtermes, Globitermes, Odontotermes, Schedorhinotermes* and *Microcerotermes*) that can be found in and around buildings and structures. Lee (2002) stated that a structure can be infested by more than one termite species at the same time or by different species one after the other.

According to Grace (1996), when different species, colonies or individuals in a colony encounter one another, social interactions of aggression and responses to aggression are known as agonistic behaviour. Numerous literatures have reported on intra- and interspecific aggression in termites (Binder, 1988; Haverty and Thorne, 1989; Pearce et al., 1990). Thorne and Haverty (1991) stated that the presence of aggression and agonistic responses in termite interactions can vary with species, population, colony, castes and individuals. When termites interact with other individuals of a different or same colony and/or species, a wide range of agonistic behaviours may be displayed. These agonistic behaviours can vary from no aggression to fierce combat (Su and Haverty 1991). Termite researchers often use aggressive behaviours to delimit boundaries among colonies of the same species (Binder 1988, Haverty and Thorne 1989, Pearce *et al.* 1990). According to Thorne and Haverty (1989), the use of natural aggressive behaviours between termite colonies in bioassays or directly in termite control, enables more detailed studies on the biology of a particular termite species. This study was conducted to investigate the agonistic behaviour between *Microcerotermes crassus* colonies from different locations and also between *M. crassus* with different termite species.

Materials and methods

Termites. Four *Microcerotermes crassus* colonies from different locations were collected; Universiti Sains Malaysia (Minden campus, Penang Island), Muka Head (Penang), Balik Pulau (Penang) and Padang

Serai (Kedah). Termites were obtained by excavating part of the nest and brought back to the laboratory for separation. Different colonies isolated were placed in different plastic containers provided with moist filter paper.

Coptotermes gestroi, *Globitermes sulphureus*, and *Odontotermes* sp. were paired with *M. crassus* in this experiment. All termite species were field-collected from Universiti Sains Malaysia, Minden campus, Penang Island. *C. gestroi* was collected from an in-ground monitoring station that was established earlier in the campus. By using the technique described by Tamashiro *et al.* (1973), the field collected termites were separated from soil debris in the laboratory. *M. crassus* and *G. sulphureus* were collected by excavating part of its nest/mound. In the laboratory, the termites were isolated from the nest and soil debris by lightly tapping the nest materials. Foraging termites of *Odontotermes* sp. were collected from underneath dead branches or dried leaves on the forest floor. Termites along with dead branches and dried leaves were gathered and brought back to the laboratory for separation. Different termite species collected were placed in different plastic containers provided with moist filter paper.

Intraspecific aggression assay. A piece of round moistened filter paper was placed in a 9-cm diameter Petri dish as the test arena, and the arena was divided half by a piece of plastic sheet. The test termites. *M. crassus* colonies were tested in pairwise tests (soldiers vs. soldiers, soldiers vs. workers, and workers vs. workers). Ten individuals (workers and/or soldiers from each colony) were selected for the test. Before the removal of the plastic sheet, termites introduced were left to acclimatize in their respective area for 10 minutes. After the plastic sheet was removed, the activity of the termites was recorded for 5 min. Interactions between the termites were observed and scored in accordance to the ranks as shown in Table 1. To differentiate different colonies, unstained termites or termites stained with 0.5% Nile Blue A or 0.5% Neutral Red were used in the study. After the recording, the dishes were covered and placed in complete darkness for 24 hours before the no survivors was recorded. Each combination was replicated five times.

Interspecific aggression assay. *M. crassus* was tested against *C. gestroi, G. sulphureus* and *Odontotermes* sp. in pairwise tests (soldiers vs. soldiers, soldiers vs. workers, and workers vs. workers). Ten termites (workers and/or soldiers) were used for each replicate. Each termite species was placed simultaneously in their respected areas on the test arena and divided by a plastic sheet. After ten minutes, the partition was removed, exposing individuals of the two termite species. Experiment was replicated five times for each combination.

Initial agonistic responses were recorded with a video recorder for five minutes and all interactions were noted according to the scores in Table 1. After that, the termites were held in the same dish and covered before being kept in the dark for 24 hours at room temperature. The no. survivors was counted after 24 hours.

Table 1: Scores for different behaviours exhibited by *Microcerotermes crassus* during encounters.

Score	Behaviour	Description
1	Antennation	Contact between antenna-antenna OR antenna-body
2	Jerking	Body moving up and down very quickly
3	Avoidance	Reorientation and increase in walking rate away from opponent
4	Chasing/Escaping	Chasing and/or being chased
5	Attack	Attempts to bite the opponent without success
6	Defecation	Deposition of a droplet of anal fluid on or near the opponent
7	Grappling	Grab, grasp and drag opponent with mandibles
8	Biting	Strong and successful bites resulting in piercing opponents body

Data analysis. Termite survivorship between different colonies/species were analysed by using paired t-test at α=0.05. Frequency of each score in percentage was calculated, and transformed into arcsine values before subjected to one-way ANOVA, and means were separated by using Tukey HSD at $p = 0.05$. All analyses were performed by using Statistix® Version 7.0 (Analytical Software, Tallahassee, Florida).

Results and discussion

Intraspecific aggression. So far, majority of the reports described aggressive behaviour between conspecific termite colonies. Intraspecific encounters among termites reported displays of immediate and evident recognition behaviour (Thorne 1982). In this study, a relatively low or no mortality was recorded between all the pairings of *M. crassus* of different colonies ($p>0.05$) after 24 hours. One or two termite mortality was recorded. There was no evidence to demonstrate that the dead individuals were caused by agonistic behaviour as all appendages were still intact. Mortality could have been caused by injuries inflicted during the handling of termites.

No evidence of aggression between all the pairings was observed and recorded. Only the occasional antennation or mutual grooming was observed. Lack of agonism between termites from different colonies among some subterranean termite species (Clement 1986, Grace 1996, Leponce *et al.* 1996, Polizzi and Forschler 1998, 1999) may be difficult to interpret.

Interspecific aggression. All interspecific pairings resulted in displays of aggression. Results showed that 24 hours after the initial encounter, no or few survivorship of *M. crassus* was recorded in their encounter with *C. gestroi*, *G sulphureus* and *Odontotermes* sp. for all pairings. Overall, interactions between worker termites of *Microcerotermes crassus* and workers of the opponent species showed that antennation behaviour was recorded at the highest among other behaviours (Table 2). Significant differences in frequency of antennation behaviour were found between *Coptotermes gestroi* and *Odontotermes* sp. with *Globitermes sulphureus*. According to Thorne (1982), worker termites can act as very useful and capable, defensive units in termite-termite encounters. Results showed significant difference of attack behaviour in *M. crassus* towards *C. gestroi* with *G sulphureus* and *Odontotermes* sp. ($p<0.05$). Other behaviours exhibited by *M. crassus* workers were not significantly different between the three opponent species ($p>0.05$).

In worker-soldier encounters, significant differences were recorded for antennation, attacking and biting behaviour ($p<0.05$). There was a distinct difference with the frequency of antennation behaviour exhibited by *M. crassus* workers versus *G sulphureus* soldiers when compared with the other two termite species ($p<0.05$). *M. crassus* workers attacked *Odontotermes* sp. more than compared to the other two species. Biting behaviour exhibited by worker *M. crassus* was significantly different between *C. gestroi* and *G sulphureus* ($p<0.05$). Remaining behaviours exhibited were not significantly different between the three opponent species.

M. crassus soldiers exhibited antennation behaviours more frequently with workers of *C. gestroi* when compared to other termite species ($p<0.05$). Pairings of soldier-worker encounters showed a significant difference in defecation behaviour exhibited towards *C. gestroi* and *Odontotermes* sp. when compared to *G sulphureus* ($p<0.05$). Less grappling and biting was exhibited by *M. crassus* soldiers towards *C. gestroi* when compared to *G sulphureus* and *Odontotermes* sp. ($p<0.05$). *M. crassus* soldiers attacked soldiers of *G sulphureus* the least among the three termite species ($p<0.05$). No or relatively low occurrences of other behaviours were exhibited by *M. crassus* soldiers when they came into contact with its opponents.

Generally, interactions between *M. crassus* and *C. gestroi* was the highest as *C. gestroi* appears to be more active, providing more opportunities in encountering each other. Less frequent behaviours were

exhibited by *M. crassus* when they met *G sulphureus* and *Odontotermes* sp. *G sulphureus* was observed to be less active in their movements when compared to *C. gestroi* and *Odontotermes* sp..

References

Binder, B. F. 1988 Intercolonial aggression in the subterranean termite *Heterotermes aureus* (Isoptera:Rhinotermitidae). *Psyche* **95**: 123-137.

Clement, J.-L. 1986 Open and closed societies in *Reticulitermes* termites (Isoptera: Rhinotermitidae): Geographic and seasonal variations. *Sociobiology* **11**: 311-323.

Grace, J. K. 1996 Absence of overt agonistic behaviour in a Northern population of *Reticulitermes flavipes* (Isoptera: Rhinotermitidaae). *Sociobiology* **28**: 103-110.

Haverty, M. I. and B. L. Thorne 1989 . Agonistic behaviour correlated with hydrocarbon phenotypes in dampwood termites, *Zootermopsis* (Isoptera: Termopsidae). *J. Insect Behav.* **2**: 523-543.

Lee, C. Y. 2002 Control of foraging colonies of subterranean termites, *Coptotermes travians* (Isoptera: Rhinotermitidae) in Malaysia using hexaflumuron baits. *Sociobiology* **39**: 411-416.

Lee, C. Y., C. Vongkaluang and M. Lenz 2007 Challenges to subterranean termite management of multi-genera faunas in Southeast Asia and Australia. *Sociobiology* **50**: 213-221.

Leponce, M., Y. Roisin and J. M. Pasteels 1996 Intraspecific interactions in a community of arboreal nesting termites (Isoptera: Termitidae). *J. Insect Behav.* **9**: 799-817.

Pearce, M. J., R. H. Cowie, A. S. Pack and D. Reavey 1990 Intra-specific aggression, colony identity and foraging distances in Sudanese *Macrotermes* spp. (Isoptera: Termitidae: Macrotermitinae). *Ecol. Entomol.* **15**: 71-77.

Polizzi, J. M. and B. T. Forschler 1998 Intra- and interspecific agonism in *Reticulitermes flavipes* (Kollar) and *R. virginicus* (Banks) and effects of arena and group size in laboratory assays. *Insect. Soc.* **45**: 43-49.

Polizzi, J. M. and B. T. Forschler 1999 Factors that affect aggression among the worker caste of *Reticulitermes* spp. subterranean termites (Isoptera: Rhinotermitidae). *J. Insect Behav.* **12**: 133-146.

Su, N. Y. and M. I. Haverty 1991. Agonistic behaviour among colonies of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), from Florida and Hawaii: Lack of correlation with cuticular hydrocarbon composition. *J. Insect Behav.* **4**: 115-128.

Tamashiro, M., J. K. Fujii, and P. Y. Lai. 1973 A simple method to observe, trap, and prepare large numbers of subterranean termites for laboratory and field experiments. *Environ. Entomol.* **2**: 721-722.

Thorne, B. L. 1982 Termite-termite interactions: Workers as an agonistic caste. *Psyche* **89**: 133-150.

Thorne, B. L. and M. I. Haverty 1991. A review of intracolony, intraspecific, and interspecific agonism in termites. *Sociobiology* **19**: 115-145

Table 2: Frequency of different behaviours (mean±SE) exhibited by *Microcerotermes crassus* towards other termite species in pairwise test over a period of 5 minutes

Score	Frequency (mean±SE)											
	worker-worker			worker-soldier			soldier-worker			soldier-soldier		
	M.c vs.			*M.c* vs.			*M.c* vs.			*M.c* vs.		
	C.g	*G.s*	*O.* sp.	*C.g*	*G.s*	*O.* sp.	*C.g*	*G.s*	*O.* sp.	*C.g*	*G.s*	*O.* sp.
1	51.6±4.1[a]	26.2±4.6[a]	17.0±5.0[a]	**18.8±2.8[a]**	**17.4±2.1[b]**	**15.2±5.5[a]**	**57.4±6.0[a]**	**10.8±1.5[b]**	**15.8±3.0[a]**	13.4±2.7[a]	13.6±1.7[a]	12.7±1.52
2	1.0±0.6[a]	0.6±0.6[a]	1.8±1.2[a]	0.2±0.2[a]	0.6±0.6[a]	0.0±0.0[a]	0.0±0.0[a]	0.0±0.0[a]	0.2±0.2[a]	0.2±0.2[a]	0.0±0.0[a]	0.0±0.0[a]
3	12.4±3.6[a]	5.8±1.7[a]	3.4±2.1[a]	10.4±0.9[a]	5.4±1.7[a]	5.0±3.0[a]	0.0±0.0[a]	0.2±0.2[a]	0.0±0.0[a]	0.6±0.4[a]	1.0±0.3[a]	0.0±0.0[a]
4	6.0±1.0[a]	0.4±0.2[a]	0.4±0.2[a]	2.4±0.2[a]	0.2±0.2[a]	1.6±0.7[a]	1.8±0.7[a]	0.6±0.2[a]	1.4±0.9[a]	1.4±0.7[a]	0.0±0.0[a]	0.3±0.3[a]
5	**39.2±4.6[a]**	**1.2±2.8[b]**	**8.8±2.6[b]**	**19.2±2.6[a]**	**6.2±1.1[c]**	**27.0±5.6[b]**	40.6±7.4[a]	4.6±0.5[a]	18.2±4.4[a]	**18.8±2.6[a]**	**10.2±1.6[b]**	**25.7±3.2[a]**
6	5.0±0.8[a]	5.4±0.9[a]	2.8±0.5[a]	10.6±1.3[a]	4.8±1.0[a]	8.0±2.0[a]	**2.0±0.3[a]**	**0.2±0.2[ab]**	**0.0±0.0[b]**	0.6±0.4[a]	0.8±0.5[a]	0.3±0.3[a]
7	2.6±0.8[a]	6.6±1.4[a]	3.6±0.7[a]	9.6±2.2[a]	3.6±0.7[a]	11.4±2.3[a]	**0.8±0.4[a]**	**1.6±0.4[b]**	**3.8±1.9[b]**	10.2±1.8[a]	7.2±1.5[a]	4.3±0.7[a]
8	7.8±1.9[a]	8.6±1.5[a]	8.4±1.4[a]	**2.6±0.4[a]**	**4.2±0.7[b]**	**3.2±0.7[ab]**	**11.4±0.9[a]**	**12.4±1.5[b]**	**14.6±5.8[b]**	4.8±1.2[a]	8.2±1.8[a]	10.0±0.6[a]

[a] Means followed by the same letter within the same column (for each pairing) are not significantly different $p>0.05$, based on Tukey HSD.

M.c - Microcerotermes crassus
C.g - Coptotermes gestroi
G.s - Globitermes sulphureus
O. sp.- *Odontotermes* sp

Attractant and Arrestant Chemicals from *Cryptomeria* for Japanese Subterranean Termite *Reticulitermes speratus* (Blattodea: Rhinotermitidae)

by

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Abstract

The monitoring-baiting system is currently commercialized to control subterranean termites in Japan, and effectively works on *Coptotermes formosanus* but not on *Reticulitermes speratus*. To improve the effect of this system, we searched for attractant and arrestant chemicals of *R. speratus*. Chips of 3 wood species; *Pinus densiflora*, *Cryptomeria japonica* and North American Spruce, Genus *Picea* were separately immersed in hexane or diethyl ether to compare the activity as attractant and arrestant. Since *R. speratus* workers responded to the hexane extract of *C. japonica*, it was further chromatographed on silica gel and successively eluted with of hexane, 10%, 30%, and 50% diethyl ether-in-hexane, and diethyl ether. Both 30% and 50% diethyl ether-in-hexane fractions caused worker aggregation significantly more frequently than the other fractions. We confirmed that several compounds were characteristic to *C. japonica* and that the Kovats Indexes were 1400-1700 and 2000-2400. Some were estimated to be sesquiterpenoid alcohols, but further purification and chemical analyses are necessary for their identification.

Key words: *Reticulitermes speratus*, *Cryptomeria japonica*, attractant, arrestant, wood extract

Introduction

Of the over 2300 termite species in the world, about 180 species are known to damage buildings and about 80 species cause significant damage (Edwards and Mill, 1986). The recent termite catalogue updated indicates over 2800 species (Constantino, 1998). Subterranean termites, including mound building and arboreal species, account for 80% of the economically important species. In Japan, two subterranean termites, *Coptotermes formosanus* and *Reticulitermes speratus*, often cause serious damage to wooden buildings including historical ones, and the damage costs reach to 100-300 billion yen every year (Yoshimura, 2003), being much higher than the fire damage costs. They, especially the latter, tend to expand the distribution to north, and recent high airtight and adiabatic houses might help such expansion (Mori, 2003).

Various soil termiticides including organophosphate and carbamates have been used to control these termites in the last half century. Such soil termiticides were topically effective but hard to eliminate the termite colonies completely. They might cause health hazard in human because of their strong toxicity. Recent popular alternative is the monitoring-baiting program (Su and Scheffrahn, 1998; Forschler and Jenkins, 2000). Monitoring stations to detect termites are placed in the soil surrounding a building, and checked regularly. The monitoring devices are placed with slow-acting baits such as the chitin synthesis inhibitor, which is less harmful for human, when termites are found in the station. This monitoring-baiting program would decrease negative environmental impacts since it requires fewer amounts of the baits by the topical application. It is already applied commercially even in Japan, and effectively works on *C. formosanus*. However, it is not successfully applied to control *R. speratus* despite their importance as the pest, because they easily disperse when the monitoring station was replaced to the baits. For the application of this technique to *R. speratus*, it is necessary to calm them down even after the replacement of the station. We, therefore, focused on chemical attractant and arrestant of *R. speratus* to gain efficiency of attractant-and-arrestant activities among chemicals extracted from several wood species. Here we report the comparison of attractant and arrestant activities among chemicals extracted from several wood species.

Materials and methods

(a) Collection and rearing of study insects

Four mature *R. speratus* colonies, hereafter named colony A, B, C, and D, were collected in Kutsuki (colony A), Shiga Prefecture in May 2008, Matsugasaki (colony B, colony C) and Yoshidayama (colony D), Kyoto Prefecture. Colony B and D were collected in September 2008. Colony C was collected in December 2007. The colonies were individually maintained in plastic containers (250×350×40 mm) supplied with water and wood pieces at 28°C under 12L/12D.

(b) Preparation and separation of wood extracts

Chips of 3 wood species; *Pinus densiflora*, *Cryptomeria japonica* and North American Spruce, Genus *Picea* separately floured, and 10g each was immersed in 200ml of hexane or diethyl ether for 24 hours at room temperature. After filtration, each extract was stored in a deep freezer at -30°C.

The hexane extract obtained from 10g of *C. japonica* was chromatographed on approximately 1g of silica gel (particular size 0.040-0.063mm, Merck, Germany), and successively eluted with 3ml of hexane, 10%, 30%, and 50% diethyl ether-in-hexane, and diethyl ether.

(c) Bioassays

A cylinder (6cm diam., 4cm height) made of a transparent sheet was placed in the center of a plastic container (250×350×40 mm), and 6 or 8 small filter papers (2cm diam., 180μm thickness, Whatman Int., England) were displayed on the circumference of a cylinder at equal intervals. The filter papers were treated with 50μl of test samples of 3 wood extracts, or 5 chromatographic fractions, and solvent (hexane). All they were impregnated with 50μl distilled water to retain moisture. Only one filter paper was not impregnated with distilled water. 100 termites were taken inside of the cylinder. The cylinder was gently removed after evaporation of the solvents, and numbers of termites staying at each filter paper were counted at the following intervals; 1, 3, 5, 10, 15, 20, 30, 40, 50, 60 min. This was repeated 9-12 times with rotating the position of the test samples. Post hoc multiple comparisons were made by using Tukey's test.

(d) Chemical analyses

Gas chromatographic (GC) analyses were conducted on a Shimadzu GC-14A gas chromatograph equipped with a flame ionization detector and an apolar capillary column InertCap 1 (GL Science, 15m length, 0.25mm i.d., and 0.25μm film thickness). Helium was the carrier gas and the column head pressure was at 98kPa. Injection was made in the splitless mode for 1 min at 300°C. The detector was also set at 300°C. The column oven temperature was kept at 60°C for 1 min, programmed to 320°C at 10°C/min, and held at the final temperature for 10 min. Gas chromatograph-mass spectrometer (GC-MS) analyses were performed on a Shimadzu QP-5050 equipped with GC-17A and an apolar capillary column, DB-1 (J & W Scientific, 30m length, 0.25mm i.d., 0.25μm film thickness). Helium was the carrier gas with the column head pressure at 100kPa. Injection was made in the splitless mode for 1min at 250°C. The detector was set at 280°C. The column oven temperature was programmed from 60°C to 300°C at 10°C/min. EI mass spectra was measured at 70eV.

Results and discussion

Fig. 1 compared the number of termites staying at filter papers treated with test samples of hexane extract 20 min after releasing the termites. Significantly larger number of termites kept staying at the filter paper that was treated with *C. japonica* extract than *P. densiflora* and Spruce extracts. As shown in Fig. 2, it was almost always observed throughout the observation periods despite colonies tested.

In contrast, such significant differences were not observed among the test samples that were extracted by diethyl ether (Fig. 3 and 4). Total numbers of termites that remained on the filter papers were apparently smaller in the diethyl ether extract than the hexane extract (Fig. 2 and 4).

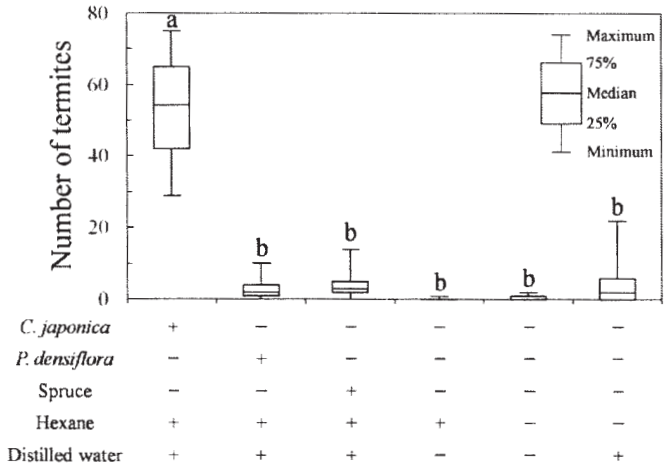

Figure 1. The numbers of termites among the three test samples; *C. japonica*, *P. densiflora*, and Spruce, at 20 min after releasing the termites (hexane extract). Each box-and-whisker diagram shows the smallest observation, lower quartile, median, upper quartile, and largest observation. Columns accompanied with the different letters are significantly different at $P = 0.001$ by one-way-layout ANOVA and subsequent ranking by Tukey-Kramer method.

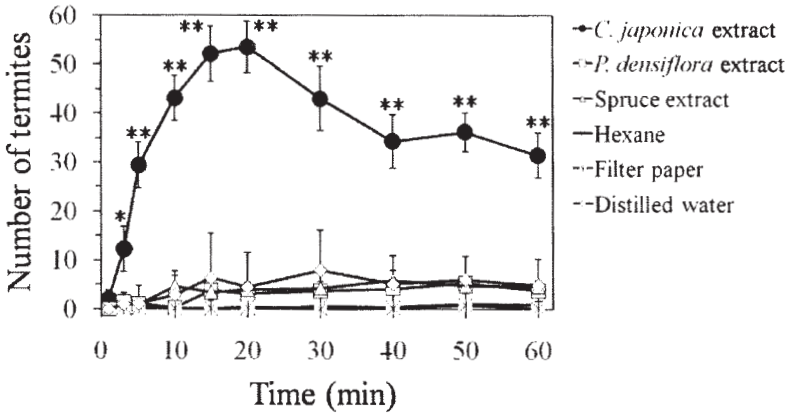

Figure 2. Transition of the number of the termites staying at each test sample for 60 min (hexane extract, colony A, n=9). Asterisks mean the averages are significantly different between *C. japonica* extract and the others at $P<0.05$ (*) and $P<0.001$ (**) by Tukey method.

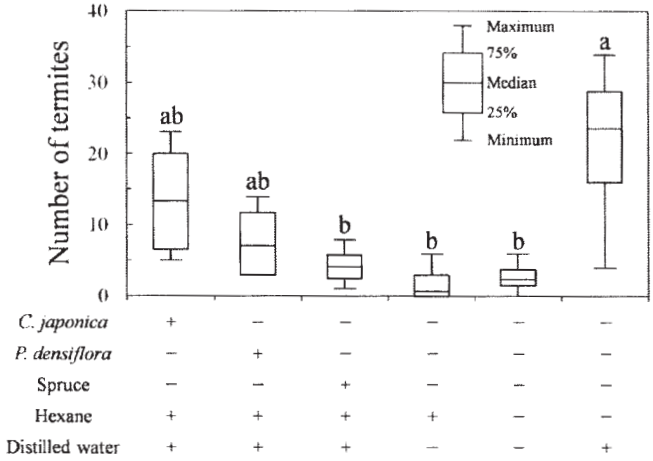

Figure 3. The numbers of termites among the three test samples; *C. japonica*, *P. densiflora*, and Spruce, at 40 min after releasing the termites (diethyl ether extract). Each box-and-whisker diagram shows the smallest observation, lower quartile, median, upper quartile, and largest observation. Columns accompanied with the different letters are significantly different at $P = 0.05$ by one-way-layout ANOVA and subsequent ranking by Tukey-Kramer method.

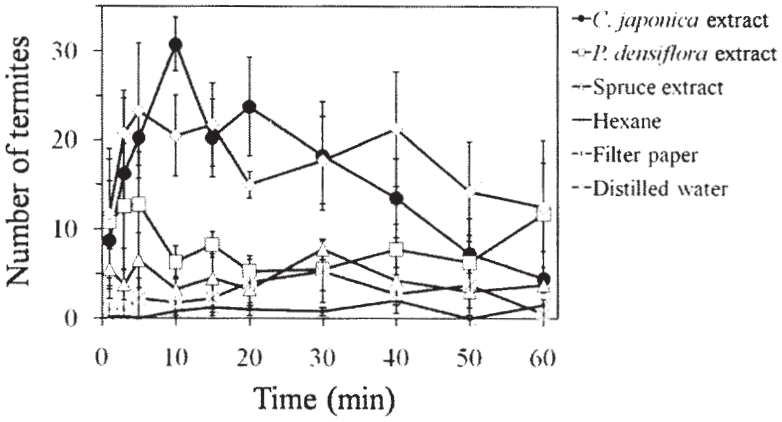

Figure 4. Transition of the number of the termites staying at each test sample for 60 min (diethyl ether extract, colony C, n=4).

Among 5 fractions obtained by silica gel column chromatography, the termites significantly preferred 30% diethyl ether-in-hexane fraction to the others, except for 50% diethyl ether-in-hexane fraction, which were treated on filter papers (Fig. 5). Its activity kept for at least 60 min.

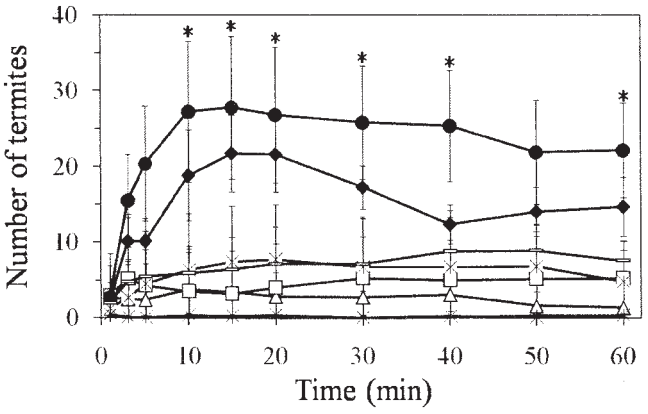

Figure 5. Transition of the number of the termites staying at each test sample for 60 min (colony A, n=9). Asterisks mean the averages are significantly different between *C. japonica* extract and the others at $P<0.05$ by Tukey method. △: hexane fraction; □: 10% diethyl ether-in-hexane fraction; ●: 30% diethyl ether-in-hexane fraction; ♦: 50% diethyl ether-in-hexane fraction; ∗: diethyl ether fraction; ×: solvent control, ▫: distilled water control, and +: filter paper.

GC analyses confirmed several peaks of which equivalent chain length (ECL) values were equivalent to *n*-alkanes of C14-C17 in the *C. japonica* extract, but neither in *P. densiflora* nor Spruce extracts (Fig. 6A, B, and C). All these three extracts contained long-chained wax components, of which ECL values were equivalent to C20-C24. Further longer-chained wax components were contained in both *P. densiflora* and Spruce. Thus, the GC profile of *C. japonica* was quite characteristic among the three samples.

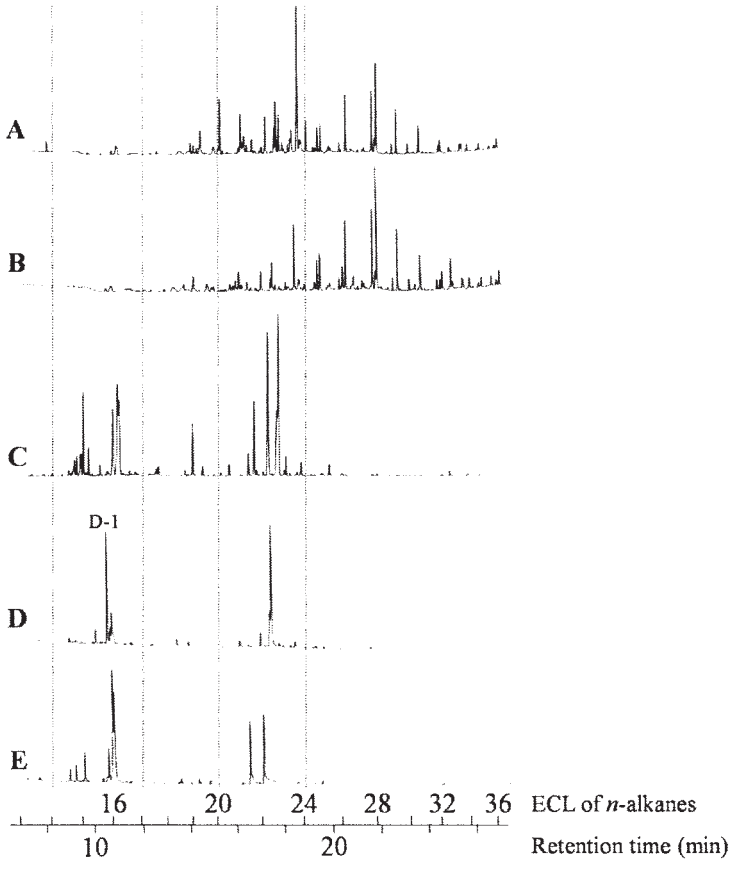

Figure 6. Gas chromatograms of the three test samples; *P. densiflora* (A), and Spruce (B), *C. japonica* (C), and two chromatographic fractions of *C. japonica* extract (30% diethyl ether-in-hexane fraction; D and 50% diethyl ether-in-hexane fraction; E). D-1 peak in the chromatogram D indicates the one of the characteristic peak of fraction eluted with 30% diethyl ether-in-hexane.

Both fractions eluted with 30% and 50% diethyl ether-in-hexane contained a few peaks corresponding to C14-C17 and C20-C24 in their ECL values (Fig. 6D and E). Of 30% diethyl ether-in-hexane fraction, the compounds corresponding to C14-C17 provided molecular ions at m/z 222 and the dehydrolized ion at m/z 204 (Fig. 7). These suggest the compounds would be sesquiterpenoid alcohols, $C_{15}H_{26}O$. Similarly, sesquiterpenoid alcohols should be also contained in the 50% diethyl ether-in-hexane fraction, though all those components were not yet completely identified.

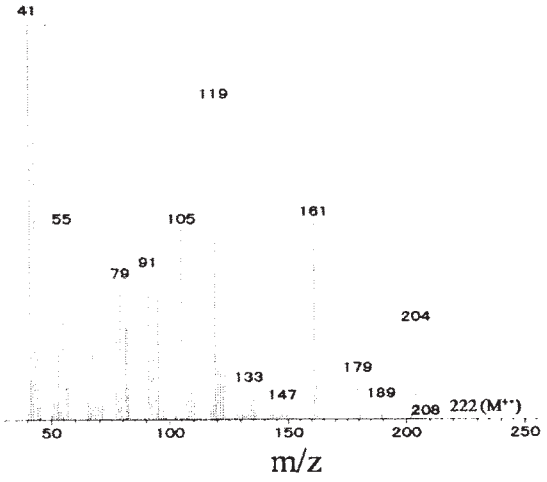

Figure 7. EI-mass spectra of peak D-1 of Fig. 6.

Conclusions

We revealed that the hexane extract of *C. japonica* contained arrestant chemicals of *R. speratus*. It is most likely that the chemicals would be alcohols because they were eluted in 30% and 50% diethyl ether-in-hexane fractions through silica gel and gave dehydrolized ions at relatively strong intensities. The compounds might involve sesqui- and di-terpenoid alcohols because of the respective Kovats Indexes that were 1400-1700 and 2000-2400 (Kovats, 1965), though further separation and analyses are necessary for their chemical identification. Our data also suggests the existence of masking substances in the diethyl ether extract, and the substances would be highly polar compounds because they were extractable by not hexane but diethyl ether. Although using polar solvent (i.e., diethyl ether) for extraction is quite general in the study of termite attractant and arrestant, it might prevent finding such chemicals because of masking effects by the highly polar compounds.

References

Constantino, R. 1998 Catalog of the living termites of the new world (Insecta: Isoptera). *Arquivos de Zoologia, museu de zoologia da universidade de Sao Paulo* **35** (2): 135-230.

Edwards, R. and A. E. Mill 1986 Termites in Buildings. Their Biology and Control. *East Grinstead: Rentokil Limited.*

Forschler, B. T. and T. M. Jenkins 2000 Subterranean termites in the urban landscape: Understanding their social structure is the key to successfully implementing population management using bait technology. *Urban Ecosystems* **4**: 231-251.

Kovats, E. 1965 Gas Chromatographic Characterization of Organic Substances in the Retention Index System. *Advances in Chromatography* **1**: 229-247.

Mori, M. 2003 The research of termite habitat in north Hokkaido in Japan. –Termite ecology and environment of habitat– *Rinsanshi dayori* **4**: 1-4 (in Japanese).

Su, N.-Y. and R. H. Scheffrahn 1998 A review of subterranean termite control practice and prospects for integrated pest management programs. *Integrated pest Management reviews* **3**: 1-13.

Yoshimura, T. 2003 Termite and Water. *Mokuzaikenkyuu shiryou* **39**: 38-47 (in Japanese).

Antennal Hygroreception of the Termite, *Coptotermes formosanus*

by

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Abstract

Owing to a large body surface-to-volume ratio in insects, environmental humidity is particularly important for survival in all insects, especially in the termites which prefer and live in high humidity habitat. We recorded electroantennograms (EAGs) to humidity changes of the termite, *Coptotermes formosanus* Shiraki and found that the termite antennae responded increasing humidity with increasing depolarizing potential. Furthermore, we found the putative hygroreceptive sensillum on the antenna by cyclopaedic survey of antennal sensilla, and characterized its external structure and distribution. These results indicated that antennae play an important role to detect environmental humidity change in the termite.

Key words: *Coptotermes formosanus* Shiraki, antenna, electroantennogram, EAG, hygroreceptor, hygroreceptive sensillum

Introduction

The termite, *Coptotermes formosanus* Shiraki is a social insect and one of the most destructive insects of houses and wood structures in Japan and United States (Yamano, 2000; Mulrooney et al., 2007). Owing to a large body surface-to-volume ratio in insects, environmental humidity is particularly important for survival in all insects, especially in the termites which live in high humidity habitat. Their social organizations are known to be largely dependent on water demands (Nakayama et al., 2005; Kulis wt al., 2008). Therefore, sensory signals about environmental humidity change probably modulate the behavior patterns of the termites. However, no neural mechanisms underlying such humidity-dependent behavioral modulation remain clear now in the termite (Merivee et al., 1999; Yokohari, 1999), while some neural mechanisms related to humidity perception have been elucidated in the cockroach (Nishikawa et al., 1995, Nishino et al. 2005). In this situation we attempted, as the first step, to record EAGs to humidity changes in the termite and to identify putative hygroreceptive sensilla. These studies on hygroreception of the termite will bring about better understanding of the humidity-dependent behavioral modulation and will be useful to prevent their invasion to houses and wood structures.

The antennae of the termite are cephalic appendixes loading sensory structures, named sensilla, which receive various kinds of stimuli, such as chemical, mechanical, thermal and hygric stimuli. On the other hand, as the workers and soldiers of most species of termites are blind, they can not use visual information in their life. Thus, the antennal sensilla must play some important roles in life of the termite. Although the structures of sensilla on the termite antennae were reported in several papers (Costa-Leonardo and Soares, 1997; Ziesmann, 1996; Prestage et al., 1963), the physiological properties of the antennal sensory system of the termite were hardly examined (Yanagawa et al., submitted).

In this present study, in order to show the detection ability of humidity change or graduation of habitat environment in the termite, we will report here EAGs to water vapor stimuli and the external structure and distribution of the putative hygroreceptive sensilla on the termite antennae.

Materials and methods

Insects

The termites, *C. formosanus*, were collected in Fukuoka, Japan and maintained in plastic boxes (49 × 36 × 32 cm) in a dark chamber at 25 °C. The termites were fed on seasoned pinewood (kuromatsu; *Pinus Thunbergii).* Worker termites were transferred from the above colonies into 90 ×

15 mm Petri dishes containing a wet paper disc and placed in a dark chamber at 25 °C for 1 to 3 weeks before use.

Recording of EAGs

An antenna was excised at scape with a small scissor after termites had been cold anesthetized on ice for 30 minutes. It was fixed on a slide glass with double-sided adhesive tape under a standard dissecting microscope. Both ends of the antenna were inserted slightly into the glass electrodes to make an electrical contact, and thereafter shielded with liquid paraffin in order to prevent fluid in the electrodes and lymph in the antenna from evaporating. The fluid in the electrodes was physiological saline for cockroaches (1.5 % 200 mM KCl0.9 %; 200 mM $CaCl_2 \cdot 2H_2O$; 0.1 % 200 mM $Na_2HPO_4 \cdot 2H_2O$; 0.9 % 200 mM $NaH_2PO_4 \cdot H_2O$) (Yamazaki and Narahashi 1959). The electrodes were made from borosilicate glass tubes (0.50 mm ID, 1.0mm OD) using a laser puller (P-2000, Sutter Instrument Co., Novato CA).

The stimulus and control air were prepared as follows. Fresh air was taken by a diaphragm pump (AP-115 IWAKI AIR PUMP, IWAKI CO., LTD, Tokyo) from the outside. The air was desiccated with silica gel and then cleaned by passing through active carbon. The flux was controlled at 1.0 liter/minute by a flowmeter en route. The cleaned air was then fed to a three-way electromagnetic valve operated by an electric pulse generator. One of the outlets was connected to a glass tube for the control stream and the other was further divided into four branches by means of glass T tubes. Each branch was connected to a small glass bottle (30 ml) after passing through a stop valve that was used to select the stimulus. In this study, one of the bottles contained 1 ml distilled water as source of water vapor and others were empty. The air passing through these bottles was fed to glass tubes for stimulation. The nozzles (3 mm in inner diameter) of the control and stimulus tubes were arranged 2 cm apart from the specimen in a concentric circle. The duration of one stimulus puff was set at 2 seconds. The specimen was exposed to the control clean and dried air during the interstimulus time. In order to keep the experimental environment clean the air near the sample was always ventilated. Temperature was kept 20-25 ℃.

Scanning electron microscopy

Termite antennae were examined with scanning electron microscopy (SEM). Samples were fixed in 4 % (v/v) osminium acid for 2 h and then dehydrated through a graded acetone series. Samples were air dried and coated with platinum-palladium (E-1030 Ion Sputter, Hitachi, Ltd., Tokyo). Observations were made with a Hitachi S-4100 SEM (Hitachi, Ltd., Tokyo).

Results and discussion

The termite antenna is moniliform and consists of three different portions; a scape, a pedicel and a flagellum. The scape is the basal segment followed by the pedicel which acts as a pivot between the scape and flagellum. The flagellum forms the rest of antenna composed by 11-13 segments. In order to examine the humidity detecting ability of the termite antenna, EAGs to humidity stimuli were recorded at 20-25 °C. Twenty-five EAGs were recorded from 5 antennae. Figure 1 shows typical EAG responses to the humidity change from dry to moist. In the EAG response to a single puff (2 seconds) of humidity increase, the gradual downward (negative) deflection appeared initially, then the potential sustained stably until the stimulus cessation and finally the sudden upward (positive) deflection appeared at the stimulus cessation.. Thereafter the potential was almost kept at the pre-stimulus level. The average EAG amplitude was 1.55 ±1.13 mV (n=25) at 1 second after stimulus-on. The amplitude of EAG to humidity change was considerably larger than that to fungal odor recorded in our previous study, where the range of amplitudes was 0.87 – 1.27 mV (Yanagawa et al. 2008). Thus, the termite antennae appear quite sensitive to humidity change.

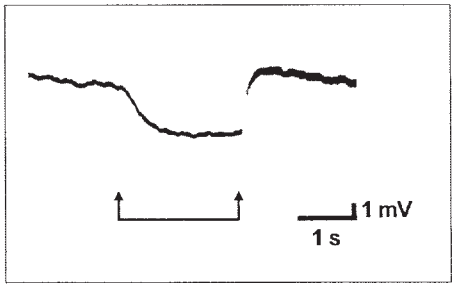

Fig. 1. EAG response to humidity change from dry to moist in the termite, *C. formosanus*. A horizontal bar with arrows under the record is the stimulation mark.

It is well known that the hygroreceptive sensillum of insects has characteristic and modality-specific structures (Yokohari, 1999). The cuticular apparatus of the sensillum has

It is well known that the hygroreceptive sensillum of insects has characteristic and modality-specific structures (Yokohari, 1999). The cuticular apparatus of the sensillum has smooth-surfaced side-wall, no pores such as olfactory pores and taste pore, and a rough-surfaced cap-like structure on its distal end of the stem. Its size is under 0.5 μm in tip diameter and under 5 μm in length. We surveyed cyclopaedically the antennal sensilla with SEM in order to find out the sensillum which satisfied the above criteria. In the results, we found the coincident sensilla the external structure of which is resembled very much with that of the hygroreceptive sensillum of *Periplaneta* (Fig.2). The sensilla distributed at one per flagellar segment and located at distal part of the segment very close to the segmental junction. Some located on ventral surface of the antennae and others on dorsal surface. Though this type of sensillum distributes in different patterns among insect species, the sensillum of the termite was distributed in a similar fashion to that of *Periplaneta*. The total number of the sensilla per antenna was about 10-12. The number of the sensillum in this termite was relatively smaller than that of other insects (Yokohari, 1999). We do not know why the amplitude of EAG to humidity change is relative large in spite of the small number of the sensilla. This is the first report of function and external structure of the antennal hygroreceptors of the termite.

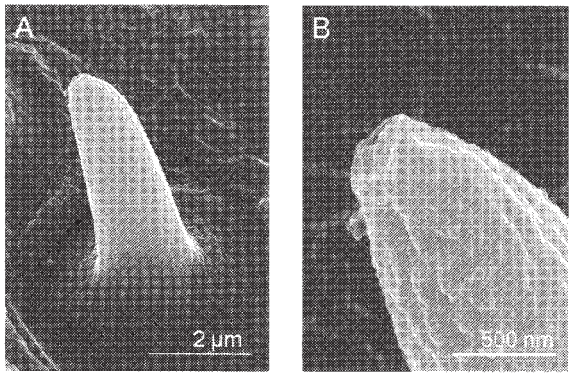

Fig. 2. SEM pictures of hygroreceptive sensillum on *C. formosanus* antennae.
A: Hygrosensitive sensillum. B: Tip of the sensillum in A.

Conclusion

The antenna of the termite *C. formosanus* responds with electrical potential change (EAG) to humidity change. The hygroreceptive sensillum has smooth-surfaced side-wall, no pores such as olfactory pores and taste pore, and a rough-surfaced cap-like structure on its distal end. The sensillum is located distal part of each segment very closed to segmental junction area, and the number of the sensillum is one per flagellar segment.

References

Costa-Leonardo, A. M. and H. X. Soares 1997 Morphological aspects of neotropical termite antenna under scanning microscopy. *Revta. bras. Ent.* **41**, 47-52.

Kulis, J., A. S. Sajap and C. Y. Loong 2008 Effect of moisture and relative humidity on survival andfeeding activity of the Asian subterranean termite, *Coptotermes gestroi* (Isoptera: Rhinotermitidae). *Sociobiology* **52** (3), 579-587.

Merivee, E., M. Rahi and A. Luik 1999 Antennal sensilla of the click beetle, *Melanotus villosus* (Geffroy) (Coleoptera: Elateridae). *International Journal of Insect Morphology* **28**, 41-51.

Mulrooney, J. E., T. L. Wagner, T. G. Shelton, C. J. Peterson, and P. D. Gerarad 2007 Historical review of termite activity at forest service termiticide test sites from 1971 to 2004. *Journal of Economic Entomology* **100** (2), 488-494.

Nakayama, T., T. Yoshimura and Y. Imamura 2005 Feeding activities of *Coptotermes formosanus* Shiraki and *Reticulitermes speratus* (Kolbe) as affected by moisture content of wood. *J. Wood*

Sci. **51**, 60-65.
Nishikawa, M., F. Yokohari and T. Ishibashi 1995 Central projections of the antennal cold receptor neurons and hygroreceptor neurons of the cockroach *Periplaneta americana*. *J. Comp. Neurol.* **361**, 165-176.
Nishino, H., M. Nishikawa, F. Yokoahari and M. Mizunami 2005 Dual, multilayered somatosensory maps formed by antennal tactile and contact chemosensory afferents in an insect brain. *The Journal of Comparative Neurology* **493**, 291-308.
Prestage, J. J., E. H. Slifer and L. B. Stephens, 1963 Thin-walled sensory pegs on the antenna of the termite worker, *Reticulitermes flavipes*. *Annals of the Entomological Society of America* **56**, 874-878.
Rocha, L., G. R. P. Moreira, and L. R. Redaelli, 2007. Morphology and distribution of antennal sensilla of *Gryon gallardoi* (Brèthes) (Hymenoptera: Scelionidae) females. *Neotropical Entomology* **36**(5), 721-728.
Yamano, K. 2000. Damage, detection and prevention. [In] "Termite and termite control " ed. by *The Japan Termite Control Association* (Tokyo), 127-140 (in Japanese).
Yamazaki T. and Narahashi T. 1959 The effects of potassium and sodium ions on the resting and action potentials of the cockroach giant axon. *J. Insect physiol.* **3**, 146-158.
Yanagawa, A., Yokohari, F. and Shimizu, S. 2008. The role of antennae in removing entomopathogenic fungi from cuticle of the termite, *Coptotermes formosanus*. *Journal of Insect Science* (In press).
Yokohari, F. 1999 Atlas of arthropod sensory receptors. Springer (Tokyo).
Ziesmann, J., 1996 The physiology of an olfactory sensillum of the termite *Schedorhinotermes lamanianus*: carbon dioxide as a modulator of olfactory sensitivity. *J. Comp. Physiol.* **179**, 123-133.

Behavioral Analysis of Tremulation and Tapping of Termites

by
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Abstract

Termites communicate with their nestmates using various types of behaviors. Some of them shake themselves back and forth / right and left, or bump their head / abdomen against foundation. In this study, we focused on tremulation (especially back and forth movement) and tapping behaviors (head bumping) in four species of termites (*Coptotermes formosanus, Reticulitermes speratus, Incisitermes minor, Zootermopsis nevadensis*). These were analyzed using a high-speed video camera, to compare the differences among species. Frequencies of the tremulation observed in *C. formosanus*, *R. speratus* and *Z. nevadensis* were almost same, 15-20 Hz, but their patterns showed a slightly difference between species. Maximum displacement amplitudes of tapping tended to be larger than those of tremulation. We observed that *I. minor* did not show tapping behaviors, but tremulation with frequency of ca. 1.0 - 1.5Hz. These results revealed that tremulation and tapping behaviors were species-specific, although the roles of tremulation are still unresolved.

Key words: termites, tremulation, tapping, species-specific behavior

Introduction

Termites attack woody structures with a large number of their nestmates, sensing environmental stimuli of plant secondary metabolites, sun-light, air-blow and so on. They integrate the information of food, nests, or enemies, and form a well-established society by communicating with nestmates.

Some insects have established sound and vibratory communications for their social and ecological interactions (Claridge, 2005 ; Cocroft, 2005: Hrncir et al., 2008). Also in termites, alarm communications have received attention, and been investigated (Stuart, 1963, 1967, 1969, 1988; Howse, 1964a, 1965). When accept strong stimuli of air-blow, flashlight or volatile chemicals, termites bump their head against nestwalls (tapping) and/or shake their bodies back and forth (tremulation). Such behaviors have been regarded as alarm communication behaviors, which inform their nestmates alarm signals by vibrating the surrounding air and/or some structures. The sounds produced by tapping the substrate with their head are audible to humans, thus they have been recorded and analyzed (Howse, 1964ab, 1965; Krishner et al., 1994). However, the movement of both tapping and tremulation are so fast that they could not have been analyzed in details with normal-speed video cameras.

In this study, we observed the tremulation and tapping behaviors of four species of termites using a high-speed video camera, comparing to reveal the differences among species.

Materials and methods

Termites

Four species of termites, *Coptotermes formosanus* Shiraki, *Reticulitermes speratus* (Shiraki), *Incisitermes minor* (Hagen), *Zootermopsis nevadensis* (Hagen) were used. A colony of *C. formosanus* was obtained from a laboratory colony that was originally collected in Okayama Prefecture, Japan and maintained in the dark at 26 $\pm$ 2 °C and approximately 65% relative humidity for over 7 years at the Forestry and Forest Products Research Institute (FFPRI). Colonies of *R. speratus*, *Z. nevadensis*, and *I. minor* were collected in Kagoshima, Hyogo, and Tokyo Prefecture, respectively, and were maintained at room temperature until use in FFPRI. Both workers and soldiers were subjected to the behavioral analysis.

Behavioral analysis

Termites were introduced into test chambers (plastic dish: 30 mm i.d.×10 mm height). Their behaviors were observed at 25 °C in a laboratory, and were recorded on a high-speed video camera (Photoron Co.Ltd., Tokyo, Japan) at 60 or 125 flames per seconds. The recorded images were analyzed with TEMA 2D software (Photoron Co.Ltd.), setting the datum point on the head, and X-Y axes as shown in Fig.1 for measuring the head movement.

All recordings were performed under LED red light (Keyence Co.Ltd., Tokyo, Japan; wavelength: 600-700nm) after stimulating them by shaking the test chamber.

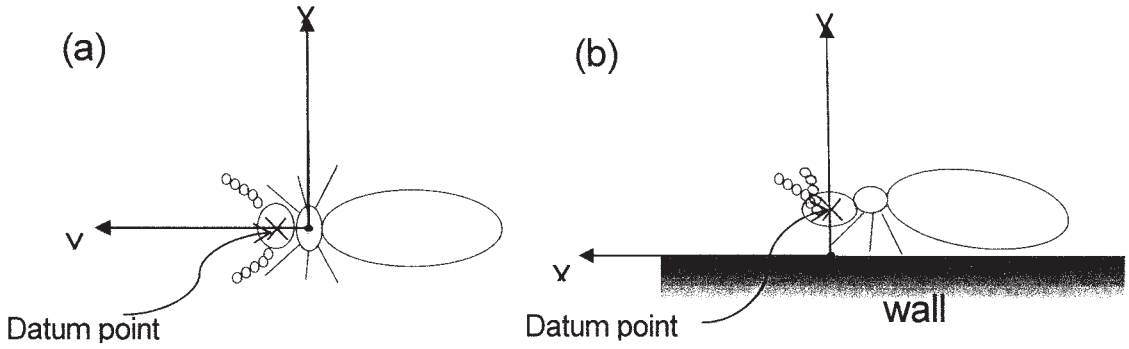

Fig. 1 Datum points and X-Y axes for tracking (a) a tremulation and (b) a tapping.
(a) A datum point (×) was settled on the head of a termite, and was tracked using a software, TEMA 2D. An origin point was set on the thorax of a termite at a time when just after a termite begun tremulation. Y-axis was positioned by a joint of an origin point and a datum point at a time when just after a termite begun tremulation.
(b) A datum point was settled on the head of a termite, and was tracked using a software, TEMA 2D. X-axis was positioned in parallel with a wall surface on which a termite tapped.

Results and discussion

Tremulation and tapping behaviors of 4 species of termites were observed, and analyzed to compare the behaviors among species (Table 1).

Table 1 Tremulation behaviors of 4 species of termites

Tested species	Body length (mm)		DA[1] (mm)		Main frequency (Hz)	
	W[2]	S[3]	W	S	W	S
C. formosanus	5.0	6.0	0.5	0.7	15-20	15-20
R. speratus	4.0	5.0	0.1	0.5	15-20	15-20
I. minor	5.0	6.0	0.3	0.2	1.5	1.0
Z. nevadensis	15.0	18.0	1.0	[4]NA	15-20	[4]NA

[1]DA: Displacement amplitude ; [2]W: Worker ; [3]S : Soldier [4]NA: Not analyzed

Workers of *C. formosanus* were trembling (Fig. 2) their heads for about 1.2 seconds with 15 cycles, and the maximum displacement amplitude (DA) were 0.5 mm. Frequency of *R. speratus* workers (Fig. 3) was 15-20 Hz in 1.5 seconds with 13 cycles, showing slightly different patterns to *C. formosanus* in tremulation. *I. minor* rocked approximately once a second with a maximum DA of 0.2 - 0.3 mm.

Z. nevadensis workers shook their bodies with DA of 1.0 mm and tapped heads with DA of 2.2 mm at 15-20 Hz for 0.3-0.5 seconds. Tapping behavior was frequently observed in *Z. nevadensis*, but was not so frequently observed in *C. formosanus* and *R. speratus*. In the case of *I. minor*, they never showed tapping behaviors during our observation. Howse (1970) has pointed out that the tapping in drywood termites is not so active. Their habitat, dry wood, is a good conductor of the vibration, and so they do not need to waste so much energy in transmitting the signal. Tapping was supposed to need more energy than tremulation.

Soldiers shook their bodies slightly larger than workers, but their frequencies and patterns were almost the same. Not all workers show tremulation and tapping, suggesting that there might be an individual difference or a role division among worker castes.

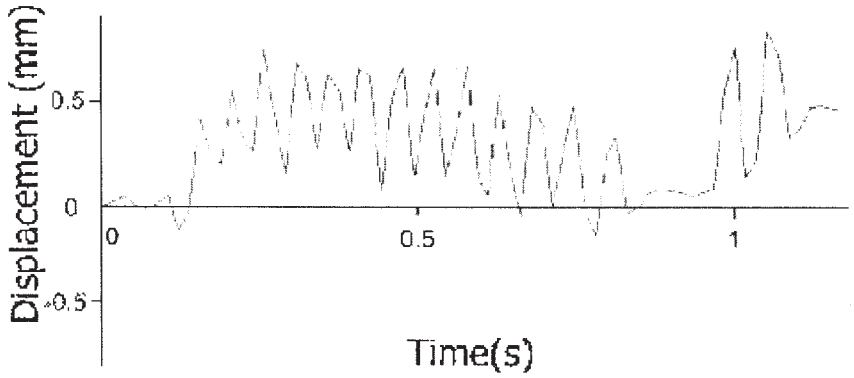

Fig. 2 Time course of a displacement (mm) of *C. formosanus* worker during tremulation

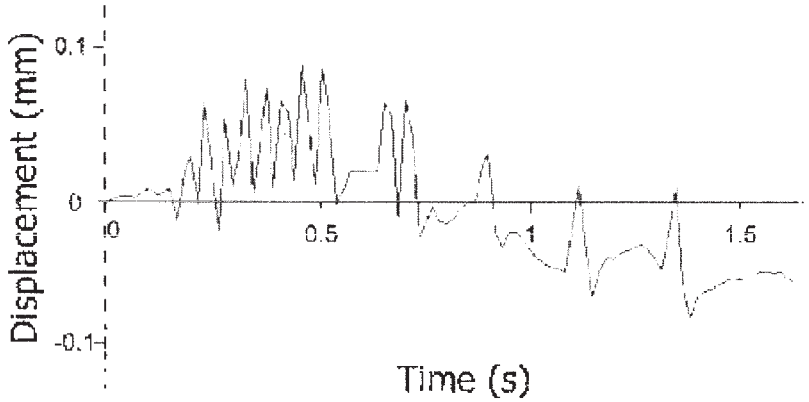

Fig. 3 Time course of a displacement (mm) of *R.speratus* worker during tremulation

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References

Claridge, M. 2005 Insect sounds and communication. Insect sounds and communication: physiology, behaviour, ecology and evolution, Drosopoulos, S. and Claridge, M. F. eds, pp. 3-10, CRC Press LLC, London.

Cocroft, R. B. and R. L. Rodriguez 2005 The behavioral ecology of insect vibrational communication. *BioScience* **55**, 323-334.

Howse, P. E. 1964a The significance of the sound produced by the termite *Zootermopsis angusticollis* (Hagen). *Animal Behaviour* **12**, 284-300.

Howse, P. E. 1964b An investigation into the mode of action of the subgeneral organ in the termite, *Zootermopsis angusticollis* Emerson, and in the cockroach, *Periplaneta americana* L.. *J. Insect Physiology* **10**, 409-424.

Howse, P. E. 1965 On the significance of certain oscillatory movements of termites, *Insectus Sociaux* **12**, 335-346.

Howse, P. E. 1970 Termites: A study in social behaviour, Hutchinson University Library, London.

Hrncir, M., A.-I. Gravel, D. L. P.Schorkopf, , V. M Schmidt and R. Zucci 2008 Thoracic vibration in stingless bees (*Melipona seminigra*): resonances of the thorax influence vibrations associated with flight but not those associated with sound production. *J. Exp. Biol* **211**, 678-685.

Kirchner, W. H., I. Broecker, and J. Tautz, 1994 Vibrational communication in the dampwood termite *Zootermopsis nevadensis*. *Physiological Entomology* **19**, 87-190.

Stuart, A. M. 1963 Studies on communication of alarm in the termite *Zootermopsis nevadensis* (Hagen), Isoptera. *Physiol. Zool.* **36**, 85-96.

Stuart, A. M. 1967 Alarm, defense and construction behavior relationship in termites (Isoptera). *Science* **156,** 1123-1125.

Stuart, A. M. 1969 Social behavior and communication, Biology of termite I, Krishna, K and Weesner, F. M. eds., pp. 193-232, Academic Press, London.

Stuart, A. M. 1988 Preliminary studies on the significance of head-banging movements in termites with special reference to *Zootermopsis angusticollis* (Hagen) (Isoptera: Hodotermitidae). *Sociobiology* **14**, 49-60.

Molecular Cloning and Expression of a Aquaporin cDNA from the Formosan Subterranean Termite, *Coptotermes formosanus*

by
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Abstract

Subterranean termites require liquid water to survive, and water is required not only for their physiological functions, but also for building nests and associated structures with optimal microclimatic conditions. We have conducted cDNA cloning of aquaporin (AQP, water channel), belonging to the major intrinsic protein (MIP) family from the digestive tract of Formosan subterranean termite, *Coptotermes formosanus*, and succeeded to obtain two clones encoding AQP homolog designated as CfAQP. Sequence analysis of *C. formosanus* aquaporin reveals two regions of NPA motifs, which are a characteristic feature of all members of the MIP family. RT-PCR analysis revealed the expression of CfAQP in the digestive tract and other tissues, suggesting that CfAQP may play important roles not only in water recycling but also other essential water transport processes in the termite.

Key words: aquaporin, *Coptotermes formosanus*, digestive tract, expression analysis

Introduction

Termites can classify 3 groups (Damp-wood termites, subterranean termites, Dry-wood termites) from their ecological features. Damp-wood termites and subterranean termites require liquid water to survive. Especially in subterranean termite, water is required for building nests and associated structures and for maintaining their optimal microclimatic conditions. There have been many studies on the utilization of water by termites, including water carrying behavior using a pair of labial gland reservoirs, water sacs, in the European subterranean termite, *Reticulitermes santonensis* (Grube and Rudolph, 1999a, b); however, the molecular mechanisms of water metabolism in termites have never been investigated in detail, in spite of the possibility that novel methods for controlling termites, such as specific inhibition of their water utilization, might be developed, based on these precise mechanisms.

Aquaporins (AQPs) are members of the major intrinsic protein (MIP) family found in mammals, invertebrates, plants and microorganisms, and facilitate the rapid movement of water across cell membranes (Agre et al., 1993; Reizer et al., 1993). The first AQP was purified from human red blood cells and shown to increase the permeability of water in *Xenopus* oocytes (Preston et al., 1992). Since then, a number of AQP as well as other members of MIP family have been identified from various species. The sequence information, water channel properties, gene expression and/or physiological roles have been studied on several insect AQPs such as *Drosophila* big brain and DRIP (Rao et al., 1990; Dow et al., 1995; Kaufmann et al., 2005), *Cicadella* AQPcic (Le Cahérec et al., 1996), *Rhodnius* RP-MIP (Echevarría et al., 2001), *Aedes* AeaAQP (Pietrantonio et al., 2000; Duchesne et al., 2003), *Pyrocoelia Pr*AQP (Lee et al., 2001), *Polypedilum* PvAqp1 and PvAqp 2 (Kikawada et al., 2008) and *Bombyx Bommo*AQP (Miyake and Azuma, 2008).

As the first step toward understanding the molecular mechanisms of water transport in termite, we report the first successful cDNA cloning of termite AQP (CfAQP) from the digestive tracts of the Formosan subterranean termite, *Coptotermes formosanus*. In addition, its expression of their transcripts in the digestive tract and other tissues are confirmed.

Materials and methods

Termites

Workers of *C. formosanus* were collected from a laboratory colony, which was maintained at room temperature (ca. 25℃) in a plastic container (420 × 740 × 325 mm in height).

Isolation of RNA

Total RNA was isolated from the whole digestive tract or nine parts (foregut, midgut, hindgut, rectum, Malpighian tubules, water sacs, fat bodies, epidermis and antennae) of 100 workers using an RNA/DNA Mini kit (QIAGEN). To avoid RNA contamination from symbiotic bacteria and protozoa, each digestive tract was

carefully dissected in 0.7% NaCl solution and then its luminal contents were thoroughly washed out with phosphate-buffered saline.

cDNA cloning and sequencing

Total RNA (ca. 5μg) isolated from the whole digestive tract of *C. formosanus* was reverse-transcribed with the *ThermoScript*™ RT-PCR System (Invitrogen) using oligo(dT)20 as a primer at 50°C for 60 min. The synthesized first-strand cDNA was used as a template for PCR amplification of partial AQP cDNA fragments encoding a central domain between two highly conserved NPA motifs, using a set of degenerate primers F1 (5'-GGDKGHCACATYAAYCCVGCSGTSAC-3') and R1 (5'-CCGAAWGWNCKRGCBGGRTTCATRCT-3'), which were respectively designed on the basis of the nucleotide sequences corresponding to the coding regions of two NPA motifs of four insect AQPs (*Cicadella viridis*, *Aedes aegypti*, *Bombyx mori*, and *Haematobia irritans exigua*). PCR was performed with an initial step of 95°C for 3 min, 30 cycles of denaturation (95°C, 1 min), annealing (50°C, 1 min), and extension (72°C, 2 min). The final elongation was at 72°C for 10 min.

The 5'- and 3'-ends of cDNA were amplified by 5' and 3' RACE methods using the Marathon™ cDNA Amplification Kit (Clontech) according to the manufacturer's instructions. For 5' and 3' RACE-PCRs, gene-specific primers, F2 (5'-CTGTGTAGTTTATCGCTGCAAGATGACA-3') and R2 (5'-TGTGGGCTGATCGTCTCGGGGCACGTCA-3'), were designed, based on the central region sequence of an aquaporin-like cDNA fragment obtained by RT-PCR.

The PCR products were characterized by agarose gel electrophoresis and purified using a QIAquick PCR purification kit (QIAGEN). The purified PCR products were ligated into pCR2.1 vector (Invitrogen), and used to transform *Escherichia coli* Top10F' competent cells (Invitrogen). For each PCR product, at least three different clones of plasmid DNA were purified using the Plasmid Midi Kit (QIAGEN) and their inserts were sequenced on both strands, using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and the ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems) in the Center for Gene Research of the Yamaguchi University.

Sequence analysis

Homology searches of both nucleic acid and the deduced amino acid sequences of *C. formosanus* AQP cDNA were performed using the BLAST program provided by the NCBI.

Hydropathy analysis (Kyte and Doolittle, 1982) and prediction of the transmembrane domain were performed using the Hydropathy Analysis Program provided by the TCDB (http://www.tcdb.org/progs/hydro.php).

RT-PCR analysis of AQP transcripts in nine different parts

For comparative analysis of the gene expression of *C. formosanus* AQP (CfAQP) among 5 parts of the digestive tract (foregut, midgut, hindgut, rectum, Malpighian tubules), total RNA (0.5μg) from each part was reverse-transcribed, and then the synthesized first-strand cDNA was amplified by PCR using the CfAQP-specific primer pair, F3 (5'-GTACAGTTTCATCTTCCGAGCCAGG-3') and R3 (5'-AGGAGCACGATCAAACTTGTTCTGC-3'). We also investigated the CfAQP gene expression in other tissues, including the water sac, fat body, epidermis and antenna by RT-PCR using F3 and R3 primers with total RNA isolated from each tissue. Because the amounts of total RNA isolated from these tissues were very small and various, different amounts of total RNA (2.5ng from water sac, 7.5ng from fat body, 0.1μg from epidermis and 29ng from antenna) were used for RT-PCR analysis. The RT-PCR products were characterized by 2% agarose gel electrophoresis.

Results and discussion

Characterization of C. formosanus *AQP cDNA*

A cDNA of 1609 base pairs (bp) encoding a putative AQP (CfAQP) with 249 amino acids (Genbank accession no. AB433197) was obtained from the digestive tract of *C. formosanus* workers. CfAQP contained two NPA motifs, which are a characteristic feature of all members of the MIP family and form a single aqueous pathway and the narrowest region of the pore (Verkman and Mitra, 2000; Zardoya, 2005). Furthermore, the AEFL sequence near the N-terminus common among orthodox AQPs (Verkman and Mitra, 2000) and C-terminal SYDF sequence found in several insect AQPs (Spring et al., 2007) were conserved in CfAQP. The NPA motifs and the aromatic/arginine (ar/R) constriction region, consisting of four amino acids (F-56, H-180, C-189 and R-195), were found to be involved in size selectivity by forming a water-selective pore by substrate size exclusion (de Groot and Grubmuller, 2001; Beitz et al., 2006). In particular, the ensemble of F, H, and R, which are highly conserved among orthodox AQPs (Kikawada et al., 2008), was also found in the

corresponding positions of CfAQP1 (F-60, H-184 and R-199), supporting its function as a water-selective channel. BLAST search result showed that all of the proteins with more than 53% amino acid sequence identity to CfAQP were insect AQPs or putative AQPs including those of *Anopheles gambiae* (64%), *A. Aegypti* (61%), *C. viridis* (59%), *H. irritans exigua* (58%) and *B. mori* (53%). In addition, the hydropathy plot profile of CfAQP was consistent with those of MIP family proteins possessing 6 hydrophobic transmembrane domains. The results indicate that CfAQP has a water-transporting function and excluded the possibility that it originated from symbiotic microorganisms inhabiting the digestive tract.

Expression analysis of C. formosanus *AQP gene in the digestive tract*

Different amounts of CfAQP cDNA fragment (627 bp) were detected in the 5 parts of the digestive tract, most abundantly in the rectum and very slightly in the hindgut.

Water homeostasis in insects is achieved by a two-part excretory system composed of secretion in the Malpighian tubules and reabsorption in the ileum and rectum (Chawn and Nicolson, 2004). In previous studies, functional insect AQPs such as *Drosophila* DRIP and *Rhodnius* RP-MIP were detected in Malpighian tubules (Dow et al., 1995; Kaufman et al., 2005; Echevarría et al., 2001). Remarkable detection of CfAQP transcripts in the rectum and Malpighian tubules suggested that CfAQP is involved in water recycling through the excretory system. Detection of CfAQP transcripts in the midgut suggested that it might contribute to water absorption from food. Interestingly, significant expression of CfAQP was detected in the foregut, where AQP gene expression has not been reported in any other insect, except for the expression of the DRIP gene at the foregut-midgut border of the *Drosophila* embryo (Kaufman et al., 2005).

In addition to the digestive tract, CfAQP transcripts were detected in the water sacs (labial gland reservoirs), epidermis and antenna. In *Bombyx mori*, AQP (*Bommo* AQP) in the silk glands, of which the origin is labial glands, contributes to stabilizing liquid silk in a native state by transporting water from hemolymph (Miyake and Azuma, 2008). Similarly, CfAQP in water sacs might contribute to water transport from hemolymph.

Thus, CfAQP may contribute not only to water recycling in the digestive tract but also to water transport processes in various tissues, except the fat body. Investigations of these processes in their relationship to the physiology and behavior of termites will provide interesting insights into water-related termite ecology and useful ideas for developing novel termite control chemicals and technologies.

References

Agre, P., G. M. Preston, B. L. Smith, J. S. Jung, S. Raina, C. Moon, W. B. Guggino, and S. Nielsen 1993 Aquaporin CHIP: the archetypal molecular water channel, *Am. J. Physiol. Renal Physiol.* **265**, F463-476.

Beitz, E., B. Wu, L. M. Holm, J. E. Schultz and T. Zeuthen 2006 Point mutations in the aromatic/arginine region in aquaporin 1 allow passage of urea, glycerol, ammonia and protons. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 269-274.

Chawn, S.L. and S. Nicolson 2004 *Insect Physiological Ecology*. Oxford University Press, New York. 243pp.

de Groot, B. L. and H. Grubmuller 2001 Water permeation across biological membranes: mechanism and dynamics of aquaporin-1 and GlpF. *Science* **294**, 2353-2357.

Dow, J. A.T., D. C. Kelly, S. A. Davies, S. H. P. Maddrell, and D. Brown 1995 A novel member of the major intrinsic protein family in *Drosophila*: are aquaporins involved in insect Malpighian (renal) tubule fluid secretion? *J. Physiol.* **489**, 110.

Duchesne, L., J. F. Hubert, J. M. Verbavatz, D. Thomas, and P. V. Pietrantonio 2003 Mosquito (*Aedes aegypti*) aquaporin, present in tracheolar cells, transports water, not glycerol, and forms orthogonal arrays in *Xenopus* oocyte membranes. *Eur. J. Biochem.* **270**, 422-429

Echevarría, M., R. Ramirez-Lorca, C. S. Hernandez, A. Gutierrez, S. Mendez-Ferrer, E. Gonzalez, J. J. Toledo-Aral, A. A. Ilundain, and G. Whittembury 2001 Identification of a new water channel (Rp-MIP) in the Malpighian tubules of the insect *Rhodnius prolixus*. *Pflugers Arch* **442**, 27-34.

Grube, S. and D. Rudolph 1999a Water supply during building activities in the subterranean termite *Reticulitermes santonensis* de Feytaud (Isoptera, Rhinotermitidae). *Insectes Sociaux* **46**, 192-193.

Grube, S. and D. Rudolph 1999b The labial gland reservoirs (Water Sacs) in *Reticulitermes santonensis* (Isoptera: Rhinotermitidae): Studies of the functional aspects during microclimatic moisture regulation and individual water balance. *Sociobiology* **33**, 307-323.

Kaufmann, N., J. C. Mathai, W. G. Hill, J. A. T. Dow, M. L. Zeidel, and J. L. Brodsky 2005 Developmental expression and biophysical characterization of a *Drosophila melanogaster* aquaporin. *Am. J. Physiol., Cell. Physiol.* **289**, C397-C407.

Kikawada, T., A. Saito, Y. Kanamori, M. Fujita, K. Śnigórska, M. Watanabe, and T. Okuda 2008 Dehydration-inducible changes in expression of two aquaporins in the sleeping chironomid, *Polypedilum vanderplanki*. *BBA Biomembranes* **1778**, 514-520

Kyte, J. and R. F. Doolittle 1982 A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* **157**, 105-132.

Le Cahérec, F., S. Deschamps, C. Delamarche, I. Pellerin, G. Bonnec, M. T. Guillam, D. Thomas, J. Gouranton, and J. F. Hubert 1996 Molecular cloning and characterization of an insect aquaporin functional comparison with aquaporin 1. *Eur. J. Biochem.* **241**, 707-715.

Lee, K .S., S. R. Kim, S. M. Lee, K. R. Lee, H. D. Sohn, and B. R. Jin 2001 Molecular cloning and expression of a cDNA encoding the aquaporin Homolog from the Firefly, *Pyrocoelia rufa*. *Korean Journal of Entomology* **31**, 269-279.

Miyake, S. and M. Azuma 2008 Developmental expression and the physiological role of aquaporin in the silk gland of *Bombyx mori*. *J. Insect Biotech. Sericol.* **77**, 87-93.

Pietrantonio, P. V., C. Jagge, L. L. Keeley, and L. S. Ross 2000 Cloning of an aquaporin-like cDNA and in situ hybridization in adults of the mosquito *Aedes aegypti* (Diptera: Culicidae). *Insect molecular biology* **9**, 407-418.

Preston, G. M., T. P. Carroll, W. B. Guggino, and P. Agre, Appearance of water channels in Xenopus oocytes expressing red cell CHIP28 protein, Science **256** (1992) 385-387

Rao, Y., L. Y. Jan, and Y. N. Jan 1990 Similarity of the product of the *Drosophila* neurogenic gene big brain to transmembrane channel proteins, *Nature* **345**, 163-167.

Reizer, J., A. Reizer, and M. H. Jr. Saier 1993 The MIP family of integral membrane channel proteins: sequence comparisons, evolutionary relationships, reconstructed pathway of evolution and proposed functional differentiation of the two repeated halves of the proteins. *Crit. Rev. Biochem. Mol. Biol.* **28**, 235-257

Spring, J. H., S. R. Robichaux, N. Kaufmann and J. L. Brodsky 2007 Localization of a *Drosophila* DRIP-like aquaporin in the Malpighian tubules of the house cricket, *Acheta domesticus*. *Comp. Biochem. Physiol.* **148A**, 92-100.

Verkman, A. S. and A. K. Mitra 2000 Structure and function of aquaporin water channels. *Am. J. Physiol. Renal Physiol.* **278**, F13-28.

Zardoya, R. 2005 Phylogeny and evolution of the major intrinsic protein family. *Biol. Cell.* **97**, 397-414.

RNA Interference in Symbiotic Protists of the Termite *Coptotermes formosanus* Shiraki through Ingestion of siRNA by the Host Termite

by
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Abstract

This study investigated RNA interference (RNAi) in symbiotic protists via voluntary feeding on small interfering RNA (siRNA) by host termite, *Coptotermes formosanus*. We used siRNAs of 21 nucleotides designed for endoglucanases of symbiotic protists, *Pseudotrichonympha grassii* (PgEG), *Holomastigotoides mirabile* (HmEG), and *Spirotrichonympha leidyi* (SlEG) to silence genes of protists. Disorganization of *P. grassii* and *H. mirabile* occurred in hindgut of termite that had fed PgEG and HmEG siRNAs, respectively, in a few days. These results provide the first examples of ptotisticidal effects from siRNA feeding by host termite. Additionally, these results contribute to novel RNAi-based termiticides because elimination of *P. grassii* and *H. mirabile* leads host termite to starvation.

Key words: RNA interference, endoglucanase, symbiotic protest, *Coptotermes formosanus*

Introduction

RNA interference (RNAi) is the mechanism through gene-specific double-stranded RNA (dsRNA) triggers degradation of homologous transcripts (Ullu *et al.* 2004). Since the first report in 1998 (Fire *et al.* 1998), RNAi has spread throughout all field of eukaryotic biology and has provided a tool to analyze gene function in a variety of organisms. Diverse processes like heterochromatin assembly and maintenance (Hall *et al.* 2002, Volpe *et al.* 2002), DNA and histone metylation (Ziberman *et al.* 2003), DNA elimination (Mochizuki *et al.* 2002), promoter silencing (Mette *et al.* 2000), and development control (Lau *et al.* 2001, Lee & Ambros 2001) are guided by small RNAs in the size range of 20-26 nucleotides. Three major classes of small RNAs have been described (Matranga & Zamore 2007): (i) small interfering RNAs (siRNAs) of ~21 nucleotides are produced through cleavage of dsRNAs. siRNAs function in defense against external nucleic acids or intermediates of viral replication; (ii) microRNAs (miRNAs) mainly regulate genes involved in developmental processes; (iii) piwi-interacting RNAs (piRNAs) of ~27 nucleotides are processed from single-stranded RNA sequences. It is believed that piRNAs function as master controllers of mobile genetic sequences called transposable elements (Brennecke *et al.* 2007).

dsRNA-mediated gene silencing in termite was reported by Zhou *et al.* siRNAs (15-25 bp) that had been enzymatically digested from synthesized dsRNAs of ~500 bp were injected into the thorax of the termite *Reticulitermes flavipes* (Zhou *et al.* 2006, Zhou *et al.* 2007), and were introduced into gut of the termite by voluntary feeding by the termite (Zhou *et al.* 2008). Both injection-based RNAi and feeding-based RNAi successfully silenced termite genes: one encoding an endogenous digestive cellulase (endoglucanse) enzyme and the other a caste-regulatory hexamerin storage protein.

RNAi can lead to the degradation of non-targeted mRNAs that happen to contain a cross-hybridizing region to siRNA trigger (Jackson *et al.* 2003, Scacheri *et al.* 2004) or to the translational silencing of unrelated transcripts by siRNAs acting as microRNAs (Doench & Sharp 2004, Birmingham *et al.* 2006). These unintended effects have been collectively referred to as off-target effects, which if not controlled for can seriously limit the utility of RNAi. Off-target sequences in long dsRNAs can lead to off-target effects and contribute to a significant false positive error rate (Kulkarni *et al.* 2006). RNAi using a mixture of various length siRNAs (15-25 bp) that were prepared by digestion of dsRNAs (~500 bp) would have high possibility of off-target effects or false positive error. To minimize off-target effect, we adopted a single (not a mixture) siRNAs of 21 nucleotides designed for symbiotic ptotists' genes: endoglucanases of *Pseudotrichonympha grassii* (PgEG), *Holomastigotoides mirabile* that is synonymous with *Holomastigotoides hartomanni* (HmEG), and *Spirotrichonympha leidyi* (SlEG). Elimination of the symbiotic protists by RNAi-based gene silencing could lead the host termite to infirmity, because the lower termite, including *C. formosanus*, can not degrade any cellulose substrates without the help of symbiotic protists in the hindgut (Yoshimura *et al.* 1993). Here, by targeting the gene fragments in the protists through siRNA feeding by the host termite, we provide indication demonstrating that silencing of genes in protists can produce lethal protisticidal effects. In addition, our findings also demonstrate that feeding-based RNAi by the host insect is the effective method to control genes of

symbiotic ptotists.

Materials and methods

Insect *C. formosanus* individuals were collected from a nest that has been maintained with blocks of *Pinus densiflora* as the food source at 26°C in our laboratory for 8 years. Worker caste termites were used for all experiments.

siRNA synthsis Table 1 provides sequence of template DNA oligonucleotide sets used for siRNA amplification. Template DNA oligonucleotides had random six nucleotides (5'-GATCAC-3') plus T7 RNA recognition sequences (5'-TAATACGACTCACTATAGGG-3') appended onto their 5' ends and 2 nucleotides (5'-TT-3') to 3' ends. For example, template DNA oligonucleotide sequences of PgEG were 5'-GATCAC TAATACGACTCACTATAGGG GTCTTACTTGGATAACGCC TT-3' for forward and 5'-GATCAC TAATACGACTCACTATAGGG GGCGTTATCCAAGTAAGAC TT-3' for reverse DNA templates, respectively. To make double strand DNA, reverse complement DNA oligonucleotides for template DNA oligonucleotides were prepared, respectively. Namely, in the case of PgEG, 5'-AA GGCGTTATCCAAGTAAGAC CCCTATAGTGAGTCGTATTA GTGATC-3' for forward DNA template and 5'-AA GTCTTACTTGGATAACGCC CCCTATAGTGAGTCGTATTA GTGATC-3' for reverse DNA template. siRNA was synthesized using a commercially available kit (*in vitro* Transcription T7 kit for siRNA synthesis, Takara-Bio, Shiga, Japan) and stored in RNase free water. Synthesized siRNAs and double-strand RNA (dsRNA) marker (Dynamarker dsRNA, BioDynamics laboratory, Tokyo, Japan) were run on 6% acrylamide gels (100 V, 3 h, 13 cm of gel length) and visualized with ethidium bromide. We designed siRNAs for endoglucanses of symbiotic protists, *P. grassii* (PgEG), *H. mirabile* (HmEG), *S. leidyi* (SlEG) and unrelated control sequence (UCS) using the siRNA Target Designer (http://www.promega.com/siRNADesigner/Default.htm) software.

siRNA feeding bioassays siRNA-mediated gene silencing was accomplished using voluntary feeding bioassay. Thirty worker caste termites were placed into 90-mm tissue culture dish (Koryo, Nara, Japan) that contained 20-mm diameter treated filter paper (Advantec, Tokyo, Japan). In advance, filter paper was treated with 100 μl of siRNA solution (125 ng/μl) or the same volume of autoclaved Milli-Q® water (Millipore, MA) alone for blank test and air-dried. Final density of siRNA was 4.0 $\mu g/cm^2$-filter paper. Assays for endoglucanases of symbiotic protists (PgEG, HmEG, and SlEG) siRNAs were carried out for 3 weeks, whereas assays for unrelated control sequence (UCS1-3) siRNA were carried out for 2 weeks. Dead termites were removed and mortality was documented every day. Feeding (mg/dish) was determined by comparing weights of paper disks before and after rearing termites.

Microscopic observation Two workers were dissected and hindgut removed from each using fine-tipped forceps every day for a week. The removed hindgut was rent on a hole-slide-glass and symbiotic protists were gently dispersed in 0.5 ml of 0.9% saline. Symbiotic protists on slide were observed using an Eclipse 80i microscope (Nikon, Tokyo, Japan) under white light. The images were captured using a DS-5MC CCD camera with a DS-U1 controller and an ACT-2U program (Nikon).

Results

siRNA synthesis As shown in Fig. 1, siRNAs of 21 nucleotides were synthesized for PgEG, HmEG, SlEG, UCS1-3. Each single-strand RNA of 19 nucleotides (shown in Table 1 as forward and reverse DNA templates) additionally had two uridylates (UU) to 3'end, e.g. PgEG siRNA was composed of 5'-GCUGUCGUACACGGUUGACUU-3' and 5'-GUCAACCGUGUACGACAGCUU-3'.

Disorganization of protists Among three species of symbiotic protists, *P. grassii* and *H. mirabile* in workers that fed on filter paper with PgEG or HmEG siRNAs were disorganized in a few days as shown in Fig. 2. Disorganization of *P. grassii* was found in two days and continued for a week when workers fed on filter paper with PgEG siRNA. Similarly, disorganization of *H. mirabile* was found in three days and continued to seven days when workers fed on filter paper with HmEG siRNA. Meanwhile, *S. leidyi* was sound throughout the observation period of a week when workers fed on filter paper with SlEG siRNA. All three species of protists were sound throughout the observation period when workers fed on filter paper with UCS1, UCS2, and UCS3 siRNAs as well as untreated filter paper.

Mortality of *C. formosanus* worker As shown in Fig.3, mortality of workers that fed on filter paper with HmEG abruptly rose in 7 days and reached about 70% in three weeks. Mortality increased gradually in workers that fed PgEG siRNA treated filter paper. Feeding on filter paper with SlEG siRNA scarcely affected mortality of workers for 21 days. In contrast to symbionts' endoglucanase siRNAs, increase in mortality of workers that

fed on filter paper with unrelated control sequence UCS1, UCS2, and UCS3 siRNAs was not larger than that of workers feeding on untreated filter paper.

Discussion

Obviously siRNAs targeting at symbiotic protists' endoglucanse had destructive effect on two kinds of protists, *P. grassii* and *H. mirabile*. RNAi mechanism in ptotists has been proposed as follows. siRNAs made a complex with an argonaute (AGO) protein The association between AGO-siRNA complexes and polyribosomes facilitates recognition of target mRNA by RISC (RNA-induced silencing complex), while the mRNA is being translated, or makes the translated mRNA for subsequent degradation in the cytoplasm. In alternative pathway, the AGO-siRNA complex might directly associate with ribosome-free mRNA and cleavage of mRNA might occur without a direct interaction between the translation and RNAi machineries (Ullu *et al.* 2004).

In protists, electroporation has been used as a means of delivering dsRNA to *Trypanosoma brucei* (Ngo *et al.* 1998). In the present study, the protists, *P. grassii*, *H. mirabile*, and *S. leidyi*, took siRNAs into their cells by self-feeding (endocytosis) on fractions of filter paper that had been impregnated with siRNAs and ingested by host termite, *C. formosanus*. Our findings demonstrate that RNAi based on feeding by host termite shows excellent potential as a functional tool for controlling the symbiotic protists. As far as we know, this is the first report of symbionts control via RNAi using host's feeding-based RNAi. Up to now, dsRNA feeding approaches to silence genes have been performed in the termite *Reticulitermes flavipes*, which fed filter paper impregnated with dsRNA for termite genes (Zhou *et al.* 2008) as well as the microbivorous nematode *Caenorhabditis elegans*, which fed the bacterium *Escherichia coli* that transcribes the recombinant dsRNA (Timmons & Fire 1998).

Outstanding increase in mortality of host termites was recorded in HmEG and PgEG siRNAs feeding. In the hindgut of termites that fed on HmEG or PgEG siRNAs, disorganization of the symbiotic protists *P. grassii* and *H. mirabile* was observed as mention above. One possible reason for this phenomenon could be that termites died of starvation caused by a decrease in symbiotic protists. However, mortality of *C. formosanus* workers in starvation was only ~15% in three weeks (Yoshimura *et al.* 1993), and starvation should not cause 30 to 70% mortality in only three weeks. Although another possible reason could be that ingredients in siRNA preparation affected health condition of host termite, this is not applicable to this case. Ingredients included in PgEG, HmEG, SlEG, UCS1, UCS2, and UCS3 siRNAs were not different from each other, because these siRNAs were prepared using same reagents and protocol. Mortality of termite that fed on unrelated control sequence UCS1-3 siRNAs was a mere 5% in two weeks, whereas mortality of termites that fed on HmEG and PgEG siRNAs was 59% and 18% in two weeks, respectively. It is possible that PgEG and HmEG siRNAs targeting endoglucanases of *P. grassii* and *H. mirabile* could be absorbed into termite through intestinal wall of termite and silence gene expression of termite through off-target effects. Absorption of siRNAs through intestinal wall would be possible, because successful dsRNA feeding approach to silence termite genes had already reported in *R. flavipes* (Zhou *et al.* 2008). However, lethal insecticidal effects by silencing of genes in protists in the present study were results of preliminary tests and ambiguity remains. Effectiveness and mechanisms of silencing genes of host insect by RNAi of symbionts should be inspected minutely in future study.

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References

Birmingham, A., E. M. Anderson, A. Reynolds, D. Ilsely-Tyree, D. Leake, Y. Fedorov, S. Baskerville, E. Maksimova, K. Robinson, J. Karpilow, W.S. Marshall & A. Khvorova. 2006 3'UTR seed matches, but not overall identity, are associated with RNAi off-targets. *Nature Meth.* **3**, 199-204.

Brennecke, J., A. A. Aravin, A. Star, M. Dus, M. Kellis, R. Sachidanandam and G. J. Hannon 2007 Discrete small RNA-generating loci as master regulators of transposon activity in Drosophila. *Cell* **128**, 1089-1103.

Doench, J. G. and P. A. Sharp 2004 Specificity of microRNA target selection in translational repression. *Genes & Development* **18**, 504-511.

Fire, A., S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver and C. C. Mello 1998 Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **391**,806-811.

Hall, I. M., G. D. Shankaranarayana, K. Noma, N. Ayoub, A. Cohen and S. Grewal 2002 Establishment and maintenance of a heterochromatin domain. *Science* **297**, 2232-2237.

Jackson, A. L., S. R. Bartz, J. Schelter, S.V. Kobayashi, J. Burchard, M. Mao, B. Li, G. Cavet and P. S. Linsley 2003 Expression profiling reveals off-target gene regulation by RNAi. *Nature Biotechnol.* **21**, 635-637.

Kulkarni, M. M., M. Booker, S. J. Silver, A. Friedman, P. Hong, N. Perrimon and B. Mathey-Prevot. 2006 Evidence of off-target effects associated with long dsRNAs in *Drosophila melanogaster* cell-based assays. *Nature Meth.* **3**, 833-838.

Lau, N.C., L. P. Lim, E. G. Weinstein and D. P. Bartei 2001. An abundant class of tiny RNAs with probable regulatory roles in *Caenorhabditis elegans*. Science **294**: 858-862.

Lee, R.C. & V. Ambros. 2001. An extensive class of small RNAs in *Caenorhabditis elegans*. Science **294**: 862-864.

Matranga, C. & P.D. Zamore. 2007. Small silencing RNAs. Curr. Biol. **17**: R789-R793.

Mette, M.F., W. Aufsatz, J. van Der Winden, M.A. Matzke & A.J. Matzke. 2000. Transcriptional silencing and promoter metylation triggered by double-stranded RNA. EMBO J. **19**: 5194-5201.

Mochizuki, K., N.A. Fine, T. Fujisawa & M.A. Gorovsky. 2002. Analysis of a piwi-related gene implicates small RNAs in genome rearrangement in tetrahymena. Cell **110**: 689-699.

Ngo, H., C. Tschudi, K. Gull & E. Ullu. 1998. Double-stranded RNA induces mRNA degradation in *Trypanosoma brucei*. PNAS **95**: 14687-14692.

Scacheri, P.C., O. Rozenblatt-Rosen, N.J. Caplen, T.G. Wolfsberg, L. Umayam, J.C. Lee, C.M. Hughes, K.S. Sharimugam, A. Bhattacharjee, M. Meyerson & F.S. Collins. 2004. Short interfering RNAs can induce unexpected and divergent changes in the levels of untargeted proteins in mammalian cells. PNAS **101**: 1893-1897.

Timmons, L. & A. Fire. 1998. Specific interference by ingested dsRNA. Nature **395**: 854.

Ullu, E., C. Tschudi & T. Chakraborty. 2004. RNA interference in protozoan parasites. Cell. Microbiol. **6**: 509-519.

Volpe, T.A., C. Kidner, I.M. Hall, G. Teng, S.I. Grewal & R.A. Martienssen. 2002. Regulation of heterochromatic silencing and histone H3 lysine-9 metylation by RNAi. Nature **297**: 1833-1837.

Yoshimura, T., J.-I. Azuma, K. Tsunoda & M. Takahashi. 1993. Cellulose metabolism of the symbiotic protozoa in termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotemitidae) I. Effect of degree of polymerization of cellulose. *Mokuzai Gakkaishi* **39**, 221-226.

Zhou, X., F. M. Oi and M. E. Scharf 2006 Social exploitation of hexamerin: RNAi reveals a major caste-regulatory factor in termites. *PNAS* **103**, 4499-4504.

Zhou, X., M. R. Tarver and M.E. Scharf 2007 Hexamerin-based regulation of juvenile hormone-dependent gene expression underlies phenotypic plasticity in a social insect. *Development* **134**, 601-610.

Zhou, X., M. M. Wheeler, F. M. Oi and M. E. Scharf 2008 RNA interference in the termite *Reticulitermes flavipes* through ingestion of double-stranded RNA. Insect Biochem. *Mol. Biol.* **38**, 805-815.

Ziberman, D., X. Cao and S. E. Jacobsen 2003 ARGONATU4 control of locus-specific siRNA accumulation and DNA and histone metylation. *Science* **299**, 716-719.

Table 1. Gene identities and sequences of the 19-length nucleotides selected for small interfering RNA (siRNA) template.

Gene identity (abbreviation)	Accession No.	Position of dsRNA template (bp)	Forward (5'→3') [a]	Reverse (5'→3') [a]
Endoglucanase of *P. grassii* (PgEG)	AB071001	333-351	GCTGTCGTACACGGTTGAC	GTCAACCGTGTACGACAGC
Endoglucanase of *H. mirabile* (HmEG)	AB071011	265-283	GGTGGGTTCGCGAGTGTAC	GTACACTCGCGAACCCACC
Endoglucanase of *S. leidyi* (SlEG)	AB189037	231-249	GAGCCTGACGGAGGATATC	GATATCCTCCGTCAGGCTC
Unrelated control sequence 1 (UCS1)			GATCGCTCGCGGCTCTTAC	GTAAGAGCCGCGAGCGATC
Unrelated control sequence 2 (UCS2)			GAATCCGCTTACCGAAATC	GATTTCGGTAAGCGGATTC
Unrelated control sequence 3 (UCS3)			GCTAACGGATTCACCACTC	GAGTGGTGAATCCGTTAGC

[a] siRNA templates additionally had random 6 nucleotides (GATCAC) plus T7 RNA polymerase recognition sequences (TAATACGACTCACTATAGGG) appended to their 5' ends and 2 nucleotides (TT) to 3' ends.

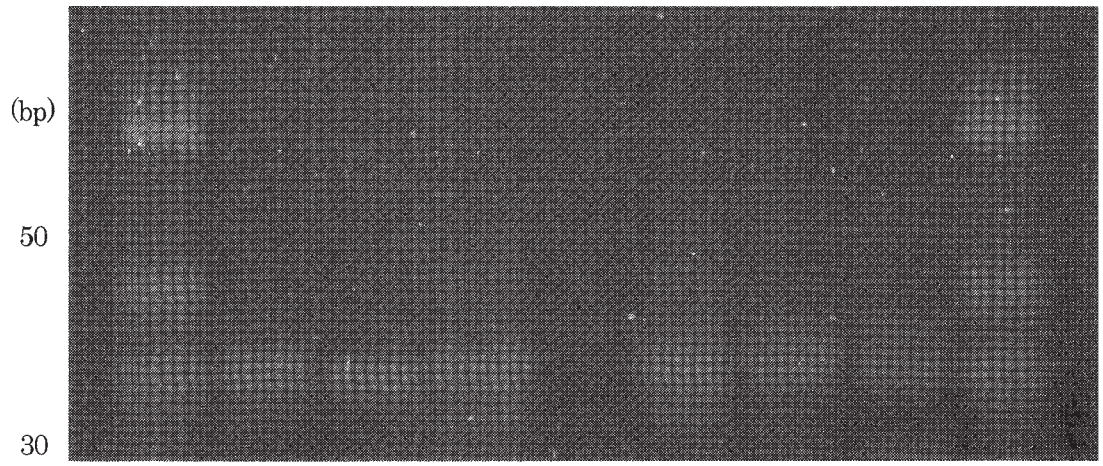

Fig. 1. Electrophoretic analysis of the synthesized siRNA.
Lane M, double-stranded RNA (dsRNA) ladder marker; lanes PgEG, HmEG, SlEG, UCS1, UCS2, and UCS3, siRNAs synthesized by T7 RNA polymerase with a pair of forward and reverse siRNA templates shown in Table 1.

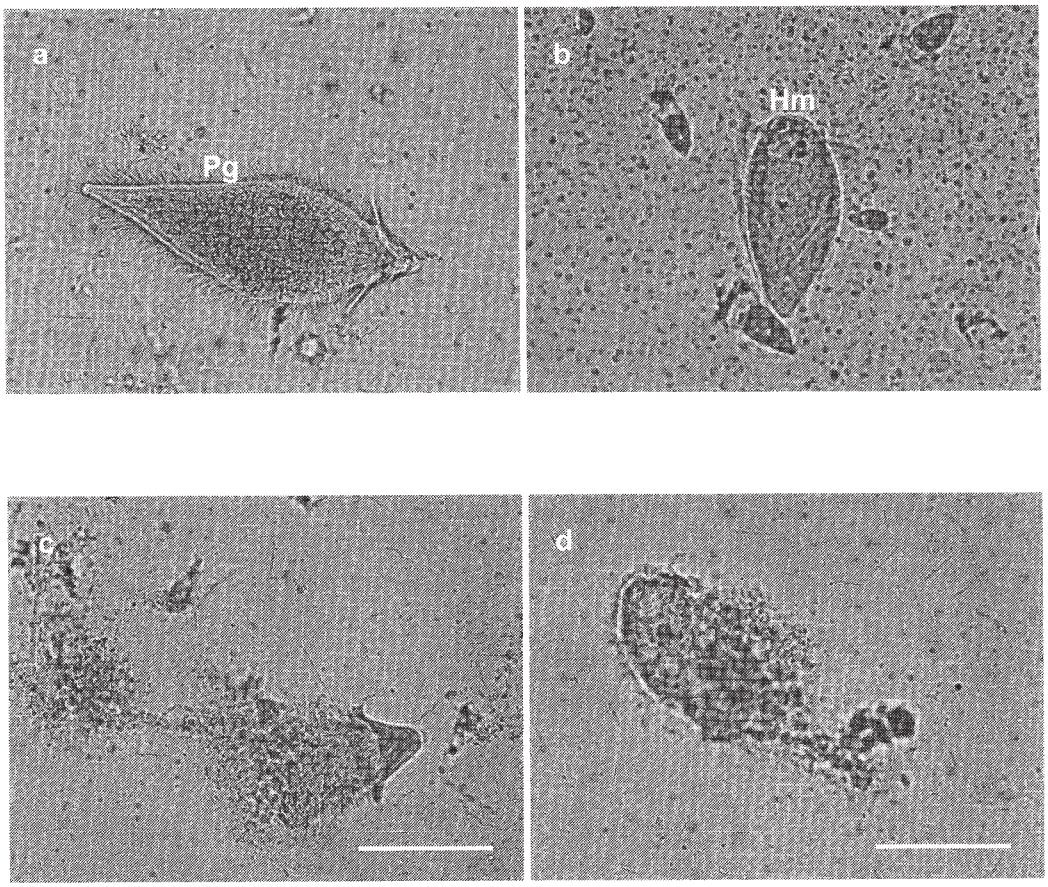

Fig. 2. Symbiotic protists in hindgut of *C. formosanus* worker.
(a, b) Protists in hindgut of worker feeding on untreated filter paper; (c, d) protists in hindgut of worker feeding on filter paper with PgEG and HmEG siRNAs.
Pg, *P. grassii*; Hm, *H. mirabile*; Sl, *S. leidyi*; Bar, 100 μm.

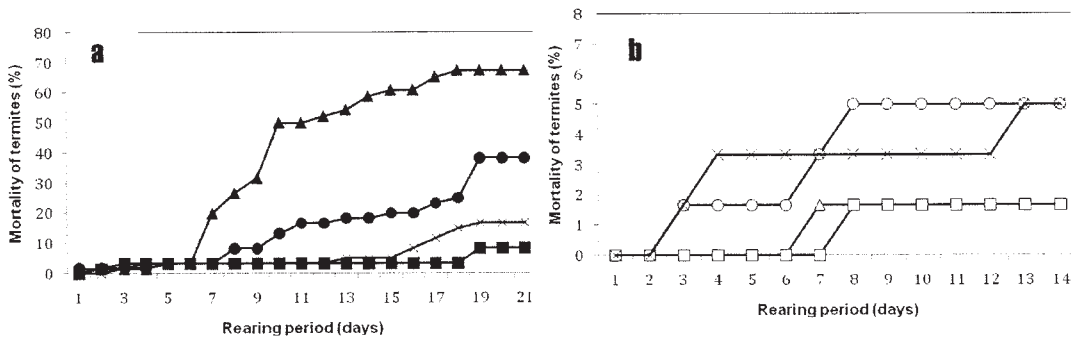

Fig. 3. Cumulative mortality of *C. formosanus* workers after feeding on siRNAs.
(a) Mortality of *C. formosanus* workers feeding on filter paper with symbiontic endoglucanase PgEG (●), HmEG (▲), SlEG (■) siRNAs, and on untreated filter paper as blank test (×) through 21 days. (b) Mortality of *C. formosanus* workers feeding on filter paper with unrelated control sequence UCS1 (○), UCS2 (Δ), UCS3 (□) siRNAs, and blank (×) through 14 days. Values are the means of two independent replicates.

Microsatellite Markers for the Asian Subterranean Termite *Coptotermes gestroi* (Wasmann)

by
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Abstract

Microsatellite markers were developed for the Asian subterranean termite, *Coptotermes gestroi* (Wasmann). We successfully isolated 14 polymorphic microsatellite loci with 5-16 alleles per locus and their observed heterozygosities ranged from 0.00 to 1.00. Eight of them are presented in this paper. These loci were shown to be useful for analyzing genetic structure and relationships of colony and population.

Key words: microsatellite loci, subterranean termite, *Coptotermes gestroi*, heterozygosities.

Introduction

The Asian subterranean termite, *Coptotermes gestroi* (Wasmann) is thought to have originated from Assam through Burma and Thailand to Malaysia and the Indonesian archipelago (Kirton and Brown 2003). It has also been brought into many regions around the world including the Turks and Caicos Islands in the Caribbean (Scheffrahn et al. 1990), and Florida in North America (Su et al. 1997). *C. gestroi* is one of the most destructive termite species in the tropics (Lee 2002; Yeap et al. 2007). In Malaysia, Thailand and Singapore, this species contributes > 85% of the total damage in buildings and structures in the urban area (Lee 2002, Lee et al. 2003).

Previous studies using mitochondrial DNA sequence have found limited genetic variation for inter-colonial comparison (Yeap et al. 2007). Polymorphic genetic markers have great potential in elucidating the details of colony organization, population structure, and relationships among introduced and native populations. To provide a sensitive tool for examining colony and population structure, we developed microsatellite markers for *C. gestroi.*

Materials and methods

Termites were collected from infested buildings and trees at various locations in Peninsular Malaysia, Singapore, Thailand, Indonesia, Philippines, Taiwan and Hawaii. Five workers from a colony of *C. gestroi* from Penang Island were used to construct the genomic library. DNA from pooled tissue was extracted using the Wizard Genomic DNA Purification Kit (Promega) after grinding in liquid nitrogen. The genomic DNA was digested with *Rsal* and ligated with MluI annealed adaptor. The ligation was then hybridized with on the Hybond N+ membrane with bound oligonucleotides consisting of 5-14 repeats of di- and trinucleotide motif (GACA, GATA, AAAT, GATG, CAA, CA, GT, AAT, CT, and AAG). Fragments that contain microsatellite were cloned into pGEM-T Easy vector (Promega). The plasmids were transformed using JM109 *E. coli* competent cells (Promega) to obtain a 100-clone library. After blue-white screening, 39 positive clones were sequenced. After elimination the overlapping sequences, 14 primer sets were designed using WEB-based Primer3 plus program (www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi). Eight primer sets were presented in this study.

Total genomic DNA of termite workers from different colonies was extracted, namely from the following locations: six from Malaysia, three from Singapore, two from Thailand, two from Indonesia, three from Philippines, three from Taiwan, and one from Hawaii. Whole bodies of workers preserved in 95% ethanol were pulverized in 1.5 ml Eppendorf tube with liquid nitrogen using a plastic pestle. DNA was extracted using CTB Tissue Extraction Kit (Intron). PCR amplification was performed in a standard 25 µl reaction volume with 2µl of total genomic DNA, 1 pmol of each forward and reverse primers, 1.5

mM $MgCl_2$, 2 mM dNTPs, and 5U/µl *Taq* DNA polymerase. All loci were amplified on a MJ Research PTC-200, Peltier Thermol Cycle, with a profile consisting of a precycle denaturation at 94°C for 2 min, followed by 35 cycles of a standard three-step PCR at 94 °C for 30 sec, 58 °C for 30 sec, 72 °C for 1 min and a postcycle extension at 72°C for 10 min. PCR products were run on 6% polyacrylamide gels and visualized with ethidium bromide. Allele lengths were scored by using ID Image Analysis Software. Observed and expected heterozygosities were calculated by using GenePop version 3.1b.

Results and discussion

Individuals from 20 different colonies of *C. gestroi* were screened for variability. Of the 8 selected primer pairs tested, all 8 loci showed variation among the entire study population with 5-16 alleles per locus (Table 1). One locus, CG4, was observed to be homozygous (heterozygosity = 0.00). On the other hand, locus CG29 showed a large number of equally frequent alleles (heterozygosity = 1.00). Among all the 8 polymorphic loci, observed heterozygosity was less than those of the expected ones in all the loci tested ($P < 0.05$, HW exact test; Raymond & Rousset 1995), except for locus CG6 and CG29. Reduced heterozygosity is expected in subterranean termites where inbreeding is common (Thorne et al. 1999).

Conclusion

The present set of microsatellite markers, with numerous polymorphic loci, provide a sensitive tool for investigating the colony and population genetic structure of both native and introduced populations of *C. gestroi.*

Acknowledgements

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References

Kirton, L. G., and V. K. Brown 2003 The taxonomic status of pest species of *Coptotermes* in Southeast Asia: resolving the paradox in the pest status of the termites *Coptotermes gestroi, C. havilandi, and C. travians* (Isoptera: Rhinotermitidae). *Sociobiology* **42**, 43-63.

Lee, C. Y. 2002 Subterranean termite pests and their control in the urban environment in Malaysia. *Sociobiology* **40**, 3-9.

Lee, C.-Y., J. Zairi, H.-H.Yap and N.-L. Chong 2003 *Urban Pest Control – A Malaysian Perspective.* Second edition. Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia. pp. 131

Raymond, M, and F. Rousset 1995 GENEPOP (Version 1.2): population genetic software for exact tests and ecumenicism. *J. Hered.*, **86**, 248-249.

Scheffrahn, R. H., N.-Y. Su, and B. Diehl. 1990 Native, introdued and structure-infesting termites of the Turks and Caicos Islands, B.W.I. (Isoptera: Kalotermitidae, Rhinotermitidae, Termitidae). *Fla. Entomol.* **73**, 622-627.

Su, N.-Y., R. H. Scheffrahn and T. Weissling 1997 A new introduction of a subterranean termite, *Coptotermes havilandi* Holmgren (Isoptera: Rhinotermitidae) in Miami, Florida. *Fla. Entomol.* **80**, 408-411.

Thorne, B.L., J. F. A. Traniello, E.S. Adams and M. Bulmer 1999 Reproductive dynamics and colony structure of subterranean termites of the genus Reticulitermes (Isoptera: Rhinotermitidae): a reveiew of the evidence from behavioral, ecological, and genetic studies. *Ethol. Ecol. Evol.*, **11**, 149-169.

Yeap, B.K., A.S. Othman, V.S. Lee and C.-Y. Lee 2007 Genetic relationship between *Coptotermes gestroi* and *Coptotermes vastator* (Isoptera: Rhinotermitidae). *J. Econ. Entomol.*, **100**, 467-474.

Table 1: Primer sequences and characteristics of 8 microsatellite loci of *Coptotermes gestroi*

Locus	*n*	Core repeat*	Primers (5'-3')	Size (bp)	*T*a (°C)	*N*a	H_O	H_E
CG4	20	$(CA)_{29}$	**F**: AAGCTTGCAGCGTTAAGTGG **R**: CCAGAGTTGGGTTTTGTGCT	167	58	5	0.00	0.82
CG6	20	$(GT)_{12}$	**F**: CACCCGTTGAAATTGACCTT **R**: AGACCGTTCCCAGCAACTTA	164	58	15	0.95	0.93
CG21	20	$(CCAA)_8(CCAT)_6$	**F**: TACCTACCGACCGAACGAAC **R**: TCCTGTTACAGCCCCAAAAG	191	58	12	0.30	0.91
CG26	20	$(CT)_6(GTCT)_7$	**F**: AAGCTCATTACGCGCAACTT **R**: GTGAAGCCTCGACAATGAGG	200	58	12	0.40	0.92
CG29	20	$(GT)_{15}(GA)_{10}$	**F**: GCTGCCTTGTCCCTTACTCA **R**: AGCACACCGCTCTACGAAGT	223	58	16	1.00	0.95
CG33	20	$(CAA)_{16}$	**F**: TTTCATCGAAAGTGCAGGTG **R**: TGTCGCATGAGGAAGATGTC	230	58	15	0.78	0.94
CG35	20	$(GAA)_3(GGA)_2(GAA)_7(GGA)_3(GAA)_6$ $(GGA)_3(GAA)_5GGA(GAA)_3(GGA)_5(GAA)_{11}$ $(GGA)_3(GAA)_8(GGA)_3(GAA)_3(GGA)_8(GAA)_{10}$	**F**: GGGCTGCACACTAAGCCTAA **R**: CACCACCACTAGTCGCTGAA	375	58	9	0.08	0.91
CG39	20	$(CAG)_3CAA(CAG)_{12}(CAA)_{13}$	**F**: CCGCGATATCAAAATTAGCAA **R**: AAGGATCGCATGTCCTTTTG	200	58	13	0.15	0.91

*Sequenced allele. *n*, number of individuals examined (only one individual per colony was genotyped); *T*a, annealing temperature; *N*a, number of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity.

Transmission of Entomopathogenic Fungus *Metarhizium brunneum* Petch and *Myrothecium roridum* Tode ex Steudel in Colony of Drywood Termites *Cryptotermes* sp. (Blattodea: Kalotermitidae) Using Vector

by
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Abstract

The transmission of entomopathogenic fungus *Metarhizium brunneum* and *Myrothecium roridum* in colony of drywood termites *Cryptotermes* sp. using vector was conducted. The proportion of vector of *Cryptotermes* sp. (10%, 20%, 30%, 40% and 50%) was treated by 10^7 conidial/ml of *M. brunneum* and *M. roridum* The result showed that there were correlation between proportion of vector and application period with mortality, LT_{50}, and consumption rate of all individual in colony. On 50% vector, after 10 days application, *M. roridum* could kill 82.5% individual in colony with LT_{50} 3.8 days and consumption rate of *Cryptotermes* sp. 0.0006gr, while *M. brunneum* just kill 70% with LT_{50} 4.4 days and consumption rate of *Cryptotermes* sp. 0.0013gr.

Key words: entomopathogenic fungus, *Cryptotermes* sp., transmission, mortality, LT_{50}, consumption rate

Introduction

In Indonesia, drywood termite *Cryptotermes* spp. is one of termite species which cause damage to wood constructions and furniture. The damage by termite attack is contrast than by other organisms (Tarumingkeng, 2000). Identification of drywood termite attack is relatively easy because the excrement of the colony is ejected from the tunnels within the wood through small holes made for the purpose.

Until now to control of termite has been applied in Indonesia such as using chemical barrier, physical barrier and baiting to protect the wood constructions from termite attack. Yusuf *et al.* (2004) mentioned that the chemical treatment is still mainly used as the termite control, and there is no termite control by the of use microorganisms (biocontrol) in Indonesia.

Currather and Hurar, 1990 in Olivera *et al.* (2003) reported that entomopatogenic fungus is the important microorganism to control any kind of insects. The research by Desyanti *et al.* (2007, 2008a, 2008b) and Ginting (2008), bio-control of termites in laboratory and field used many species of entomopatogenic fungi: *Metarhizium brunneum*, *Metarhizium anisopliae*, *Beauveria bassiana* and *Myrothecium roridum* effective for *Coptotermes* spp. and *Schedorhinotermes javanicus* Kemmer. The pathogenicity tests with *M. brunneum* and *M. roridum* for drywood termite was also done by Desyanti *at al.* (2008a), but their transmission in colony is not known yet. Using microorganisms as agents to control drywood termites *Cryptotermes* sp., will be useful as an alternative technology of termite control to replace the use of chemical compounds that has been widely applied for a long in Indonesia.

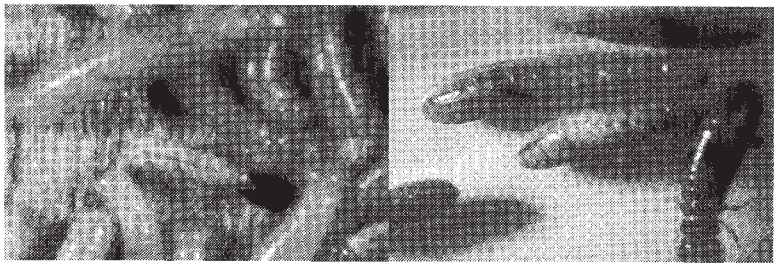
To control of termites needs special technique because their behaviors and life are hidden in dark condition. They communicatewith *sensory* (taught and test) and with chemical such as pheromones, volatile chemical compounds. Beside that, those social insects have levels of different castes and functions for their life activities in their colonies. The all social interaction of termite are generally known as *grooming, trophallaxis* and *cannibalistic* (Pearce 1997). So that, their behaviors could be useful to get success to control termite activity with vector inoculated by entomopathogenic fungus.

In this experiment, we would like to use vector inoculated by *M. brunneum* and *M. roridum* as agents to transmit infected propagulee of both species on colony of *Cryptotermes* sp

Materials and methods

Termites

The used termites in this research are Drywood termite *Cryptotermes* sp. from Forest Products Research Institute Bogor Indonesia. For use as vector, termites were stained with 0.05% (wt/wt) Nile-blue A by forced feeding of stained paper (Whatman no. 42, Θ 125 mm for 5 days (Fig. 1)



(a) **(b)**

Fig. 1. Drywood termites *Cryptotermite* sp. (a) and blue *Cryptotermes* sp. as vector (b)

Fungus isolate and preparation

Species of fungi used in this research are *Metarhizium brunneum* and *Myrothecium roridum*. The all isolates were store in room temperature until use. The data of fungi species are shown in Table 1.

Table 1 Species of entomopathogenic fungi from sand inoculums

Isolates	Host or inoculum's source	Species of fungi	geography origin (years)
1. Mb-Ps	Sand	*Metarhizium brunneum*	Bogor (2004)
2. My-Ps	Sand	*Myrothecium roridum*	Padang (2006)

For bio-assay, the Species of fungi were cultured in medium SDAY, after 3 weeks suspensions of fungi were prepared by additional of 2 ml sterilized aquadest contained 0,05% Triton X-100. The Petri dish was sought to get the conidia and dilute in the sterilized aquadest to get the dilution. The haemocytometer was used to count the total of conidia.

Filter paper was placed in a box (2x2x2cm) together with 10 nymphs of termites *Cryptotermes* sp. The vectors according with the treatments (0%, 10%, 20%, 30%, 40% and 50% from total colony population) were sprayed with 0.5 ml of conidium's suspension 10^7 conidia/ml and than take place between the healthy populations (untreated individual) of each colony in laboratory. The box was placed in a plastic container and keep in the dark condition for 10 days. The dead termites were evaluated every day and termite's mortality was calculated. Beside mortality, LT and consumption activity also evaluated.

Results and discussion

Mortality (%)

The results are indicated that the transmissions of entomopatogenic fungus (*M. brunneum* and *M. roridum*) with proportion vectors were shown in Table 2 and Fig. 1. The all treatments, mortality greater with level proportion of vector and application times. Proportion of vector 50% infected by fungi (*M. roridum*) can caused mortalities highest, but not significant with used proportion vector 40% and 30% treated by *M. roridum*, and proportion vector 50% treated by *M. brunneum*. Generally used proportion vector treated by *M. roridum* can caused mortality of termites were higher than treated by *M. brunneum*.

Table 2 Mortality of drywood termites *Cryptotermes* sp. Treated with proportion of vectors inoculated by *M. roridum* and *M. brunneum* 10 days after inoculation

Species	Vectors				
	10%	20%	30%	40%	50%
M. roridum	25 cde	35 bcd	70 a	70 a	82.5 a
M. brunneum	12.5 de	52.5 abc	65 ab	67.5 ab	70 a

Mean followed by the same letter are not significantly different at 0.05 level of confidence according DNMRT test. (Control mortalities were 0%). This treatments Mean with 5 replications

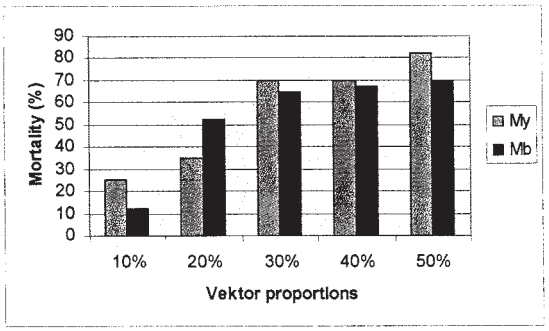


Figure 1. Mortality of drywood termite *Cryptotermes* sp. Using vectors inoculated by *M. roridum* (My) and *M. brunneum* (Mb), 10 days after inoculated (control mortality was 0%)

This is my be that transmission of *M. roridum* and *M. brunneum* from vector to uninfected termites (trans-contamination), effective by termite's behavior *grooming* and *trophallaxis*, but only effective before the all vectors were die. The transmission can continuous if the fungi produce conidium's on termite's cadaver. Yoshimura *et al.* (1992) mentioned that was estimated new fungus conidia can produce needing the times about 5 days after the insects die.

Lethal Time (LT)

Letal Time (LT_{50}) drywood termites *Cryptotermes* sp. treated with varies proportion of vectors inoculated by *M. roridum* more lower (3.8 days) than *Metarhizium brunneum* (4.4 days). In observed about it's patogenisity *M. roridum* also caused mortality more higher, this was balance with LT result are lower. The fungi have higher patogenisity will causing mortality in sort times.

Consumption rate (gr)

Proportion of *Cryptotermes* sp.'s consumption rate indicated that vector proportion have negative correlation with proportion consumption of *Cryptotermes* sp. Generally, The heigher proportion of vector caused lower proportion of consumption rate. This is estimated the vectors had transfer the entomopatogenic fungi to another individual in colony, so that it was causing the termite not health and fitful , their proportion of consumption rate and activities were downs. Sari *et al.* (2004) reported the defensive activities of consumption (*antifeedant*) are indicated by weigh loss of specimen. When loss of specimen lower defensive activities of consumption weighs is height.

The consumption rate also was become on the treatments until 50% of vector proportion that were estimated before the untreated termite was contaminated by vector, they were active to attack the specimen. But after the infected propagule was transmit, termite's activities and consumption downs and the and termite death.

Tanada and Kaya (1993) mentioned that period of insects death by entomopatogenic fungi generally not sign by another symptom in early infection. After infection and spreads infective propagule inside their body, the insect's activities become slow. The same of symptoms also shown from termites, the end infection, termite was loss its power, stay in site and than die. Also it was reported that period of infection until the insect die varied 3 - 12 days, this is depend about the size of insect body.

Conclusions

The transmission of entomopathogenic fungus *Metarhizium brunneum* and *Myrothecium roridum* in colony of drywood termites *Cryptotermes* sp. using proportion of vector of *Cryptotermes* sp. (10%, 20%, 30%, 40%, and 50%) was treated by 10^7 conidial/ml of *M. brunneum* and *M. roridum* in laboratory. This study resulted that there were correlation between proportion of vector and application period with mortality, $LT_{50,}$ and consumption rate of individual in colony. On 50% vector, after 10 days application, *M. roridum* could kill 82.5 individual in colony with LT_{50} 3.8 days and consumption rate of *Cryptotermes* sp. 0.0006gr, while *M. brunneum* just kill 70% with LT_{50} 4.4 days and consumption rate of *Cryptotermes* sp. 0.0013gr.

References

Desyanti, Zulyusri, Yumarni 2008a Effectiveness' of Entomopathogenic Fungi Species *Metarhizium brunneum*

and *Myrothecium roridum* against Drywood Termite *Cryptotermes* sp. (Isoptera: Kalotermitidae). Report of Hiba Berasing Research, department of Study and Culture Republic of Indonesia.

Desyanti and T. Santoso 2008b The Virulence of Isolates of Entomopatogenic Fungus *Myrothecium roridum* Tode ex Steudel (Deuteromycotina: Hyphomycetes) Against Subterranean Termites *Coptotermes* sp. (Isoptera: Rhinotermitidae). Proceeding of TRG (5), 103-106

Desyanti, Y. Hadi, S. Yusuf and T. Santoso 2007 Effectiveness' of Some Entomopathogenic Fungi Species as Bio-control Agent to Subterranean Termite *Coptotermes gestroi* WASMAN (Isoptera: Rhinotermitidae) Using Contact and Baiting Methods. *Journal of Tropical Wood Science and Technology* **5**(2), 68-77

Finney D. J. 1971 *Probit Analysis*. Ed ke-3. Combridge: University Press.

Ginting, S. 2008 Pathogenicity of several isolates of entomopathogenic fungi toward subterranean termite *Coptotermes curvignathus* Holmgren and Schedorhinotermes javanicus Kemmer (Isoptera: Rhinotermitidae). Thesis of Post Graduate Student of Bogor Agricultural University

Oliveira C. N de, P. M. O. J Neves and L. S. Kawazoe 2003 Compatibility between the entomopathogen fungus *Beauveria bassiana* and insecticides used in coffee plantations. *Sci. agric* **60**(4).[connecting].mailto:Scientia@esalq.usp.br [24 januari 2004].

Pearce, M. J. 1997 *Termite: biologi and management*. New York: CAB International Publisher.

Sari, K., W. Syafii, K. Sofyan and M. Hanafi 2004 Anti termite properties of resin Damar Mata kucing from *Shorea javanica*. *Journal of Tropical Wood Science and Technology* **2** (1), 8-15.

Steel, R. G. D., J. H. Torrie 1993 *Prinsip dan Prosedur Statistik: suatu pendekatan biometrik*. Sumantri B, penerjemah: Jakarta: PT Gramedia Pustaka Utama.

Tanada, Y., H. K. Kaya 1993 *Insect Pathology*. Sandiago: Academic Press, INC. Harcourt Brace Jovanovich Publisher.

Tarumingkeng, R. C. 2000 *Manajemen Deteriorasi Hasil Hutan*, Topik-topik Terpilih. Jakarta: Ukrida Press.

Yoshimura, T., K. Tsunoda, M. Takahashi and Y. Katsuda 1992 Pathogenicity of An Entomopathogenous Fungus, *Conidiobolus coronatus* TYRRELL. And MACLEOD, to *Coptotermes formosanus* Shiraki. *Jpn. J. Environ. Entomol. Zool.* **4**(1), 11-16.

Yusuf, S. 2004 Current Termite Management in Indonesia. TRG 1, Pacific Rim Termite Research Group; Malaysia, 8-9 March.

Evaluation Method of Particulate Materials as a Physical Barrier against Termite Attack

by
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Abstract

Recently the more attention is paid to the less- or non-chemical methods for termite control. As a non-chemical treatment, physical barriers using particulate materials have been investigated in terms of environmental safety, cost effectiveness, and duration of performance, and practically used in some countries. In this study, to establish evaluation method for particulate materials as a physical barrier, laboratory- and field-testing method was investigated. In the laboratory test using U-shape glass container, pelletized stone of 2.00 to 3.35mm prevented *Coptotermes formosanus* Shiraki from penetrating. In the field test using the concrete enclosures, three particulate materials of palletized stone of 2.00 to 3.35mm, crushed glass of 2.00 to 3.35mm, and glass beads of 1.40 to 2.36mm with 100mm-thickness prevented termites from penetrating for two years, though most of the particulate materials of 50mm-thickness were penetrated by termites from the four corners of concrete enclosures.

Key words: physical barrier, particulate material, pelletized stone, termite attack, penetration behavior

Introduction

In Japan, the damages by the subterranean termites (*Coptotermes formosanus* Shiraki and *Reticulitermes speratus* (Kolbe)) are serious problem. Recently, the more attention is paid to the methods of the termite controls of less- or non-chemical. As one of the non-chemical treatment, physical barrier using particulate materials as basalts (Tamashiro 1991), granites (French 1989, 1993), and gravels (Somnuwat 1995) were investigated.

It was showed that the relationship between particle size and termite body size is an important factor in controlling tunneling activity of subterranean termites, and the crushed volcanic cinders of 0.85 to 2.36 mm in diameter prevented *Reticulitermes hesperus* from penetrating (Ebeling 1957). Tamashiro and co-workers indicated that the particles of particular sizes (1.70 to 2.40 mm in diameter) prevented *C. formosanus* from penetrating (Tamashiro 1991). Su and co-workers investigated the penetration of the sand barrier consisting of crushed quartz rocks and fossilized coral by *C. formosanus* in laboratory- and field-testing (Su 1991, 1992). These results show that it is important to investigate the relationships between the size of termites and particles to evaluate the effects of the physical barrier using particles.

On the other hand, recycled materials using sludge, glass, or stone were tested as physical barrier and it was recommended to use the particulate materials of smooth surface and spherical shape as a physical barrier (Yanase 2000, 2005).

In Japan, the performances of particulate materials as a physical barrier have been examined using the method of evaluating termiticides, because there have not been a standard method for the physical barrier. In this study, to establish a method of evaluating particulate materials as a physical barrier against subterranean termites, laboratory- and field-testing method were investigated.

Materials and methods

In the laboratory test, pelletized stone of specified sizes were prepared by sieving using a series of wire sieves (JIS Z 8801, 1994) to obtain seven groups of uniform size ranging 1.00 to 1.18, 2.00 to 3.35, 3.35 to 4.00mm in nominal diameter respectively, and the soil component (under 1.00 mm) was used as a control. It was reported preliminary that pelletized stone with the size of 2.00 to 3.35mm prevented *Coptotermes formosanus* Shiraki from penetrating by the method using clear acryl tube (Fig. 1)(Yanase 2005). In this study, U-shape glass container consisting of two glass tubes, shown in Fig. 2, was prepared for penetration test. Pelletized stone of 50mm thickness was put into one glass tube on the sandy loam soil of 40mm thickness and a block of Japanese red pine (*Pinus densiflora* Siebold & Zucc) was put on the layer of pelletized stone. 300 workers and 30

soldiers of *C. formosanus* and moist disk papers were put into the other glass tube. After three weeks from inserting termites, the penetration length was measured and the penetration behavior of termites into pelletized stone was observed. The penetration test was replicated three times for each size of the pelletized stone and the soil.

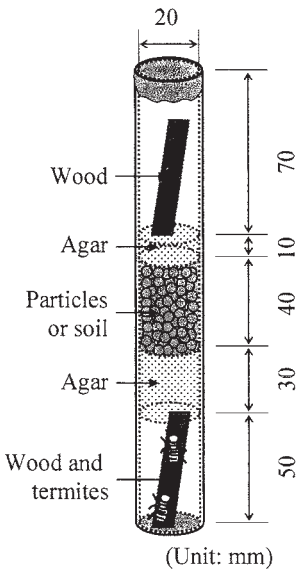


Fig. 1 Setup of termite penetration test. (Yanase 2005)

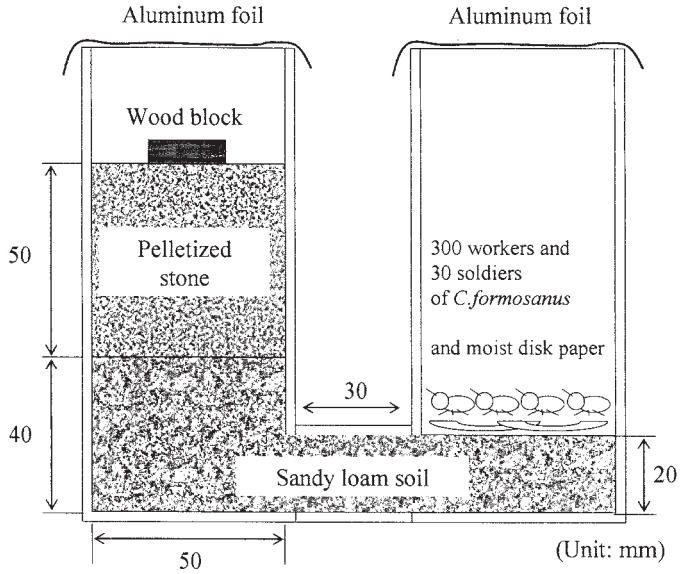
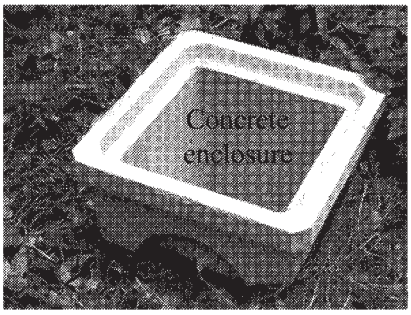
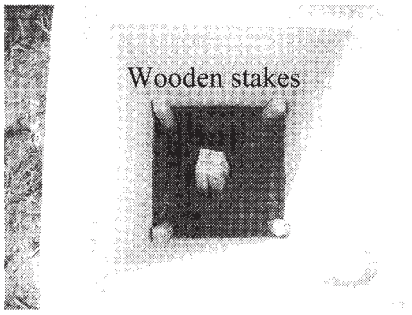


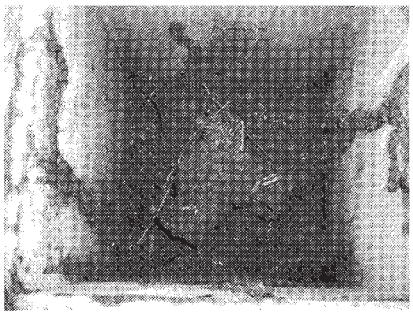
Fig. 2 Setup of termite penetration test using U-shape glass container.



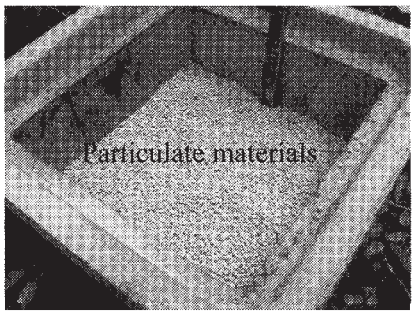
(a) Locate the concrete enclosure near the termite colony



(b) Insert wooden stakes



(c) Infestation in the concrete enclosure



(d) Install the particulate materials

Fig. 3 Penetration test in the field using concrete enclosure.

In the field test, the concrete enclosures set near termite colony were used. So it was desirable that termites attacked the physical barrier continuously. Concrete enclosures of 300 by 300 by 420(H) mm were located near the colonies of *C. formosanus* and eight wooden stakes of Japanese red pine were inserted into a concrete

enclosure for assembling termites (Fig. 3). Three particulate materials of palletized stone of 2.00 to 3.35mm, crushed glass of 2.00 to 3.35mm, and glass beads of 1.40 to 2.36mm were prepared, so these materials prevented termites from penetrating in the laboratory test. Particulate layers of 100 or 50mm-thickness and soil of 100mm-thickness as a control were set on the soil inside the enclosure after covering the wooden stakes by soil. The termite activity was checked periodically until termites visit the inside area of enclosure and start to infest the wooden stakes. Penetration of termites into physical barrier was checked periodically.

Results and discussion

In the laboratory test, pelletized stone of 2.00 to 3.35mm prevented termites from penetrating, shown in Table 1, and the particles of 1.00 to 1.18mm were tunneled by termites and the particles 3.35 to 4.00mm were penetrated through. The result that pelletized stone of 2.00 to 3.35mm was effective as a physical barrier was obtained by using both U-shape glass container. Furthermore the problem found in the acryl tube test that the layer of particulate materials were collapsed by tunneling agar layer (Fig. 1) was not found in the U-shape glass container test, and long-term test over one month in the laboratory was possible since termite mortality was low.

Table 1 Penetration length of termites for a layer of pelletized stone.

Particle size (mm)	Penetration length (mm)			n [a]
	Sample 1	Sample 2	Sample 3	
1.00 - 1.18	50 [b] (1) [c]	50(2)	50(1)	3
2.00 - 3.35	11	25	21	0
3.35 - 4.00	50(1)	50(1)	50(1)	3

a) Total numbers of the samples penetrated by termite.

b) 50 mm denotes that the layer was penetrated by termite.

c) Days required for termite penetration.

In the field test, the results of penetration test after two years from inserting particulate materials were shown in Table 2. The particulate materials of 100mm-thickness prevented termites from penetrating for two years, though all of the concrete enclosures with soil were penetrated by *C. formosanus* within one year. It was found in the layers penetrated by termites, the wooden stakes set at the corners of enclosure were fully consumed by termites in the test period and the particle layer has been collapsed, allowing the termites to crawl up from the collapses. This situation will not occur in the practical house basement, and suggests the necessity to improve test method. For preventing the collapse of particles, another test was also conducted that the soil layer of 100mm-thickness on the top of wooden stake was set in the concrete enclosure.

Table 2 Penetration of termites for two years in the field test.

Particulate materials	Particle size (mm)	Thickness (mm)	n [a]	Total numbers of sample
Pelletized stone	2.00-3.35	100	1	5
	2.00-3.35	50	5	6
Crushed glass	2.00-3.35	100	1	3
Glass beads	1.40-3.35	100	0	3
	1.40-3.35	50	1	1

a) Total numbers of the samples penetrated by termite.

Conclusions

The result that pelletized stone of 2.00 to 3.35mm prevented termites from penetrating was obtained in the laboratory test using U-shape glass container, and corresponded to the result using acryl tubes. the problem found in the acryl tube test that the layer of particulate materials were collapsed by tunneling agar layer using acryl pipe were not found using U-shape glass container, and a long-term test over one month in the laboratory

was possible. In the field test the performance of the particle layer was also evaluated, though most of the particulate materials of 50mm-thickness were penetrated by termites from the four corners of concrete enclosure. This was because the layer of particle collapsed at the corners where the wood stakes was consumed by termites and cavitated. For preventing the collapse of particles, the soil layer of 100mm-thickness on the top of wooden stake can be a solution. It was achieved that termites attacked physical barrier continuously by using only the concrete enclosure that termites inhabited.

References

Ebeling, W. and R. J. Pence 1957 Relation of particle size to the penetration of subterranean termites through barriers of sand or cinders. *Journal of Economic Entomology* **50**, 690-692.

French, J. R. J. 1989 The case for non-chemical termite barriers in termite control. *The International Research Group on Wood Preservation* Document No.: IRG/WP 1381.

French, J. R. J. and B. Ahmed, 1993 Termite physical barriers: Is retrofitting with Granitgard an option?. *TheInternational Research Group on Wood Preservation* Document No.: IRG/WP 93-40011.

Tamashiro, M., J. R. Yates, R. T. Yamamoto and R. H. Ebesu 1991 Tunneling behavior of the formosan subterranean termites and basalt barriers. *Sociobiology* **19**, 163-170.

Somnuwat, Y., C. Vongkaluang, T. Yoshimura, K. Tsunoda and M. Takahashi 1995 Tunneling of subterranean termites, *Coptotermes getsroi* Wasmann and *Coptotermes formosanus* Shiraki into gravel physical barriers. *Japan Journal of Environmental Entomology and Zoology* **7**(3), 3-19.

Su, N.-Y., R. H. Scheffrahn and P. M. Ban 1991 Uniform size particle barriers: a physical exclusion device against subterranean termites (Isoptera: Rhinotermitidae). *Journal of Economic Entomology* **84**, 912-916.

Su, N.-Y., R. H. Scheffrahn and P. M. Ban 1992 Penetration of sized-particle barriers by field populations of subterranean termites (Isoptera: Rhinotermitidae). *Journal of Economic Entomology* **85**(6), 2275-2278.

Yanase, Y., M. Shibata, Y. Fujii, S. Okumura, K. Iwamoto, T. Nogiwa, T. Yoshimura and Y. Imamura 2000 Feasibility of termite control using crushed cement-stabilized sludge (Polynite) as a physical barrier and acoustic emission (AE) monitoring. *The International Research Group on Wood Preservation*, Document No.: IRG/WP 00-10381.

Yanase, Y., Y. Fujii, S. Okumura, T. Yoshimura and Y. Imamura, M. Ishida, H. Kawaguchi and T. Okumura 2005 Application of particulate materials to a physical barrier against termites: Effects of size, shape and surface properties of particles on the penetration behavior of termites. *Journal of the Society of Materials Science, Japan* 54(4), 387-391. (in Japanese).

Microwave Technology as a Non-Destructive Termite Control Method – Preliminary Results –

by
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Abstract

Feasibility of microwave technique was discussed with three economically important Japanese termite species , *Coptotermes formosanus* Shiraki, *Reticulitermes speratus* (Kolbe), and *Incisitermes minor* (Hagen) in the laboratory, since the technique is expected to be an environmentally friendly alternative to the chemical treatment Two levels of microwave, 2.45 GHz and 5.8 GHz.were applied in this study. The effects of direct microwave irradiation (termite mortality or repellency) were first evaluated by at 50 mW/cm^2 and 100 mW/cm^2 for one hour. Although microwave irradiation raised 5.0-5.3℃ of body surface temperature of termites, no effect on termite mortality was observed. When an indirect irradiation applied to, dry-wood termites, *I. minor* within wood specimens at 100mW/cm^2, the positioning (distribution) of termite survivors was remarkably affected. Termite mortality was significantly different between 2.45 GHz and 5.8 GHz. Exposure for 45 min at 5.8 GHz was required to kill more than 50% termites within the specific size of specimens, whereas 2.45 GHz could kill only less than 10%. However, in case of thicker specimens, 2.45 GHz irradiation resulted in the higher mortality than that of 5.8 GHz irradiation for the shorter period of exposure. These results seem to support the feasibility of microwave irradiation as an on-site control measure if irradiation conditions are promptly selected.

Key words: microwave irradiation, drywood termite, non-destructive, frequency, power density

Introduction

Termites are known as a group of serious pests in the world. The damage caused by subterranean and dry-wood termites has an important economic effect. Chemical treatments, including soil treatment, wood impregnation, or fumigation have been widely used over the last few decades. However, an increased public concern about unfavorable effects would not support the massive use of insecticides any more.

Microwave heating is based on the conversion of the absorbed electromagnetic energy throughout the irradiated material to the thermal energy. Conventional heat treatments are labor intensive because the heating periods depend on the thermal conduction of materials, whereas microwave can quickly raise the center temperature because its heating process doesn't depend on the thermal conduction. As a result, the control of some pests such as termites by microwave energy has been studied by several researchers (Webber et al. 1946, Vadivambal et al. 2007, 2008 , Halverson et al. 1996, Nelson et al. 1972, Mahroof et al. 2003, Watters 1976). Lewis and Haverty (1996) studied use of microwave as one of the non-chemical control methods for termites. Lewis (2000) found that the western dry-wood termite, *Incisitermes minor* (Hagen), died if being irradiated at 500W for more than 90s. The author also reported if the microwave method is applied in the field, safety evaluations of microwave device are also needed to determine the effects on other non-target organisms.

The main object of this research was development of non-destructive termite control method by using microwave energy. Therefore, maximum power density was 100 mW/cm^2 and two levels of frequencies 2.45 GHz and 5.8 GHz were selectively studies based on practical applicability in the field.

Materials and methods

Termites. Mature workers of three species of termites, *Coptotermes formosanus* (Shiraki), *Reticulitermes sperutas* (Kolbe) and *Incisitermes minor* (Hagen), were used for the direct irradiation test. *C. formosanus* and *I. minor* were obtained from laboratory colonies maintained at the Research Institute for Sustainable Humanosphere (RISH) of Kyoto University. *R. spreratus* workers were collected from some field colonies on the Uji Campus of Kyoto University. Number of termites used were 30 for *C. formosanus*, 50 for *R. speratus* and 15 for *I. minor* per irradiation test. These numbers were determined to make the volume of termites similar among termite species. For the indirect irradiation test, same numbers of *I. minor* were used.

Wood specimens. Sugi (*Cryptomeria japonica* D. Don) specimens with a size of 100 (L)×100 (T) (mm), and 10, 20, 30, 40, 50 (R) (mm) were used as in the indirect irradiation test. These dimensions were determined

on the basis of the area to irradiate at a certain amount of power density at both frequencies. The test specimens were basically obtained from sapwood portion. All specimens were preconditioned for one week to adjust their moisture contents.

Microwave. Figure 1 shows the experimental apparatus for the microwave irradiation system used in the present experiments. This system was set up to heat only target materials, termites or wood samples. Frequencies used in this study were 2.45 GHz and 5.8 GHz, and power densities were 100 mW/cm^2 and 50 mW/cm^2 at each frequency, the latter was used for only direct irradiation test. The electric power output (P) was measured by power meter (437B, HEWLETT PACKARD, U.S.), and power density (D) was calculated as follows:

$$D \approx \frac{P}{\frac{\lambda}{4} \times \frac{\lambda}{2}} \left(mW / cm^2\right)$$

Termites were exposed to microwaves in 30dB shielded room at Solar Power Station/Satellite Laboratory (SPSLAB) of Kyoto University. The room temperature was kept at 27□ during irradiation.

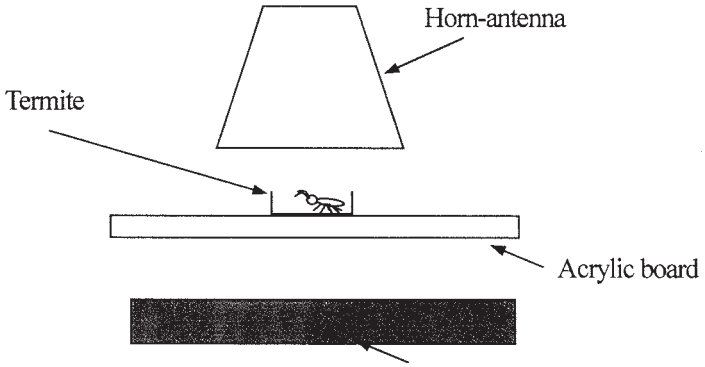

Fig.1. Experimental apparatus: Method of microwave irradiation

In direct irradiation test, termites were put in a plastic petri dish (⌀52 mm), microwave was irradiated from upside of the petri dish. The distance between the bottom of Hone-antenna and the petri dish was about 300 mm. The test termites were exposed to microwave for 15 to 60 min. Surface temperatures of termites before and after irradiation were measured by radiation thermometer (IT-550L, HORIBA Ltd., Japan) for determination of thermal difference. In addition, mortality after exposure was assessed on Whatman No. 2 filter paper saturated with 0.4ml distilled water in a 56 mm-diameter petri dish for 3 weeks in a termite culturing room.

In the indirect irradiation test, *I. minor* in a 52 mm-diameter plastic petri dish was sandwitched between two wood specimens. Microwave energy was emitted from upside of the surface of upper specimen (Fig. 2). for 60 min. The thermal difference inside and surface of wood specimens were measured by using K-type thermocouples and the radiation thermometer. Mortality of termites during exposure was counted regulerly. At the same time, the differences of moisture contents between before and after irradiation were determined for each specimen.

Determination of repellent effect. To determine the effect of termite repellency caused by microwave energy, two types of test containers made of petri-dishes and aluminum meshes (Fig. 3) were used. As show in Fig. 3, the left container was to form the shielded area in the outer part of a petri dish, and the right container was for preparing the area in its central area. By using these materials, it was possible to create the different shielded area in the same irradiation space. One hundred workers of *C. formosanus* were used as test insects. Frequencies used were 2.45 GHz and 5.8 GHz, and the irradiation time was 60 min. Power density output was 100 mW/cm^2. During irradiation, the activity of termite was recorded by a digital video camera (HDR-HC3, SONY). On the basis of the shot image, the number of termites in the unshielded area was counted. In addition, analysis of variance (ANOVA) (p<0.05) was done to check the significance between the behavior of irradiated termites and non-irradiated termites. Comparison of means and frequency was done using Tukey's test (α<0.05).

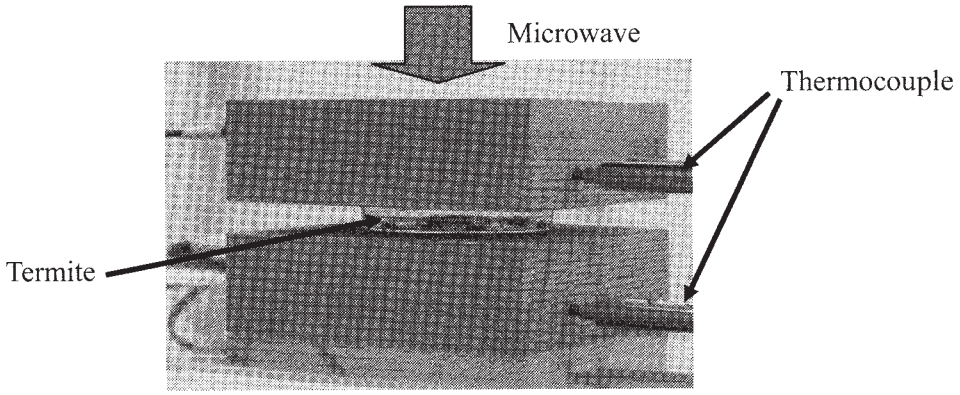

Fig.2. Method of irradiation to wood specimens within *I. minor*

Determination of repellent effect. To determine the repellent effect in microwave energy, two types of test containers made of petri-dishes and aluminum meshes (Fig. 3). As show in Fig. 3, the left container was used to prepare the shielded area in the outer part of a petri dish, and the right container was used for preparing the area in its central area. In the shielded area, microwave energy was presented at about 10 % of the output energy. Their materials were added to 90mm-diameter plastic petri dish, and they were exposed to microwave energy in same method for the direct irradiation test. One hundred workers of *C. formosanus* were used as test insects. Frequencies used were 2.45GHz and 5.8GHz, and the irradiation time was 60min. Power density output was 100mW/cm^2. During irradiation, the temperature of shielded room was kept at 27°C, the activity of termite was observed in dark by recording images with a digital video camera (HDR-HC3, SONY). On the basis of the shot image, the number of termites in the unshielded area was counted. In addition, analysis of variance (ANOVA) (p<0.05) was done to check the significance between the behavior of irradiated termites and non-irradiated termites. Comparison of means and frequency was done using Tukey's test (α<0.05).

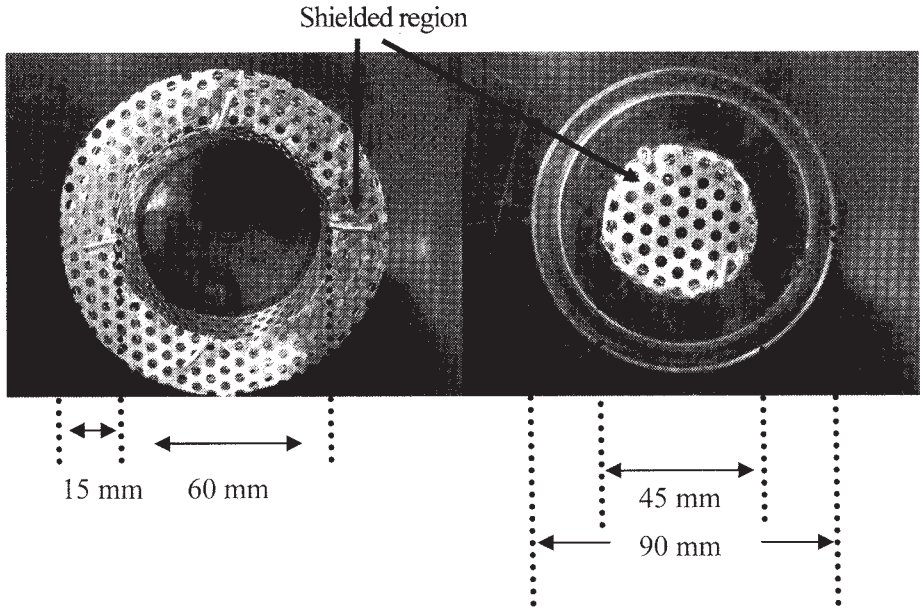

Fig.3. Materials for microwave shielding

Results and discussion

Thermal difference of termite. In the direct irradiation test, thermal difference based on surface temperature of termite measured before and after irradiation was recognized as variations in body temperature of termite. The obtained results suggested that microwave energy used in this study could not raise the temperature high enough to kill termites.

Mortality after direct irradiation. There was no difference in termite mortality between exposed and unexposed termites, regardless of termite species. It was suggested that microwave energy did not physiologically affect termite. Lewis et al. (1996) reported that the mortality of *I. minor* at 4 weeks after irradiation was over 90% by using higher microwave energy. At higher microwave energy, if in the direct

irradiation, termite mortality may be strongly higher. There is a possibility that termite is affected indirectly, if the body temperature is raised to near the lethal temperature.

Indirect irradiation. The temperature inside the wood specimens increased with exposure time. The indirect resulted in higher mortality than that of the direct irradiation. Irradiation for 60 min at 2.45 GHz was not enough to raise the wood temperature, whereas at 5.8 GHz for same period, that was raised to more than 60℃.

Repellency. At both 2.45GHz and 5.8GHz, the workers of *C. formosanus* were less susceptible to microwave energy. Non-irradiated termites (control) tended to move from the outer part to the central part of the petri dish in two shielded regions, and the number in non-shielded region reached an equilibrium level as time went by. The average number of irradiated termites at 2.45 GHz in non-shielded region was significantly different from that of non-irradiated termites, if the outer part was shielded. Results of ANOVA showed there was significant difference in the average number of movement to unshielded area between irradiated and non-irradiated termite in case of shielding outer part (F=5.42; df=2, 228; P<0.01). Tukey's tests showed that at 2.45GHz, the average number of that was significantly lower than non-irradiated termite. However, there was no significant difference in other results. Shayesteh and Barthakur (1996) who studied stored-product insects reported the movement towards the surface from inside the medium during irradiation. The effects of microwave on termites were observed only as the body temperature elevation by direct irradiation in the present study. Therefore, the statistical analysis explains that the change in behavior of termite is affected by microwave heating. In the direct irradiation, the average thermal difference of *C. formosanus* between before and after irradiation at 2.45GHz was 3.2℃. It is, therefore, possible that microwave irradiation at 2.45 GHz, 100 mW/cm^2 had some influence on *C. formosanus* except the heating effect.

Conclusions

The present results indicated that high absorption efficiency of wood can cause high mortality of termites by direct irradiation and indirect irradiation tests. At 2.45 GHz, the thin wood specimens were not enough heated, whereas it resulted in complete mortality in case of thicker specimens. 5.8 GHz irradiation could raise the temperature within specimens, however that frequency was not attributed to uniform heating in whole irradiation area. Disinfestation of wood with termites in by using microwave seems applicable at a adequate frequency considering penetration depth in accordance with wood dimension. The effect of microwave depends only on heating of termites, and is related to termite repellency, although the termites are less sensitive to the existent of electromagnetic field.

References

Halverson, S. L., W. E. Burkholder, T. S. Bigelow, E. V. Nordheim, and M. E. Misenheimer 1996 High-power microwave radiation as an alternative insect control method for stored products. *Journal of Economic Entomology* **89**, 1638-1648.

Lewis, V. R., and M. I. Haverty 1996 Evaluation of six techniques for control of the western drywood termite (Isoptera: Kalotermitidae) in structures. *Journal of Economic Entomology* **89**, 922-934.

Lewis, V. R., A. B. Power, M. I. Haverty 200 Laboratory evaluation of microwaves for control of the western drywood termite. *Forest Products Journal* **50**, 79-87.

Mahroof, R., B. Subramanyam, J. E. Throne, and A. Menon 2003 Time-mortality relationship for *Tribolium castaneum* (Coleoptera: Tenebrionidae) life stages exposed elevated temperatures. *Journal of Economic Entomology* **96**, 1345-1351.

Nelson, S. O. 1972 Possibilities for controlling stored-grain insects with RF energy. *Journal of Microwave Power* **7**, 231-237.

Shayesteh, N. and . N. Barthakur 1996 Mortality and behaviour of two stored-product insect species during microwave irradiation. *Journal of Stored Products Research* **32**, 239-246.

Vadivambal, R., D. S. Jayas, and N. D. G. White 2007 Wheat disinfestation using microwave energy. *Journal of Stored Products Research* **43**, 508-514.

Vadivambal, R., D. S. Jayas, and N. D. G. White 2008 Determination of mortality of different life stages of *Tribolium castaneum* (Coleoptera: Tenebrionidae) in stored barley using microwave. *Journal of Economic Entomology* **101**, 1011-1021.

Watters, F. L. 1976 Microwave radiation for control of *Tribolium confusum* in wheat and flour. *Journal of Stored Products Research* **12**, 19-25.

Webber, H. H., R. P. Wagner, and A. G. Pearson 1946 High frequency electric fields as lethal agents for insects. *Journal of Economic Entomology* **39**, 487-498.

Chlorantraniliprole (DPX E2Y45): New Chemistry and Novel Mode of Action Insecticide for Global Termite Control

by
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Abstract

Chlorantraniliprole, discovered and patented by DuPont scientists, is a novel insecticide that affects termite muscles. This compound is a classic nonrepellant that provides very effective control of termite colonies. These research data presented here will summarize current laboratory and field trials from around the globe for termite control with multiple species. Efficacy has been documented in both laboratory and field studies globally. Development of chlorantraniliprole as a liquid termite product has also been documented in the United States under experimental use permit with professional pest management applications. The product has been branded as DuPont™ Altriset™ 18.4%SC. Development of this product will allow professional termite applicators another choice for termite control with new chemistry, novel mode of action, a reduced environmental foot print as well as superior termite efficacy.

Key words: chlorantraniliprole, DPX E2Y45, anthranilic diamides, termite control

Introduction

Chlorantraniliprole, coded as DPX E2Y45 by DuPont Crop Protection, represents new chemistry and a novel mode of action for global termite control. This new active ingredient has been branded by DuPont Crop Protections as Rynaxypyr® and by DuPont Professional Products (the non-crop business) as Calteryx™. (Anonymous, 2007). This compound is in a new class of insecticides called the anthranilic diamides (Lahm et. al. 2005, Cordova et al. 2006) Chlorantraniliprole controls insect pests through activation of insect ryanodine receptors (RyRs), a new mode-of-action for insect control. This activation of ryanodine receptor channels leads to internal calcium store depletion, that impairs regulation of muscle contraction. Insects exposed to chlorantraniiliprole exhibit general lethargy and muscle paralysis followed by death. Furthermore, chlorantraniliprole is remarkably selective for insect over mammalian RyRs and this selectivity is a key attribute of the low mammalian toxicity. This compound also has a favorable environmental profile (Bassi et al. 2007).

In 2008, chlorantraniliprole was registered by the US Environmental Protection Agency as a Reduced Risk pesticide for use on peaches, pears, lettuce, tomatoes, apples and turf. A full description of the registration of chlorantraniliprole by the EPA was published (Environmental Protection Agency, 2008). Four products are currently registered globally, DuPont™ Coragen® for vegetable crops, DuPont™ Altacor® Insect Control for tree and vine crops, DuPont™ Prevathon® for rice insects and DuPont™ Acelepryn™ Insecticide for turf and ornamental noncrop applications. The global termite brand name is DuPont™ Altriset™ formulated as a water based suspension concentrate 18.4% SC. This product has passed the two year lab test at the United States Forest Service laboratory and has been installed in the concrete slab and ground board test sties in AZ, FL, MS and SC. In addition, numerous laboratory and field studies are being conducted to document termite efficacy. Field sites have been established in the United States, Australia, Japan and Thailand.

In addition, chlorantraniliprole has been evaluated around homes in the Unites States under the federal EPA experimental use permit (EUP) program. Application to homes occurred in 2008. Professional pest management applications were conducted with supervision by university professors in 10 states with different termite species, environmental conditions and construction types. The inspection data from these EUP homes has demonstrated superior efficacy of chlorantraniliprole against key termite species.

Materials and methods

Laboratory and field methods are described in various papers and literature. Data will be presented from these studies and a brief method section will be included. Most of these laboratory and field study designs are known and accepted protocols from the termite research community.

Results and discussion

Laboratory data has shown that chlorantraniliprole is effective on termites at low concentrations. Data will be presented from various laboratories including the DuPont Stine-Haskell Research Center, various university cooperators and government agencies, including the United State Forest Service. The ability of chlorantraniliprole to demonstrate termite transfer between colony members has been shown in a series of laboratory experiments. These data will be discussed.

Field data collected from sites around the world will show effective termite control under a variety of environmental conditions. These field sites will include applications around homes in the USA that will show termite control with several key termite species.

Conclusions

Laboratory and field results have documented that chlorantraniliprole is an effective liquid termiticide. This novel chemistry and unique mode of action will allow pest management professionals to utilize a new termite control product. The superior efficacy coupled with low mammalian toxicity and low environmental impact will separate chlorantraniliprole from existing liquid termiticides currently on the market.

References

Anonymous 2007 DuPont™ Rynaxypry™ Insect Control Technical Bulletin. DuPont Crop Protection. E. I. du Pont de Nemours and Company, 12 pages.

Bassi, A., R. Alber, J. A. Wiles, J. L. Rison, N. M..Frost, F. W. Marmor and P. C. Marcon. Chlorantraniliprole: a novel anthranilic diamide insecticide. Proceedings of the XVI International Plant Protection Congress 2007. Vol 1: 52-59.

Cordova, D., E. A. Benner, M. D. Sacher, J. J. Rauh, J. S. Sopa, G. P. Lahm, T. P. Selby, T. M. Stevenson, L. Flexner, S. Gutteridge, D. F. Rhoades, L. Wu, R. M. Smith and Y. Tao 2006 Anthranilic diamides : A new class of insecticides with a novel mode of action, ryanodine receptor activation. Pesticide Biochemistry and Physiology 84 (2006): 196-214.

Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances 2008 Pesticide Fact Sheet: Chlorantraniliprole. April, 2008 1-77 pages.

Lahm, G. P., T. P. Selby, J. H. Freudenberger, T. M. Stevenson, B. J. Myers, G. Seburyamo, B. K. Smith, L. Flexner, C. E. Clark and D. Cordova 2005 Insecticidal anthranilic diamides: A new class of potent ryanodine receptor activators. Bioorganic & Medicinal Chemistry Letters 15 (2005): 4898-4906.

Utilization of Bifenthrin and Impralit as Plywood Preservatives against Drywood Termites *Cryptotermes cynocephalus*

by
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Abstract

This research aimed to evaluation the quality of plywood made from Meranti wood with bifenthrin and impralit preservatives in the adhesives of plywood (glueline treatment) with different concentration. Evaluation the quality of plywood included the physical properties (moisture content, water absorption and thickness swelling), internal bonding and durability of plywood against drywood termite *Cryptotermes cynocephalus*. Bifenthrin and impralit preservatives was dipping in the adhesives with concentration 0%, 2%,4% and 6% . Urea formaldehyde was used as the adhesives with catalyst ammonium chloride. Result of the research showed that average moisture content value was 8.89 – 9.68%, thickness swelling was 3.62 – 6.12%, and water absorption was 21.57 – 27.26% respectively. Meanwhile the internal bonding value was range between 2.14 – 6.76 kg/cm^2. The plywood properties from the research is fulfilled the SNI (1991), FAO (1966) and JIS (1994) Standard. The durability test with drywood termites *Cryptotermes cynocephalus* showed that the plywood is resistance. Mean weight loss value of plywood is 5.76 – 12.35% and the mortality value was range between 44 – 80%. From the research, it is concluded that plywood with bifenthrin preservatives has a physical properties and internal bonding which can fulfilled the FAO (1966), Indonesian Standard (1991) and JIS (1994) standard. The best value was from plywood with bifenthrin preservatives in concentration 6%.

Key words: drywood termites, *Cryptotermes cynocephalus*, bifenthrin, impralit, plywood

Introduction

Biological degradation is a main factor that degrades the durability of wooden product. Among all factors leading to biodegradation, termites are most damaging wooden products worldwide (Chang & Cheng 2002).It is also known that termites cause damage to a variety of materials ranging from fabrics, such as particleboard, plywood, MDF and etc. (Sakasegawa *et al.* 2003). Plywood is the one of the wood panel product which become a major product in export commodity in Indonesia. Demand of plywood increasing as well as the growth of development in urban areas. Effort to sustainability the plywood production, the industries try to used the unknown species as raw material, especially the wood with durability in lower class. To increased the properties of plywood, especially on the durability properties, preservatives is needed (Shams *et al.* 2003). This research aimed to evaluation the utilization of bifenthrin and impralit in the adhesives as plywood preservatives. The quality of plywood then evaluated both on physical properties (moisture content, water absorption and thickness swelling), internal bonding and durability against drywood termite *Cryptotermes cynocephalus*.

Material and methods

Plywood was made in plywood factory, PT. Alas Kusuma Pontianak West Kalimantan. Plywood consisted of face, core and back (three ply) from Meranti wood. bifenthrin and impralit preservatives was dipping in the adhesives with concentration 0%, 2%,4% and 6% (v/v). Urea formaldehyde was used as the adhesives with catalyst ammonium chloride. The quality of plywood then evaluated both on physical properties (moisture content, water absorption and thickness swelling) and internal bonding. Evaluation the durability of plywood against drywood termite *Cryptotermes cynocephalus* was held according to Indonesia National Standard. The samples measured were 50 mm x 25 mm x 15 mm, and put in glass box. On the top of sample were put 50 nymph of *C. cynocephalus*. The sample then put on dark room for 30 days. Weight loss of sample were measured in the end of evaluation.

Results and discussion

Moisture Content

The properties of plywood are summarized in Table 1. Plywood average moisture content value were 8.89%-9.58%, meanwhile the average value in plywood without preservatives were 9.35%. The highest moisture content was on plywood with Impralit preservatives with concentration 2% and the lowest was on

plywood with Bifenthrin preservatives with concentration 6%. The concentration and preservatives were significance influenced on moisture content value. This moisture content value were fulfilled the JIS Standard, Indonesian Standard and FAO Standard.

Table 1. The physical properties of plywood

Preservatives	Concentration (%)	Moisture Content (%)	Thickness Swelling (%)	Water absorption (%)	Internal bonding (kg/cm^2)
Bifenthrin	0	9.35	5.15	23.66	3.84
	2	9.46	4.72	24.50	5.54
	4	9.23	4.58	22.69	6.15
	6	8.89	3.62	21.57	6.76
Impralit	0	9.35	5.15	23.66	3.84
	2	9.68	6.12	27.26	2.14
	4	9.42	4.68	23.85	5.09
	6	9.26	4.62	21.83	6.26
Indonesian Standard (1991)		Max 14	Max 20	Max 30	Min 6
FAO Standard (1966)		6-12	5-15	15-25	2-12
JIS Standard (1994)		5-13	Max 12	Max 25	Min 3,1

Thickness Swelling

The average of thickness swelling value were 3.62%-6.12%, and the average value on plywood without preservatives were 5.15% (Table 1). The highest thickness swelling was on plywood with impralit preservatives with concentration 2% and the lowest was on plywood with bifenthrin preservatives with concentration 6%. This thickness swelling value were fulfilled the JIS Standard and Indonesian Standard, meanwhile only plywood made with impralit preservatives with concentration 2% can fulfilled the FAO Standard.

Water absorption

The highest water absorption was on plywood with Impralit preservatives with concentration 2% and the lowest was on plywood with bifenthrin preservatives with concentration 6%. The average of water absorption value were 21.57%-27.26%, meanwhile the average value in plywood without preservatives were 23.66% (Table 1). Water absorption value was influenced by the effectivity of internal bond between veneer and adhesives. This water absorption value were fulfilled the JIS Standard, Indonesian Standard and FAO Standard.

Internal bonding

The average of internal bonding value were 2.14%-6.76%, and the average value on plywood without preservatives were 3.84% (Table 1). The highest internal bonding was on plywood with bifenthrin preservatives with concentration 6% and the lowest was on plywood with impralit preservatives with concentration 2%. This thickness swelling value were fulfilled the FAO Standard. Plywood with bifenthrin preservatives with concentration 4% and 6% and plywood with Impralit preservatives with concentration 6% can fulfilled the Indonesian Standard. All of plywood can fulfilled the JIS Standard except the plywood with impralit preservatives with concentration 2%.

Durability plywood

The properties of plywood durability are summarized in Table 2. The average of weight loss value were 5.76%-12.35%, meanwhile the average value in plywood without preservatives were 14.32%. The highest weight loss was on plywood with impralit preservatives with concentration 2% and the lowest was on plywood with bifenthrin preservatives with concentration 6%. The concentration and the preservatives were significance influenced on weight loss value. According to Indonesian Standard the plywood with bifenthrin and impralit preservatives were resistance against drywood termites *C. cynocephalus* attack.

The average of termite mortality value were 44%-80%, meanwhile the average value in plywood without preservatives were 14%. The highest termite mortality value was on plywood with bifenthrin preservatives with concentration 6% and the lowest was on plywood with impralit preservatives with concentration 2%. From the result, it was obvious that by increasing the concentration of preservatives, will decreasing the weight loss of plywood and increasing the mortality of drywood termites.

Table 2. The durability properties of plywood

Preservatives	Concentration (%)	Termite Mortality (%)	Weight Loss (%)	Indonesian Standard
Bifenthrin	0	14	14.32	Susceptible
	2	44	11.62	Resistance
	4	68	8.34	Resistance
	6	80	5.68	Resistance
Impralit	0	14	14.32	Susceptible
	2	46	12.35	Resistance
	4	62	9.28	Resistance
	6	76	6.67	Resistance

Conclusion

Bifenthrin and impralit preservatives were increasing the plywood properties, included moisture content, thickness swelling, water absorption and internal bonding. The plywood were fulfilled the JIS Standard, Indonesian Standard and FAO Standard. Plywood were resistance against drywood termites *Cryptotermes cynocephalus* attack. Utilization of bifenthrin preservatives with 6% concentration resulting the highest termite mortality and the lowest weight loss of plywood.

References

Chang, S.-T. and S. S. Cheng, 2002 Antitermitic Activity of Leaf Essentials Oils and Components from *Cinnamomum osmophleum*. *J. Agricultural and Food Chemistry* **50**, 1389-1392.

Sakasegawa, M., K. Hori and M. Yatagai 2003 Composition and Antitermite Activities of Essentials Oils from *Melaleuca species*. *J. Wood Science* **49**, 181-187.

Shams, Md. I., H. Yano and S. Kawai 2003 Phenolic Resin Impregnated Veneer/Wafer Overlaid Particleboard. *Wood Research* No. 90, 17-18.

Field Efficacy of Silafluofen as Soil Termiticides in Phuket: Thailand

by
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Abstract

Silafluofen EC was evaluated for its efficacy as a soil termiticide in Phuket:Thailand where subterranean termite activity has been extremely active since 2005. The Standard Test Method used in this evaluation was "The Modified Ground Board Test". The same formulation of the product as currently used in Japanese market at concentrations of 0.1% and 0.15% was used in this field trial.

Results as appeared by means of visual rating of the wooden baits from test plot showed that very limited damage was observed on the baits collected from the plots treated by both concentrations of the tested product after 3 years, confirming the effectiveness of silafluofen at the concentrations of 0.1% and 0.15% in providing good barricade to prevent the underground tunneling of subterranean termites. These test results were consistent with those obtained from field tests in Japan where a similar but somewhat different ground board test method has been adopted.

Key words: silafluofen EC., soil termiticide, modified ground board test, Phuket:Thailand, subterranean termite.

Introduction

Termite control receives a very high attention from home owners and pest control professionals worldwide including Thailand because of the severity of the damage caused by termites which jeopardize both the economy and safety of human life. To assess the effectiveness of termiticides, efficacy test methods have generally been established in accordance with circumstances of each nation such as termite species, environments and structures of constructions. At the TRG 3 conference in 2006, we reported on "Laboratory efficacy test methods of termiticides in Thailand and evaluation of silafluofen products by the methods" and discussed regarding differences in Thai and Japanese standard laboratory test methods.

Field efficacy test methods of termiticides are considered to be more important as they are directly associated with actual treatment conditions. In Thailand, Royal Forest Department (RFD) is a Government Institute responsible for the evaluation of termiticides prior to the registration application paper can be forwarded to Thai FDA for registering. RFD announced 2 types of standard field efficacy test methods, 1) STAKE TEST and 2) MODIFIED GROUND BOARD TEST as Standard Methods. The latter test represents field conditions of buildings with slab on the ground. On the other hand, a test method for soil treatment in Japan has been determined as JWPA-S 13 (Japan Wood Preserving Association Standard No. 13).

Silafluofen (Katsuda *et al.* 1986, 2005, Minamite *et al.* 1990, Nakayama *et al.* 1998, Vongkaluang and Katsuda 2006), a termiticidal ingredient containing silicon, is characterized by low fish toxicity and chemical stabilities under sunlight, in the soil and under alkaline environments in addition to high termiticidal activities and low mammalian toxicity. Due to these excellent properties, silafluofen has been widely used as termiticides such as EC formulations for soil treatment and oil formulations for timber treatment since 1991 in Japan.

Field trial of silafluofen EC on the market in Japan were conducted in Thailand according to the Thai standard modified ground board test method. The test results are reported and compared with those obtained from the Japanese JWPA-S 13 method.

Materials and methods

1. Thailand: MODIFIED GRUOND BOARD TEST.

(1) Test procedures:

1) Install a test plot of 1×1×0.2m with concrete blocks.
2) Fill and compact the installed concrete plot with river sand.
3) Dilute the test termiticide to required concentrations.
4) Evenly spray the dilution on the surface of the soil, 5 L per 1 plot.
5) Put PVC sheet on the surface of the treated soil.

6) Pour concrete (8 cm thick) on PVC sheet leaving only a hole (Φ10cm) around the PVC pipe (Φ10cm×10cm height) in the middle of the ditch.
7) Cut out the PVC sheet inside the PVC pipe.
8) Put rubberwood bait (5×5×2.5cm) inside the PVC pipe.
9) Cover the pipe.

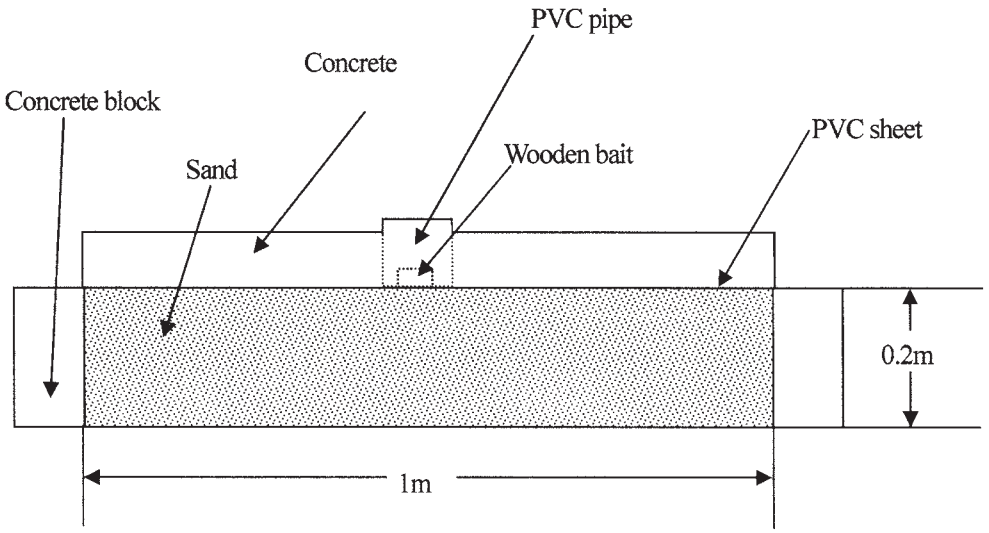

Fig.1 Standard modified ground board test method.

(2) Evaluation of effectiveness
Evaluation of test termiticides is made by comparing the results on the wooden baits in treated and untreated (Control) soil.

2. Japan : JWPA-S 13
JWPA-S 13 method is illustrated in Fig.2 as reference.

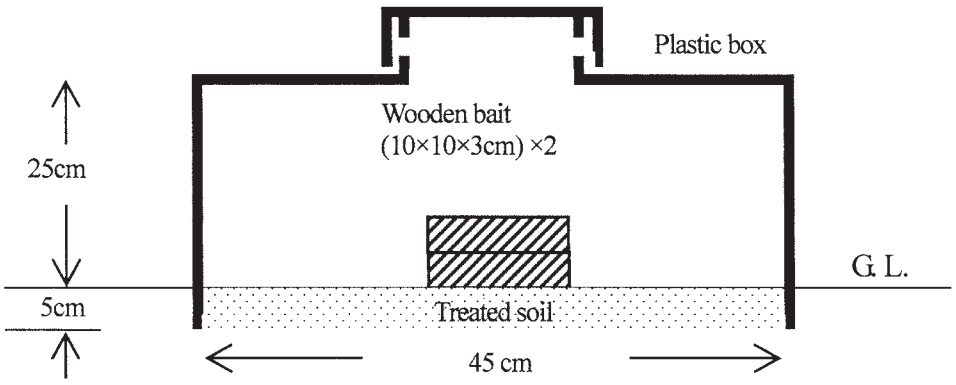

Fig.2 JWPA-S 13 Standard test method.

Results and discussion

1. Test results in Thailand
- Test duration : November 2005~November 2008
- Test location : Phuket Province : Thailand
- Test results : Table 1

Table 1 Test results of dilutions of silafluofen termiticide (after 3 years)

Test termiticide	Damage found on wooden bait (%)				Average damage (%)
	1	2	3	4	
Silafluofen 0.10%	0	0	0	0	0
Silafluofen 0.15%	10	0	0	0	2.5
Control	100	90	5	90	71.25

- Silafluofen at the concentrations of 0.10% and 0.15% performed well when used under the condition of the Modified Ground Board Test to prevent the underground tunneling of subterranean termites.

2. Test results in Japan
   - Test place : Test sites of Architectural Research Association in Kagoshima
   - Test results : Table 2

Table 2 Test results of dilutions of silafluofen termiticide (after 7 years)

Test termiticide	Damage found on wooden bait
Silafluofen 0.10%	No damage
Silafluofen 0.15%	No damage
Control	Serious damage after 1 year (replaced with new wooden bait)

- Silafluofen-based termiticides showed high residual efficacy against termites for a long period of time.

References

Katsuda, Y., H. Hirobe and Y. Minamite (Assignee: Dainihon Jochugiku) 1986 Insecticide and miticide containing arylalkylsilicon compounds, and process for producing thereof. JP 61-87687.

Katsuda, Y., Y. Minamite and K. Nakayama 2005 "Silafluofen"and its termiticidal properties. Proc. 2nd Conference of Pacific Rim Termite Research Group, 70-74.

Minamite, Y., T. Kanzaki, Y. Katsuda and K. Nishimoto 1990 Application of a novel silaneophane (Hoe-498) to termiticides. *Jpn. J. Environ. Entomol. Zool.* **2**, 117-122.(in Japanese).

Nakayama, K., Y. Katsuda and K. Nishimoto 1998 Development of silafluofen and its application to termite control. *Wood Preservation* **24**(4), 16-23 (in Japanese).

Vongkaluang, C. and Y. Katsuda 2006 Laboratory efficacy test methods of termiticides in Thailand and evaluation of silafluofen products by the methods. Proceedings of the Third Conference of Pacific Rim Termite Research Group, 71-74.

The Resistance of Pine Wood from Timber Estate against Termite at Various Levels of Tree Age

by
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Abstract

Resistance of particular wood species depends on various kinds of factors such as extractive content, wood specific gravity, tree ages at the moment of its cutting/feeling, growth rate of tree, origin of tree from natural forest or plantation forest, tree species variety, and environment factor, like temperature and humidity, which in all can affect wood durability. This research aimed to look into wood resistance against dry-wood termite and subterranean termite, based on levels of tree ages. Wood species as used was pine (*Pinus merkusii* Jungh et de Vriese.) with tre ages varying at 17, 12,23, 27 and 28 years. The pine tree grows at Sukabumi region. The size of pine wood sample for dry-wood termite and subterranean termite in laboratory experiment measured consecutively 5 cm x 2.5 cm x 2 cm and 2.5 cm x 2.5 cm x 0.5 cm. Meanwhile, the size of sample for field test was 20 cm x 2 cm x 2 cm. Laboratory results revealed that the resistance of pine wood with tree ages at 21 – 28 years, against dry-wood termite belonged to class III (Indonesia National Standard 01-7207-2006). However, pine wood with tree ages at 17 and 28 years fell into class V. Meanwhile for the field test, the destruction in pine wood with 17 year tree age reached the highest (100 %), while the lowest was in pine wood with 27 year tree age (84 %).

Key words: pine wood, dry-wood termite, subterranean termites, laboratory, field test.

Introduction

Values of particular wood species are determined by its specific characteristics, among others wood resistance against wood-destroying organisms. Related with such, various wood species afford different resistances against kinds of organisms that attack. Even, in the same wood species, its resistance is not similar against different organisms, i.e. dry-wood termites and subterranean termites. Besides, resistance of particular wood species is affected by age of tree, when it is felled; growth rate of trees; tree origin whether it is from natural forest or from plantation forest; and physical-chemical properties of wood itself. All those factors can significantly affect wood resistance and hence its durability (Martawijaya, 1996).

One of the wood species from plantation forest is pine wood which finds much uses for merchant woods, in addition to other wood species such as sengon, mahoni, and teak. The success of Indonesia's plantation forest (IPF) is not unrelated with its management uses. There are three kinds of IPF wood uses that comprise merchant wood, fiber wood, and energy wood. Each of those uses requires different specific criteria. For merchant wood, as example, it will be preferred woods with characteristics such as attractive decoration, ease in working, high strength and dimensional stability, and effective resistance against wood-destroying organisms (Anonim, 1992). Establishment of IPF actually has been started since 1985. Then it is assumed that there must have been IPF trees with ages of about 20 years. According to Karnasudirdja and Kadir (1989), tree rotation of IPF's wood merchant ranges about 10-30 years. In the past, IPF which was established in Indonesia, was intended to meet the needs of raw material for pulp and paper industries. However, with appropriate management and handling, production of IPF'wood besides being allocated for pulp/paper industries, can also comply with the raw material needs for merchant-wood industries (Anonim, 1992).

Pine wood presents one of the several species used for IPF establishment, and its trees are planted much by the community as well as by the company. Pine wood has been used a lot as raw material for plywood, furniture, and hand-craft items. In general, natural durability of pine wood belongs to class IV; however, it is not yet know definitively whether each of the various ages of its trees afford as similar resistance against particular organism as it is against another organism. In relevance, therefore, it is necessary to conduct research regarding resistance of pine wood against two different wood-destroying organisms, i.e. dry-wood termites and subterranean termites (both in the laboratory test), and field graveyard test.

Materials and methods

Materials

Main material as used was wood of pine (*Pinus merkusii Jungh* et de Vries) species with 5 levels of its tree ages, i.e. 17 years, 21 years, 23 years, 27 years, and 28 years. This wood was procured from IPF area under the administration of the State Forest Enterprise (Perum Perhutani) situated in Cianjur (West Java). Meanwhile, the test organisms were dry-wood termites (*Cryptotermes cynocephalus* Light.) and subterranean termites (*Coptotermes curvignathus* Holmgren).

Methods

Preparing test wood specimens

Pine wood at five age levels was each cut to test specimens measuring 5 cm in length x 2.5 cm in width x 2 cm in thickness (for resistance test against dry-wood termites), measuring 2.5 cm in length x 2.5 cm in width x 0.5 cm in thickness (for resistance against subterranean termites), and measuring 20 cm in length x 2.5 cm in width x 2 cm in thickness (for field or graveyard test).

Specimens of pine wood which had been prepared in their intended sizes (as specified above) were used in the test for the resistance against those two organisms (dry-wood termites and subterranean termites). The testing procedures were referring to those of modified ASTM (Anonim, 1995) and Indonesian National Standard (SNI) No. 01-7207-2006 (Anonim, 2006).

Wood resistance against dry-wood termites in laboratory test

Five pine-wood specimens for each of the five age levels (17-28 years) were prepared with sizes specified for the test against dry-wood termites (5 cm x 2.5 cm x 2 cm) and then placed in contact with a glass tube (3 cm in height and 1.8 cm in diameter), one specimen for one glass tube. Wood specimen was placed horizontally under the glass tube vertically installed on it such that the larger surface of specimen was in contact with the lower mouth (hole) of glass tube. In other to make the contact between lower glass-tube and upper specimen-surface airtight for the possible small air-spaces, those spaces was sealed using wax. Afterwards, inside the glass tube were inserted as many as 50 dry-wood termites of the worker type. In this way, therefore, the termites could not move out of the glass tube during the test. Instead, the termites would eat wood specimens thereby leaving a kind of tunnels on the specimen surface. This test arrangement was allowed to proceed for 12 weeks. In this test, those five pine-wood specimens as described previously were regarded as replicates.

After 12 weeks, the examination or assessment was carried out on the wood specimens covering percentage of wood-weight losses, percentage of survival termites, and degree of attack by termites. The data were used to determine the resistance class of pine wood with various age levels using the classification of wood resistance as described in Table 1 (Anonim, 2006). In addition, to assess the degree of attack by termites, the related criteria by the AWPA were also consulted (Anonim, 1972).

Table 1. Classification of wood resistance against the attack by dry-wood termites (*Cryptotermes cynocephalus* Light)

Class	Resistance criteria	Weight loss (%)
I	Very resistant	< 2.0
II	Resistant	2.0-4.4
III	Moderate	4.4-8.2
IV	Poor	8.2-28.1
V	Very poor	> 28.1

Source: Anonim (2006)

Wood resistance against subterranean termites in laboratory test

Five pine-wood specimens for each of the age levels (17-28 years) were also prepared with sizes specified for the test against subterranean termites (2.5 cm x 2.5 cm x 0.5 cm) and then placed into the glass bottles, one specimen for one bottle. Inside the bottle, the wood specimen was places lengthwise rather vertically such that one of the widest specimen-surface leaned against the inner wall of the bottle. Further, into the bottle was put 200 grams of wet sand with moisture content of 7% (below its water-holding capacity). Subsequently, into the bottle were put as many as 200 healthy, active subterranean termites (*Coptotermes curvignathus* Holmgreen). Afterwards, the arrangement test (i.e. wood specimen, wet sand, subterranean termites, and bottle) was stored in dark room and then let for 12 weeks. In this test similar to dry-wood termites, those five pine-wood specimens were also regarded as replicates.

After 12 weeks, the examination or assessment was carried out on the wood specimens covering also percentage of wood-weight losses, percentage of termite survival, and degree of attack by termites. The data were used to determine the resistance class of pine wood with various age levels using the classification of wood resistance as described in Table 2 (Anonim, 2006). In addition, to assess the degree of attack by termites, the related criteria by the AWPA were consulted as well (Anonim, 1972).

Table 2. Classification of wood resistance against the attack by subterranean termites (*Coptotermes curvignathus* Holmgreen)

Class	Resistance criteria	Weight loss (%)
I	Very resistant	< 3.52
II	Resistant	2.52-7.50
III	Moderate	7.50-10.96
IV	Poor	10.96-18.94
V	Very poor	18.94-31.89

Source: Anonim (2006)

Wood resistance against subterranean termites using the field test

Pine-wood specimens for each of the age levels (17-28 years) were prepared with sizes specified for the field (graveyard) test against subterranean termites (20 cm x 2.5 cm x 2 cm) and then buried vertically, with the upper portion about 5 cm below the soil-ground surface. The burial was carried in Bogor, and lasted for 3 months. Afterwards, examination that comprised wood-specimen failure was carried out the modified ASTM procedures (Anonim, 1995).

Results and discussion

In detailed scrutiny, resistance of test pine wood specimens against dry-wood termites appeared to be significantly different from that against subterranean termites, as described in the following.

Resistance against dry-wood termites in laboratory test

In laboratory test, resistance of test pine wood specimens against dry-wood termites was assessed according to each age level of those specimens. Results of the resistance test (i.e. survival termites, weight loss, and degree of attack) that lasted for 12 weeks are presented in Table 3.

Table 3. Weight loss, survival termites, and degree of attack on test pine wood specimens due to the attack by dry wood termites

No	Age levels of wood specimens (years)	Weight Loss (%)	Resistance class	Survival termites (%)	Degree of attack	
					K (%)	T
1	28	7.12	III	11.2	8.4	B
2	27	7.12	III	24	5.6	B
3	23	7.12	III	24	8.2	B
4	21	7.11	III	15.6	12	B
5	17	8.28	IV	31.6	16.6	C

Remarks: K = condition of test wood specimens; T = level

Referring to the test as above (Table 3), there were differences as well as similarities in pine wood resistance based on age levels. Pine woods with ages of consecutively 21 years, 23 years, 27 years, and 28 years belong to class III, based on weight loss. This is because all weight losses for those ages (21-28 years) were more than 4.4% and less than 8%, in the range of 7.11-7.12%. Meanwhile, weight loss of pine wood at 17 year age was 8.28% (greater than 8%) indicating that this wood is less resistant compared to those with 21-28 years old, and therefore in resistance could be regarded as class IV (Anonim, 2006).

Wood resistance, in this regard pine species, against dry-wood termites can also be specified by the degree of attack and percentage of survival termites (Table 3). For pine wood specimen at 17-year age, it revealed the highest degree of attack, i.e. 16.6% and regarded as level C compared to the others at ages of 21-28 years, i.e. 5.6-12% and as level B. In addition, in order to scrutiny resistance of pine woods at their different age levels, it can also be assessed from the percentage of survival termites. In pine wood with 17 year age, percentage of survival termites was the highest (31.6%), while the wood with 28 years old revealed the lowest percentage (11.2%). Concurrently, in pine woods with 23 year and 27 year age, the percentages of termite survival were similar (24%); and with 21 years, the percentage was 15.6%. Again, this confirmed that

resistance of 17-year old pine wood against dry-wood termites was the lowest followed in increasing order by the woods with ages at consecutively 27 and 27 years, 21 years, and 28 years.

Resistance Against Subterranean Termites in Laboratory Test

Assessment on resistance test against these termites and parameters as used were similar to those as previously described in the test against dry-wood termites, both in laboratory tests. Results of the resistance test that lasted for 6 weeks are shown in Table 4.

Table 4. Weight loss, survival termites, and degree of attack on test pine wood specimens due to the attack by subterranean termites

No	Age levels of wood specimens (years)	Weight Loss (%)	Resistance class	Survival termites (%)	Degree of attack	
					K (%)	T
1	28	23.78	V	59.6	78	D
2	27	11.23	IV	23.8	27	C
3	23	11.78	IV	27.8	43	C
4	21	11.55	IV	17.5	18	C
5	17	26.23	V	60	78	D

Remarks: K = condition of test wood specimens; T = level

Viewing results of the test (Table 4), there were also differences as well as similarities in pine wood resistance based on age levels. Pine woods with age levels of 17 years and 28 years revealed similar resistance, and therefore at both ages, they could be judged belonging to class V (level D), and it also conformed to the SNI (Anonym, 2006). At those two ages, wood resistance was the lowest with weight losses above 18.94% (i.e. 23.78-26.23%). Meanwhile, for pine woods with age levels at 21-27 years, their weight losses were smaller than those at 17 years and 28 years, i.e. 11.23-11.78%. These losses were above 10.94% but below 18.49%, and therefore those pine woods also according to the SNI (Anonim, 2006) could be categorized as class IV (level C).

Similar to the cases for dry-wood termites, wood resistance of pine species against subterranean termites could also be specified by the degree of attack and percentage of living-termites (Table 4). It could be indicated that pine wood with ages of 27 years and 28 years sustained the highest degree of attack, i.e. 78% (ludged as level D, compared to those with the other ages (21-27 years), i.e. 18-43%, as level C. Besides, resistance of wood pines at their different age levels could also be determined by the portion (percentage) of survival termites (Table 4). Pine wood with ages 17 years revealed the highest survival termites, followed in increasing order by pinewoods with ages consecutively 28 years, 23 years, 27 years, and 21 years (as the lowest). In all, this again confirmed that there are two resistance group of pine wood against subterranean termite attack, i.e. less resistant (17 years and 28 years) and more resistant (21-27 years).

Extractive content inside the wood can significantly affect wood resistance against wood-destroying organisms. In relevant, Rachman, *et al.* (2007) reported that the extractive content in pine wood at 17 years old was 2.22%, at 28 years 2.26%, and at 21 years 2.52%. Further, Martawijaya (1996) stated that extractive content directly affected wood durability, in that wood durability of particular species increased with extractive content. In the case of teak wood, it turned out teak woods from Myanmar and from Thailand contained extractive of consecutively 0.88% and 0.56%, while extractive of teak wood from Java was lower (0.35%). As a result, teak wood from Myanmar and Thailand was more durable against subterranean termites compared to that fro Java.

Resistance Against Subterranean Termites with the Graveyard Test.

The field or graveyard test lasted for 3 months, afterwards the resistance of pine wood specimens with ages in the range of 17-28 years was examined, and the results were presented in Table 5.

Table 5. Resistance of pine wood at various age levels against subterranean termites on the field (graveyard test)

No.	Age levels of wood specimens (years)	Degree of attack (%)
1	28	97
2	27	84
3	23	94
4	21	92
5	17	100

Based on the assessed that data, it could be summed up that the degree of attack in pine wood with 17 year age was the highest (100%), which further tended to diminish reaching 21 years old and afterwards instead increase (at 23 years). At 27 years, the degree of attack was the lowest (84%), and then at 28 years increased again (97). According to Martawaijaya (1996), it was stated that there was significant correlation between wood durability and tree ages. In the case of teak wood, specimens with varying ages revealed different durability. Teak wood from tree with 20 year age belonged to durability class IV. Further, durability of teak wood with older ages tended to increase, i.e. 23 years and 30 years (both as class III), 57 years (class II). However, at 59 years durability decreased (as class III), while at 75 years it increased again (class II). To sum up, extractive content in wood, in addition to tree ages, can affect its durability against wood-destroying organisms. For the case of pine (*Pinus sylvestris*) wood, it was found a kind of extractive in its inside called pynosylvine, a substance that enabled the wood to resist the fungi attack. Possibly, that plynosylvin substance was also effective providing pine wood resistance against termites. Related with such, extractive in wood can be repellent and toxic to wood-destroying organisms (Martawijaya, 1996).

Conclusions

Resistance of pine wood against the attack by dry-wood termites in laboratory test was affected by pine tree ages. At 17 year age, wood resistance belonged to class IV. The resistance increased at 21 year age (durability class III); and until 28 year age, the resistance remained practically unchanged (durability class still III).

Resistance of pine wood against the attack by subterranean termites in laboratory test was affected also by pine tree ages. At 17 year, wood resistance belonged to class V. The resistance increased at 21-27 year age (in all, durability class III); and afterwards at 28 year age, the resistance instead decreased (durability class V again).

Resistance of pine wood against the attack by subterranean termites with the field (graveyard) test was affected also by pine tree as well. At 17 year age, the degree of attack was the highest (100%). At 21 years, the degree of attack was the lowest (92%), which further increased at 23 year age (94%). At 27 years, however, the degree of attack decreased (84%), which further increased again at 28 year age (97%)

References

Anonim. 2006 Uji ketahanan kayu dan produk kayu terhadap organisme perusak kayu. SNI 01.7207-2006. Badan Standardisasi Nasional.BSN. Jakarta

Anonim. 1995 Designation D 2278. Standard Test Method for Field Evaluation of Wood Preservatives in Round Post-Size Specimens. Annual Book of ASTM Standards. Vol. 04.10 p. 331 –435.

Anonim. 1972 Standard Method for Laboratory Evaluation to Determine Resistance to Subterranean Termites. American Wood Preservers' Association Standard. AWPA.

Karnasudirdja, S. and K. Kadir. 1989 Suatu kajian mengenai kegunaan jenis kayu HTI untuk pertukangan. Prosiding Diskusi Sifat dan Kegunaan Jenis Kayu HTI. Badan penelitian Dan Pengembangan Kahutanan, Jakarta.

Martawijaya, A. 1996 Keawetan Kayu dan Faktor Yang Mempengaruhinya. Petunjuk Teknis. Pusat Penelitian dan Pengembangan Hasil Hutan dan Sosial Ekonomi Kehutanan.

Rachman. O, N. Hadjib, Jasni, A. Santoso, S. Rullyati and J. Malik. 2007 Penetapan Daur Teknis Kayu HTI Untuk Bahan Baku Kayu Pertukangan. Laporan Hasil Penelitian (Tidak diterbitkan). Pusat Penelitian dan Pengembangan Hasil Hutan. Bogor.

Termite Resistance of Some Woods from Natural and Plantation Forests in South Kalimantan Indonesia

by
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Abstract

Five woods form natural forest and six woods from plantation forest or multy porpuse tree species from South Kalimantan were tested to (1) subterranean termite in the field or graveyard test, (2) subterranean termite (*Coptotermes curvignathus* Holmgren) in laboratory, and (3) dry wood termite (*Cryptotermes cynocephalus* Light) in laboratory, with five replication for each test. Termite resistance class was classified regarding to weight loss percentages of subterranean termite test and dry wood termite test, and the class was classified into five classes with class one is the most resistant and class five is very susceptible attacked. The result showed that Ulin wood (*Eusideroxylon zwageri*) from natural forest was the most resistant to termite attack and the woods from natural forest belonged to class I-IV for subterranean termite and class I-III for dry wood termite, and wood from plantation forest namely Terap (*Arthocarpus odoratissimus* blanco) is resistant against the attack of both termites, and the wood from plantation forest belonged to class II-V for subterranean termite and class I-IV for dry wood termite.

Key words: natural forest, plantation forest, subterranean termite, dry wood termite, graveyard test, laboratory test, resistance class.

Introduction

The decrease of logs production from natural forest in South Kalimantan causes high price of wood products, such as furniture, building material, wooden ship and handicraft. At present the wood industry has been in the gloomy situation because of the shortage of raw material from natural forest. In the attempt to meet the demands of logs for small scale wood industry, the woods from plantation forest and community forest have been utilized. Some tree species from community forest belong to multi purpose tree species which are cultivated for fruits, latex and flowers, and if they are not productive anymore because of old age, the woods of these trees can be used as raw material for wood industries. Nevertheless, generally these tree species produce the woods unsuitable for processing into wood products.

In South Kalimantan until middle 2007 the forest plantation area for pulp tree is planned to 318,585 ha and has been planted 235,038 ha, for building material is planned 202,365 ha and has been planted 44,881 ha, the other purpose is 68,500 ha (Ministry of Forestry, 2008). Furthermore it was stated that in 2007 the wood for all purposes from plantation forest was produced 245,250 m^3, and this amount will be increasing in line to forest plantation activity.

Since it takes a long time to grow trees until they reach large diameter boles, many young trees have been utilized for raw material of wood industries. One of wood properties which is important for wood utilization is the resistance against termite attack, especially in the tropical area the attack of termite is very broadly intensive, and it implies a huge amount of economic loss for rehabilitation, as Yoshimura and Tsunoda (2005) mentioned that in Indonesia the economic loss of various buildings by termite attack was around US$ 200-300 million. To extend service life of wood products, wood preservative can be applied for those woods (Lusyiani, 2001).

In conjunction with above description, a research has been conducted to examine the natural resistance of wood species from natural forest were Ulin, Ketapi, Alau, Red-Meranti and Sumpung and from community forest and plantation forest were Durian, Terap, Rubber-wood, Sungkai, Pantung and Sengon. Most of the wood species have been used for construction and furniture in South Kalimantan.

Materials and method

Materials

The woods from natural forest were Ulin (*Eusideroxylon zwageri*), Sumpung (*Swintonia glauca*), Alau (*Araucaria* spp), Meranti Merah (*Shorea accuminata*), and Ketapi (*Sandoricum koecapi*), and woods from

community forest and plantation forest were Durian (*Durio zibethinus*), Pantung (*Dyera costulaca*), Rubber-wood (*Hevea brasiliensis*), Terap (*Arthocarphus odoratissimus*, Sengon (*Paraserianthes falcataria*), Sungkai (*Peronema canescens)* and Mangga Lokal (*Mangifera* spp). These woods were more than 10 years old, and the wood specimens were heartwood.

Dry wood termite test

Five wood samples sized 5.0 cm by 2.5 cm by 2.0 cm (length, width and thickness), on the centre of it sample a glass tube (3 cm height by 1.8 cm diameter) was placed, and 50 worker termites were introduced in the glass tube. The samples were then put in a dark room for 12 weeks. At the end of the test, wood failure, wood weight loss and termite mortality were determined, the test procedures were according to SNI (2006).

Graveyard test

Five wood samples sized 20 cm by 5.0 cm by 2.0 cm (length, width and thickness) were vertically buried at 10 cm depth to the soil ground in Bogor during three months. At the end of the test, sample failure was determined.

Laboratory test for subterranean termite

Five wood samples sized 2.5 cm by 2.5 cm by 0.5 cm (length, width and thickness) were put in and touc hed to the jam pot, and in each jam pot was put 200 g of sand (7 % moisture content) and 200 healthy and active worker termites, and the jam pots were put in the dark room for six weeks. Each week the bottles were weight and if moisture content of the sand reduced 2 % or more, water was added to reach moisture content standard. At the end of the test, wood failure, wood weight loss and termite mortality were determined (SNI, 2006).

Results and discussion

Dry wood termite test

After twelve weeks test period the percentages of termite mortality, attack degree, and wood weight loss are shown at Table 1.

According to Table 1 the woods from natural forest have a higher resistant to dry wood termite attack comparing to woods from plantation forest which were indicated by higher termite mortality, lower attack degree and lower wood weight loss. Based on percentage of wood weight loss, the resistance classification to dry wood termite can be evaluated referring to SNI (2006), and the results shows that resistance class of wood from natural forest are varied in class I and class III with the average of class II, and wood from plantation forest or community forest are class I, class III and class IV with the average of class III. Compared to Martawijaya *et al.* (1981 and 1989) the resistance class of five woods to dry wood termite can be mentioned that the results of this test was higher for ulin and durian, lower for sengon, and same result for ketapi and sungkai.

Table 1. Failure and weight loss of wood sample, and mortality of dry wood termite

No	Wood species	Termite mortality (%)	Attack degree (%)	Weight loss (%)	Resistance class (Martawijaya *et al.*)
A	Natural Forest Wood				
1	Ulin (*Eusideroxylon zwageri)*	91.2	4.2	1.84	I (II)
2	Alau (*Araucaria* spp)	83.6	16.0	1.90	I
3	Sumpung (*Swintonia glauca)*	73.2	8.0	1.92	I
4	Red Meranti (*Shorea acuminata*)	44.0	34.0	4.87	III
5	Ketapi (*Sandoricum koecapi*)	71.2	16.0	4.69	III (III)
	Average	72.64	15.64	3.04	II
B	Plantation Forest Wood				
1	Terap *(Arthocarpus odoratissimus*)	87.6	6.6	1.91	I
2	Sungkai (*Peronema canescens)*	88.0	26.0	4.71	III (III)
3	Pantung (*Dyera costulata*)	49.6	39.0	5.04	III
4	Durian(*Durio zibethinus*)	60.0	35.0	4.86	III (IV)
5	Mangga (*Mangifera* spp)	48.8	30.0	4.61	III
6	Rubber-wood (*Hevea brasiliensis*)	38.8	40.0	13.60	IV (III)
7	Sengon (*Paraserianthes falcataria)*	37.6	40.0	12.18	IV
	Average	58.63	30.94	6.70	III

Ulin wood from natural forest has high specific gravity, i.e. 1.04 in average and ranged 0.88 -1.19 and with high silica content of 0.5 %, and classified to durability class I (Martawijaya *et al.* 1989), so that the wood

is very hard naturally. It causing termites could not scatter the wood, so they did not get food and died. It was shown by mortality test reached 83 – 91 %, small weight loss percentage 1.84 – 1.90 %, and attack degree 4.2 % only. Referring to Martawijaya *et al.* (1981 and 1989) and Hadi *et al.* (1992) some the other woods have lower specific gravity and lower silica content, durian 0.57 and 0.1 %, red meranti 0.51 and 0.29, sungkai 0.63 and 0.4 %, and sengon 0.33 and 0.2 % respectively.

Graveyard test

After three months test period, the percentage of attack degree is shown at Table 2. According to Table 2 the average of attack degree of the wood from natural forest was 31.5 % and the wood from plantation forest or community forest was 75.0 %, these values indicated that the wood from natural forest was more resistant than from plantation or community forest.

Similar to dry wood termite test, ulin wood was very resistant and it was not attacked at all or 0 % attack degree and this result matched to Martawijaya *et al.* (1989) ulin wood belong to durability class I. Compare to durian and rubber-wood from plantation forest the attack degrees were 100 %, and referring to Martawijaya *et al.* (1981) both woods were classified to durability class IV-V.

Another species from plantation forest, terap wood was moderately resistant which had attack degree 25 % only. Terap wood is very popular in the village because the unripe fruit is for vegetable and its ripe fruit as fresh fruit. The tree grows naturally in the yard of house, fast growing species and has dense leaves becoming for shading tree, and the wood colour is yellowish white.

Table 2. Attack degree of graveyard test.

No	Wood species	Attack degree (%)
A	Natural Forest Wood	
1	Ulin (*Eusideroxylon zwageri)*	0
2	Alau (*Araucaria* spp)	28
3	Sumpung (*Swintonia glauca)*	13
4	Red Meranti (*Shorea acuminata*)	85
	Average	31.5
B	Plantation Forest Wood	
1	Terap *(Arthocarpus odoratissimus*)	25
2	Sungkai (*Peronema canescens)*	70
3	Pantung (*Dyera costulata*)	80
4	Durian(*Durio zibethinus*)	100
5	Rubber-wood (*Hevea brasiliensis*)	100
	Average	75.0

Subterranean termite in laboratory test

After six weeks test period the percentages of wood failure, wood weight loss, and termite mortality are shown at Table 3.

According to Table 3 the woods from natural forest have a higher resistant to subterranean termite attack comparing to woods from plantation forest which was indicated by higher termite mortality, lower attack degree and lower wood weight loss. Based on percentage of wood weight loss, the resistance classification to dry wood termite can be evaluated referring to SNI (2006), and the results shows that resistance class of wood from natural forest are varied in class I, class II and class IV with the average of class III, and wood from plantation forest or community forest are class II, class III, class IV and class V with the average of class IV.

On the subterranean termite test, wood which had high specific gravity and density constantly showed its natural endurance about termite attack, but it is not a guarantee that the wood from natural forest has better resistant to termite attack. The wood resistant to bio-deterioration depends on amount and chemical composition of extractive content. The woods from plantation forest, i.e. terap, sungkai and durian had better termite morality value comparing to red-meranti. At the moment people plant durian getting the fruits, and after the tree is becoming old people can utilize the wood for any purpose. Like wise sungkai which has precious wood texture and widely planted as hedge suitable recommended to be more expanded

Table 3. Failure and weight loss of wood sample, and mortality of subterranean termite.

No	Wood species	Termite mortality (%)	Attack degree (%)	Weight loss (%)	Rsesitance class
A	**Natural Forest Wood**				
1	Ulin (*Eusideroxylon zwageri)*	100	1.8	2.47	I
2	Alau (*Araucaria* spp)	85.9	20.0	4.52	II
3	Sumpung (*Swintonia glauca)*	100	5.0	7.42	II
4	Red Meranti (*Shorea acuminata*)	22.7	54.0	20.86	IV
		77.15	20.2	8.8175	
B	**Plantation Forest Wood**				
1	Terap *(Arthocarpus odoratissimus*)	100	5.0	3.82	II
2	Sungkai (*Peronema canescens)*	69.8	27.0	12.46	III
3	Pantung (*Dyera costulata*)	20.2	65.0	15.12	IV
4	Durian(*Durio zibethinus*)	68.7	20.0	15.52	IV
5	Mangga (*Mangifera* spp)	48.8	30.0	14.80	IV
6	Karet (*Hevea brasiliensis*)	8.5	77.0	25.52	V
7	Sengon (*Paraserianthes falcataria)*	23.1	65.0	23.10	V
		48.44	41.29	15.76	

Conclusion

1. The natural forest woods have the resistant against termites in the range of resistance classes of I – IV and average II.
2. The woods of plantation or community forest have the resistance classes against termites in the range of durability II – V and average IV.
3. Terap wood has high resistance to termites attack, while sungkai and durian woods have moderate resistance to termites attack, and these species is recommended for plantation and community forests.

References

Hadi, Y. S.; N. Hadjib and Jasni, 2002 Resistance of Polystyrene Wood to Marine Borer and Subterranean Termite. Proceedings of The 6 Th Pacific Rim Bio- Based Composites Symposium and Workshop on The chemical Modification of Celluloses. Portland, Oregon, USA. Vol. 2, pp 528-534.

Lusyani 2001 Dry wood Attack and Resistance of 3 Woods from Plantation Forests in South Kalimantan Indonesia. Journal Sylva, Forest Product and Technology, Forestry Faculty, Lambung Mangkurat University. Vol.1. pp. 1-5.

Martawijaya, A., I. Kartasujana, K. Kadir and S. A. Prawira 1981 Atlas Kayu, Jilid I. Forestry Research and Development Center, Ministry of Forestry, Jakarta.

Martawijaya, A., I. Kartasujana, Y. I. Mandang, S. A. Prawira and K. Kadir 1989 Atlas Kayu, Jilid II. Forestry Research and Development Bureau, Ministry of Forestry, Jakarta.

Ministry of Forestry 2008 Data of plantation forest. Directorate General of Forest Products.

SNI (National Standard Bureau) 2006 Uji ketahanan kayu dan produk kayu terhadap organisme perusak kayu. Badan Standarisasi Nasional Indonesia.

Yoshimura, T. and K. Tsunoda 2005 Termite Problems and Management in Pacific-Rim Asian Region. IAWPS 2005 International Symposium on Wood Science and Technology, Volume I: Oral Presentation, Pacific, Yokohama, Japan, 27-30 November 2005, p. 316-317.

Polystyrene and Acetylated Woods Resistance to Biodeterioration

by
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Abstract

Acetylated and polystyrened woods of mindi (*Melia azedarach*) and sugi (*Cryptomeria japonica*) were tested for their resistace to (1) subterranean termite (*Coptotermes curvignathus* Holmgren), (2) dry wood termite (*Cryptotermes cynocephalus* Light), and (3) white rot fungus *Schizophyllum commune* in the laboratory. Weight percent gain of acetylation was 19.9% for mindi and 24.4 % for sugi, and polymer loading of polystyrene for mindi was 41.0 % and 74.9% for sugi. Wood sample sizes were 0.8 cm by 2 cm in cross section by 2.5 cm in longitudinal direction for subterranean termite test and 5 cm for dry wood termite and fungal tests. Density of mindi was 0.43 g/cm^3 and sugi 0.34 g/cm^3, and replication of wood sample was five. The results showed that mindi wood had better resistance to subterranean termite and dry wood termite attacks, but both wood species had similar resistance to fungal attack. Polystyrene and acetylation were effective in increasing resistace to subterranean termite, dry wood termite and fungal attacks. Polystyrene wood was more resistant to subterranean termite and dry wood termite attacks than acetylated wood, while both modified woods had similar resistance to fungal attack.

Key words: acetylated and polystyrened woods, dry wood termite, subterranean termite, white rot fungi, termite resistance class

Introduction

In the last decade Indonesian wood industry has been supplying about 60% from plantation forest, it was a great change because previuosly the logs was supplied dominantly from natural forest (calculated data from Ministry of Forestry, 2007). About two million hectares of fast growing species has been developed with cutting cycle of 10-15 years, e.g. mangium (*Acacia mangium),* sengon (*Paraserianthes falcataria),* tusam (*Pinus merkusii),* and mindi *(Melia azedarach)* (Nurrochmat and Hadi, 2005 and Anonymous, 2005). Mindi tree has been planting by community and Perum Perhutani because of fast growing, for example in Bogor the tree of 10 years age has 10 meters height branch free and 38 cm diameter at breast height (Balitbang Kehutanan, 2001). The specific gravity of mindi wood is 0.53 (0.42-0.65), shrinkage from green to oven dry weight is 3.3% in radial and 4.1 in tangential directions, strength class II-III equal to mahagony and red meranti woods, and durability class IV-V or not resistant.

Wood from plantation forest generally has a lot of juvenile wood and the wood is inferior in physical-mechanical properties and durability comparing to mature wood. Even the existing housings in Indonesia were built with mature wood, but in 2000 the economic loss of various buildings by termite attack was around US$ 200-300 million (Yoshimura and Tsunoda, 2005), apparently the loss will be increased in the future if the juvenile wood from plantation forest is not preserved prior to use for building materials.

To extend service life of timber, it can be done through wood preservation, i.e. fill in poison chemical or preservative to the wood. Currently this technique is very commonly applied, but it has side effect to contaminate environment and poison for the human being and other living organisms. Hadi *et al.* (2005) stated that CCA (Chromated Chlor Arsen) was very effective for wood preservation purpose before 2000 era, but in early 21st century it was banned in almost any country because of poison to living organisms and environment, and the subtitute was looked for even it is not equal effectiveness. Acetylation is a way to preserve wood and environmentally sound, Rowell (2008) stated that dimensional stability, resistance to decay fungi and destructive insects, as well as other positive performance improvements can be greatly increased by reacting wood of different geometries with acetic anhydride (acetylation) resulting in a new generation of value added wood-based products that perform very well in adverse environment. Hadi *et al.* (1995) resulted acetylation of the flakes increased the resistance to dry wood termite, subterranean termite, and fungal attacks. Furthermore, Temiz *et al.* (2006) revealed that acetylated and heat-treated wood samples showed better decay resistance against *P. placenta* and *C. versicolor* than silicon treatments, and Brelid *et al.* (2000) mentioned that the resistance to fungal decay of acetylated wood with an acetyl content of about 20% is of the same magnitude as for CCA treated wood at a high retention

level 10.3 kg/m^3.

Polystyrened wood also can be considered for wood preservation, Hadi *et al.* (2002 and 2003) impregnated mono styrene to pine, albizia and rubber-wood wood and polymerized with heat at 60 °C during 24 hours, and then tested to *Trametes versicolor* white rot fungi and *Tyromyces palustris* brown rot fungi, subterranean termite, and marine borer, and the results showed that pine had the most resistant to white rot and brown rot fungal attacks, and polystyrene wood and Impralite CCB preserved wood had the same resistant to brown and white rot fungal attacks, subterranean termite, and marine borer, and they had much better resistant than untreated wood. The other researchers Yildiz *et al.* (2005) stated that at full and half loading levels of polystyrene maritime pine (*Pinus pinaster* Ait.) and poplar (*Populus x. euramericana* cv. I-214) helped decreasing weight losses due to white and brown rots fungi attacks, and Devi *et al.* (2003) found that biodegradability of polystyrene wood was improved on treatment with styrene/styrene-GMA, also Baysal *et al.* (2007) stated that boric acid and borax mixture pretreatment for polystyrene wood got total resistance against white and brown rots fungi attacks.

The purpose of this work is to determine the resistance of acetylated and polystyrened mindi *(Melia azedarach)* and sugi (*Cryptomeria japonica*) woods to subterranean termite, dry wood termite and fungal attacks in laboratory tests.

Materials and mthods

Materials

The wood species of mindi *(Melia azedarach)* from Bogor Indonesia and sugi (*Cryptomeria japonica*) from Japan were used to determine termite attack resistance. Air dry woods were vacuumed at 600 mmHg for 30 minutes and followed by immersion in monomer styrene and then pressure at 10 kg/cm^2 for 30 minutes. The wood samples were wrapped with aluminum sheet and put into oven at 100 °C for 24 hours. After opening the aluminum cover, the sample woods were weighed for polymer loading calculation. For acetylation process, the wood samples were oven dried and then immersed in acetic anhydride for 24 hours, and followed by wrapping with aluminum sheet and put into oven at 120 °C for 24 hours. After opening the aluminum cover, the sample woods were weighed for weight gain calculation. For comparison purpose, wood without treatment was included as control. The sample size was 0.8 cm by 2 cm in cross section by 2.5 cm in longitudinal direction for subterranean termite test and 5 cm for dry wood termite test, and amount of samples for both tests was five.

Subterranean termite test

Five wood samples were put in and touched to the jam pot, and in each jam pot was put 200 g of sand (7% moisture content) and 200 healthy and active worker termites (*Coptotermes curvignathus* Holmgren), and the jam pots were put in the dark room for six weeks. Each week the bottles were weight and if moisture content of the sand reduced 2% or more, water was added to reach moisture content standard. At the end of the test, wood weight loss and termite mortality were determined (National Standard Bureau, 2006).

Dry wood termite test

On the center of its sample a glass tube (3 cm height by 1.8 cm diameter) was placed, and 50 worker termites (*Cryptotermes cynocephalus* Light) were introduced in the glass tube. The samples were then put in a dark room for 12 weeks. At the end of the test, wood failure degree, wood weight loss and termite mortality were determined, the test procedures were according to National Standard Bureau (2006).

Fungal test

Schizophyllum commune white rot fungi was inoculated in the glass boxes using potato dextrose agar media until it grew adequately. The wood samples were put in the glass boxes, and after two months of inoculation at 22 to 28 °C and 80 to 90% relative humidity weight loss percentage was determined.

Data analysis

Factorial 2 by 3 in completely randomized design was used to analyze the data, the first factor was wood species namely mindi and sugi, and the second factor was treatment to wood namely control, acetylation and polystyrene.

Results

Density of mindi was 0.43 g/cm^3 and 0.34 g/cm^3 for sugi, and polystyrene polymer loading of mindi reached 41.0% and sugi 74.9%, and weight percent gain of acetylated wood for mindi was 19.9% and sugi 24.4%, sugi wood was easier penetrated with styrene or acetic anhydride because sugi has lower density compared to mindi, and this result was similar with Hadjib *et al.* (2000) mentioned that lower density wood had more polymer loading of polystyrene. The results of wood samples resistance to subterranean termite and dry

wood termite both in laboratory tests are discussed in the following discussion.

Subterranean termite test

Weight loss of wood sample, termite mortality and resistance class after six weeks of subterranean termite test are shown in Table 1. Mindi had better resistant than sugi due to wood weight loss and termite mortality, mindi control had 10.3% wood weight loss but sugi 45.4%, and termite mortality of mindi control was 61% but sugi 4% only, and due to National Standard Bureau (2006) mindi blongs to resistance class III or moderate and sugi class resistance V or very not resistance. Mindi has higher density than sugi resulting mindi is potentially more resistant than sugi as Arango *et al.* (2006) mentioned based on the six hardwood species indicate a significant inverse association between percentage mass loss and specific gravity or with other term higher specific gravity wood has more resistant to *Reticulitermes flavipes* termite.

Table 1. Wood weight loss and termite mortality of subterranean termite test.

Respond	Mindi			Sugi		
	Control	Acetylated	Polystyrene	Control	Acetylated	Polystyrene
Wood Weight Loss (%)	10.34	5.88	1.34	45.38	17.37	6.94
Resistance Class	III	II	I	V	IV	II
Termite Mortality (%)	61.3	90.0	100	3.8	18.9	68.1

Wood treatment with acetylation or polystyrene was highly significant affecting wood weight loss and termite mortality, and the treated woods were much better resistant to subterranean termite than control wood. Furthermore it can be mentioned that polystyrene wood was better resistant than acetylated wood to subterranean termite attack, and regarding to resistance class (National Standard Bureau, 2006) acetylated wood increased one class and polystyrene wood increased two-three classes.

Dry wood termite test

Weight loss of wood sample, termite mortality and resistance class after twelve weeks of dry wood termite test are shown in Table 2. Mindi had better resistant than sugi due to wood weight loss and termite mortality, mindi control had 6.87% wood weight loss but sugi 16.88%, and termite mortality of mindi control was 46.4% but sugi 22.0% only. Regarding to resistance class classifiaction against dry wood termite attack (National Standard Bureau, 2006), mindi belongs to class III or moderate and sugi class IV or not resistant.

Acetylation or polystyrene could increase dry wood termite attack resistance which was indicated by lower wood weight loss, higher resistance class and higher termite mortality compared to control wood. Based on wood weight loss polystyrene wood was more resistant than acetylated wood, and regarding to resistance class (National Standard Bureau, 2006) acetylated wood increased one class and polystyrene wood increased two-three classes, but if termite mortality as reference both modified woods had similar resistant to dry wood termite attack.

Table 2. Wood weight loss and termite mortality of dry wood termite test.

Respond	Mindi			Sugi		
	Control	Acetylated	Polystyrene	Control	Acetylated	Polystyrene
Wood Weight Loss (%)	6.87	3.66	1.39	16.88	6.25	1.61
Resistance Class	III	II	I	IV	III	I
Termite Mortality (%)	46.4	70.8	68.8	22.0	95.2	83.6

Fungal test

Weight loss and moisture content of wood sample after two months fungal test are shown in Table 3. Mindi and sugi had similar resistant to fungal attack which was indicated by wood weight loss of mindi at 1.48% and sugi at 1.33%, and moisture content of mindi was 32.5% and sugi 30.0%.

Table 3. Wood weight loss and moisture content of fungal test.

Respond	Mindi			Sugi		
	Control	Acetylated	Polystyrene	Control	Acetylated	Polystyrene
Wood Weight Loss (%)	1.48	0.42	0.70	1.33	0.33	0.54
Moisture Content (%)	32.5	14.7	13.9	30.0	13.0	10.9

Acetylation or polystyrene could increase fungal attack resistance which was indicated by lower wood

weight loss and lower moisture content compared to control wood, and both modified woods were similiar resistant to fungal attack. To clarified the resistance of both modified woods to fungal attack, it could be suggested that the test period should be longer than two months or at least three months.

Conclusios

From discussions above, it could be concluded that :

1. Mindi wood had better resistance to subterranean termite and dry wood termite attacks, and both wood species had similar resistant to fungal attack.
2. Polystyrene or acetylation were effective to increase subterranean termite, dry wood termite, and fungal attacks resistance, while polystyrene wood was better resistant to subterranean termite and dry wood termite attacks than acetylated wood, and both modified woods had similar resistant to fungal attack.

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References

Anonymous. 2005 Pelatihan Persemaian Mindi (*Mindi Nursary Training*). RUAS, Majalah Kehutanan dan Lingkungan (*Magazine of Forestry and Environment*), Perum Perhutani Unit 1 Cental Java, Edition 05/V/November 2005, p. 17.

Arango, R. A., F. Green, K. Hintz, P. K. Lebow, R. B. Miller. 2006 Natural durability of tropical and native woods against termite damage by *Reticulitermes flavipes* (Kollar). International Biodeterioration & Biodegradation 57 (2006) 146–150.

Balitbang Kehutanan. 2001 Mindi (*Melia azedarach* L.). Ministry of Forestry Jakarta.

Baysal, E., M. K. Yalinkilic, M. Altinok, A. Sonmez, H. Peker and M. Colak. 2007 Some physical, biological, mechanical, and fire properties of wood polymer composite (WPC) pretreated with boric acid and borax mixture. Construction and Building Materials (21) 1879–1885.

Brelid, P. L., R. Simonson, O. Bergman and T. Nilsson. 2000 Resistance of acetylatede wood to biological degradation. Holz als Roh-und Werkstoff, Vol. 58(5): 331-337.

Devi, R. R., I. Ali and T.K. Maji. 2003 Chemical modification of rubber wood with styrene in combination with a crosslinker: effect on dimensional stability and strength property. Bioresource Technology (88) 185–188.

Hadi, Y.S., I. G. K.T. Darma, F. Febrianto and E.N. Herliyana. 1995 Acetylated rubberwood flakeboard resistance to biodeterioration. Forest Products Journal Vol. 45(10): 64-66.

Hadi,Y. S., N. Hadjib and Jasni. 2002 Resistance of polystyrene wood to marine borer and subterranean termite. Proceedings of The Sixth Pacific Rim Bio-Based Composites Symposium and Pre-symposium Workshop on Chemical Modification of Cellulosics", Portland, Oregon State University, USA, 10-13 Nov. 2002, p: 528-534.

Hadi,Y. S., I.G. K.T. Darma, N. Hadjib and Jasni. 2003 Polystyrene wood resistance fungal attack. International Conference on Forest Products, Chungnam National University, Daejon, South Korea, 21-24 April 2003, p. 494-497.

Hadi, Y. S., M. Westin and E. Rasyid. 2005 Resistance of furfurylated wood to termite attack. Forest Products Journal Vol. 55 (11): 85-88.

Hadjib, N., Barly, Y. S. Hadi and I.G.K.T. Darma. 2000 Physical and mechanical properties of three polystyrene Indonesian woods. Proceeding The Fifth Pacific Rim Bio-Based Composites Symposium, Canberra, Australia, 10-13 December 2000, p.687.

Ministry of Forestry. 2007 Forestry Statistics of Indonesia 2006. Jakarta.

National Standard Bureau. 2006 Wood and wood products resistance test to wood destroying organism. Indonesian National Standard.

Nurrochmat, D. R. and Y. S. Hadi. 2005 Case Studies on the Politics of Logging in Asia and Europe: Case Study on Indonesia. Lecture delivered at 12th ASEF University "Asia-Europe Environmental Co-operation: Towards Sustainable Forest Management", Univ. of Brunei Darussalam, Bandar Seri Bengawan, 13 July 2005.

Rowell, R.M. 2008. Use of Acetylation for Production of Decay Resistant Wood Composites. Proc eedings of International Symposium on Wood Science and Technology, IAWPS2008, Harbin, P.R. China, 27-29 Sep 2008, p. 401-402.

Temiz, A., N. Terziev, B. Jacobsen and M. Eikenes. 2006 Weathering, water absorption, and durability of silicon, acetylated, and heat-treated wood. Journal Applied Polymer Science 102 (5): 4506-4513.

Yildiz, U. C., S. Yildiz and E. D. Gezer. 2005 Mechanical properties and decay resistance of wood–polymer composites prepared from fast growing species in Turkey. Bioresource Technology (96) 1003–1011.

Yoshimura, T. and K. Tsunoda. 2005 Termite Problems and Management in Pacific-Rim Asian Region. IAWPS2005 International Symposium on Wood Science and Technology, Volume I: Oral Presentation, Pacifico, Yokohama, Japan, 27-30 November 2005, p. 316-317.

Termiticidal Performance of Zinc Borate-Incorporated Particleboard

by
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Abstract

The termite resistance of zinc borate-incorporated particleboard was examined. Particleboards (300 by 300 by 15 mm), which were prepared from particles of mixed wood species generated from demolished construction materials were incorporated with zinc borate at target contents 0, 1, 1.5 and 2% of particle weight. An in-line treatment method was utilized to introduce the powdered chemical during the blending stage. ICP analysis indicated that the amount of zinc borate was not lost during board manufacturing. Standard static bending tests demonstrated that there was no significant loss in mechanical properties. The relative termiticidal efficacy increased with the content of zinc borate.

Key words: zinc borate-incorporated particleboard, ICP analysis, bending strength, termite resistance

Introduction

Wood-based composites has been increasingly produced over the past few decades due to some reasons, e.g. depletion of high quality wood, development of new composite technologies, and widespread acceptance of wood composites for constructional applications. Wood-based composites, however, require protection from the effects of moisture, weather, biological agents (decay fungi, insects, and marine borers) and fire when used in the exposed outdoor environments. It has been reported that these composite products are experiencing failures due to fungal and insect attack (Eaton and Hale 1993; Schmidt 1991; Chung et al. 1999; Shupe and Dunn 2000). The protection of composites is a topic that continues to receive considerable attention from researchers and manufacturers (Gardner et al. 2003). Preservative process during composite production which is known as in-process or in-line treatment has been documented as an effective method for protecting composites. Protection throughout the cross section of composite and processing (cutting, drilling etc.) at anytime are thought to be advantageous features of the in-process method when compared with other preservative methods. In recent years, a great deal of research and development work has been conducted with regard to the incorporation of borate into wood-based composites (Laks and Manning 1995). Inorganic borate systems, particularly with zinc borate, have an established commercial track record due to their low cost, efficacy against fungi and insects, low mammalian toxicity, minimal environmental impact, high compatibility with most manufacturing processes and fire retardancy at higher retentions. Tsunoda et al. (2002) who applied the in-process incorporation of zinc borate to medium density fiberboards, reported that 1 and 1.5 % BAE significantly reduced mass losses due to termite attack without any undesirable effects on mechanical and physical properties.

The aim of the current investigation was to examine feasibility of zinc borate incorporation in particleboards so that the effectiveness of zinc borate retention levels on termite attack in the laboratory termite tests and effects on mechanical properties were determined.

Materials and methods

Test boards were prepared from particles of mixed wood species generated from demolished construction materials for recycling purposes. The particles were air dried to approximately 7.3% moisture content. Powdered zinc borate was added to the blender at target contents 0, 1, 1.5 and 2% of particle weight. Those were 0, 0.853, 1.280 and 1.707 %BAE (Boric Acid Equivalent), respectively. Then a polymeric diphenylmethane diisocyanate (pMDI) was sprayed onto the particle-zinc borate mixture. The additional level of resin was 10% based on the oven-dried weight of the particles. The mixture was transferred into a forming box to form mats prior to hot pressing. The mats were hot-pressed at 160°C for 10 minutes. Single layered particleboards were manufactured with dimensions of 300 x 300 x 15 mm. The targeted density of the boards was 700 kg/m^3. No water repellent chemicals were utilized during the particleboard manufacturing. Three replicate boards were manufactured for each treatment. All boards were conditioned at ambient conditions for 4 weeks before they were cut into specimens for bending and termite tests.

Zinc borate contents in the boards were analyzed using Inductively Coupled Plasma (ICP) spectrometer

(Model SPS 7800, Seiko Instruments). A small sample of each board was milled and digested according to American Wood Preservers' Association (AWPA) A7-04 standard (AWPA, 2006). Both zinc and boron amounts were measured so that zinc borate contents were calculated from the two measured figures.

The static bending properties, modulus of rupture (MOR) and modulus of elasticity (MOE), of the boards were evaluated according to the Japanese Industrial Standard (JIS) for particleboards (JIS A 5908, 2007). Three samples were obtained from each board representing nine replicates for each retention level. Results were analyzed by ANOVA (MyStat version 12).

Termiticidal performance of the boards was evaluated according to JIS K 1571 (2004) with a minor modification of sample size. Five replicate samples (20 x 13 x 15
mm) for each treatment level were randomly selected for the termite test. Sugi (*Cryptomeria japonica* D. Don) sapwood samples (10 x 10 x 20 mm) were also includd in the test as reference materials. Individual sample was exposed to 150 workers and 15 soldiers of *Coptotermes formosanus* Shiraki for 21 days. Details of the termite test should be referred to the JIS K 1571. In addition, the same test units were exposed to termite test conditions without termites to correct weight changes associated with unforeseen causes other than termite activity. Relative efficiency against termite attack (RET) was calculated as {[(mean percent mass loss of the untreated specimens) – (mean percent mass loss of the treated specimens)] / (mean percent mass loss of the untreated specimens) x 100}.

Results and discussion

ICP analysis of zinc borate in particleboards: Figure 1 shows that percent BAE values calculated from ICP data of elemental boron and zinc seem to coincide fairly well with target contents with small variations in zinc borate contents among samples as indicated error bars.

Effect of zinc borate incorporation on the mechanical and physical properties: Incorporation of zinc borate did not appear to have any negative effect on mechanical properties within the range of retentions tested. Statistical analysis did not show any significant difference in modulus of rupture (MOR) and modulus of elasticity (MOE) among contents of zinc borate incorporated in particleboards.

Termiticidal performance of zinc borate-incorporated particleboards: The relative termiticidal efficacy increased with the content of zinc borate (Figure 2). Mass loss data suggested that 2% zinc borate could be an upper limit of threshold value, since the zinc borate content level suppressed mass loss less than 3% in the no-choice laboratory test.

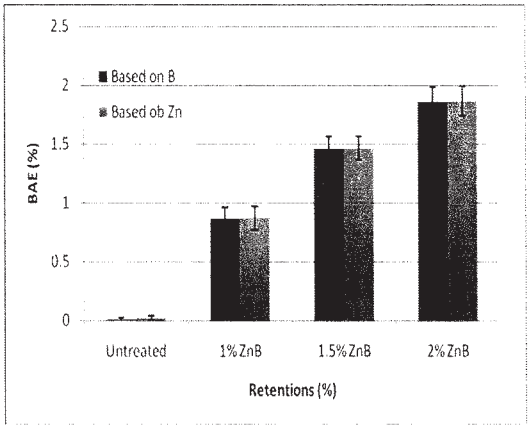

Figure 1 Mean percent BAE (boric acid equivalent) in zinc borate- incorporated particleboards based on ICP analyses (error bars indicate ± standard deviation of 3 replicates).

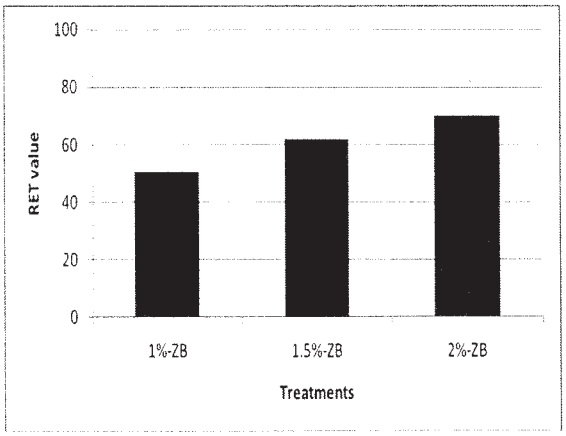

Figure 2 Relative efficiency against termite (RET) of zinc borate-incorporated particleboards (mean of five replicates)

Conclusions

Incorporation of zinc borate by in-process method proved to be feasible in protecting particleboards from termite attack. ICP analysis demonstrated that the amount of zinc borate was not lost during board manufacturing, and no detrimental effect on MOR and MOE was seen. These were common to the results with oriented strand boards and medium density fiberboards (Laks and Manning, 1995; Tsunoda et al., 2002). Although the low water solubility of zinc borate is preferable as a wood protecting chemical, field evaluations are needed to determine the persistence of efficacy and the leaching resistance of the biocide from treated particleboards.

References

American Wood Preservers' Association 2006 Standard for wet ashing procedures for preparing wood for chemical analysis, *AWPA Book of Standards* A7-04, AWPA, Selma, AL.

Chung, W.-Y., S.-G。 Wi, H.-J.Bae, and B.-D. Park 1999 Microscopic observation of wood-based composites exposed to fungal deterioration, *Journal of Wood Science*, **45**, 64-68.

Eaton, R. A. and M.D.C. Hale 1993 *Wood: Decay, pests and protection*, Chapman & Hall, London, 546pp.

Gardner, D. J., C. Tascioglu, and M. E. Wälinder 2003 Wood composite protection. In: *Wood Deterioration and Preservation: Advances in Our Changing World.* B.Goodell, D.D. Nicholas, and T.P. Schultz, eds; American Chemical Society, Washington D.C., pp. 399-419.

JIS K 1571 1998 Quantitative standards and testing methods of wood preservatives. *Japanese Industrial standard (JIS)*, Japanese Standards Association, Tokyo.

JIS A 5908 2007 Particleboards, *Japanese Industrial Standard (JIS)*, Japanese Standards Association, Tokyo.

Laks, P. E. and M. J. Manning 1995 Preservation of wood composites with zinc borate. *The International Group on wood Preservation. Doc.* No. IRG/WP/95-30074.

Schmidt, E.L. 1991 A resin-compatible copper naphthenate to preserve aspen composites. *Forest Products Journal* 41(5):31-32.

Shupe, T. F. and M. A. Dunn 2000 The Formosan subterranean termite in Louisiana: Implications for the forest products industry. *Forest Products Journal* **50** (5), 10-18.

Tsunoda, K., H. Watanabe, K. Fukuda and K. Hagio 2002 Effect of zinc borate on the properties of medium density fiberboard. *Forest Products Journal* **52** (11/12) 62-65.