



**TRG 2**

# **Proceedings**

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Annex



# **International Research Groups and Conferences on Termites**

by

Kunio Tsunoda

Research Institute for Sustainable Humanosphere, Kyoto University, Uji, Kyoto 611-0011, Japan

## **Abstract**

There are some international research groups (societies) and conferences, which are held regularly on termite biology and management. International Union for the Study of Social Insects (IUSI), International Congress on Entomology (ICE) and International Conference on Urban Pests (ICUP) deal with approaches to scientifically understand termite biology. International Research Group on Wood Protection (IRG) has a working party to discuss both termite biology and management. Entomological Society of America is not a true international scientific society, but many entomologists seem to participate in the annual meeting from many countries.

Federation of Asian and Oceania Pest Managers Associations (FAOPMA) is strongly linked with pest management industry in the Pacific Rim regions. National Pest Management Association (NPMA) arranges annual meeting in the US and approximately 3,000 participants including a few hundreds of foreigners enjoy lectures on pest managements and industrial exhibition.

Pacific Rim Termite Research Group is a unique society, which originates from Asia-Pacific region and focuses on termites only. Expanded activities are expected to promptly develop both termite management business and collaborative research among scientists and countries as well.

**Key words:** IUSI, ICE, ICUP, IRG, FAOPA, NPMA, ESA

## International Research Groups and Conferences on Termites

**Kunio Tsunoda**

Research Institute for Sustainable Humanosphere  
Kyoto University

★ **International Union for the Study of Social Insects (IUSSI)**

★ **The International Research Group on Wood Protection (IRG)**

★ **Federation of Asian and Oceania Pest Managers Associations (FAOPMA)**

★ **International Congress on Entomology (ICE)**

★ **International Conference on Urban Pests (ICUP)**

★ **National Pest Management Association (NPMA)**

★ **Entomological Society of America (ESA)**

## International Union for the Study of Social Insects (IUSSI)

- ★ The International Union for the Study of Social Insects was founded in 1951 to facilitate communication among social insect researchers worldwide.
- ★ International congress of IUSSI is held every 4 years.
- ★ The next international congress of IUSSI will be held in the Omni Shoreham Hotel, Washington D.C., USA, July 30 - August 4, 2006.

2002 - Sapporo, Japan  
1998 - Adelaide, Australia

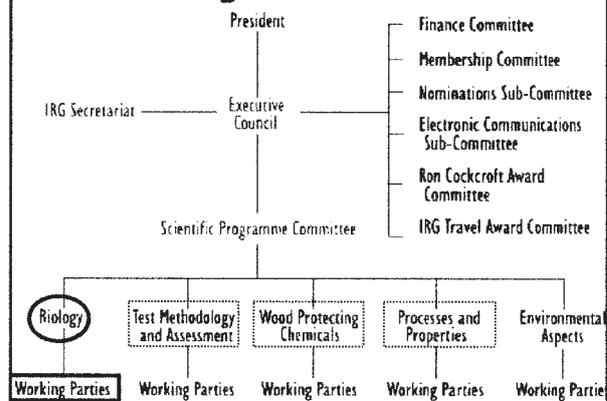
### IUSSI Sections



## The International Research Group on Wood Protection (IRG)

- ★ The International Research Group was established in January 1969 by the OECD.
- ★ Meetings have been held annually since then.
- ★ The group consists of 5 sections.
- ★ Each group has working parties to discuss specific subjects.

### IRG - Organization Structure



## Federation of Asian and Oceania Pest Managers Associations (FAOPMA)

- ★ The Federation was founded by a number of industrial leaders from Asian countries in 1987.
- ★ The first general meeting and inauguration ceremony of the federation (formerly known as FAOPCA) was held on November 7, 1988 in Tokyo (47 people from eight countries).
- ★ Current country/regional members: Australia, China, Hong Kong, India, Korea, Malaysia, New Zealand, Philippines, Singapore, Taiwan.

## Federation of Asian and Oceania Pest Managers Associations (FAOPMA)

- ★ The 16th FAOPMA Convention and Exhibition was held in Mumbai (Bombay) India on November 25- 26, 2004.
- ★ The 15th FAOPMA meeting was held in Makati, The Philippines, on November 18-20, 2003.
- ★ The 14th FAOPA meeting was held in Yokohama on November 10-12, 2002
- ★ The 17th FAOPMA meeting will be held in Korea in 2005.

## International Congress on Entomology (ICE)

**ICE 2008**

International Congress on Entomology  
Durban, South Africa 2008

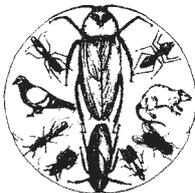
**APCE 2005**

The 5th Asia-Pacific Congress of Entomology in  
Jeju Island, Korea, October 18-21, 2005

## International Conference on Urban Pests (ICUP)

In 1990 several urban entomologists decide that the discipline of urban entomology would benefit from international conference.

A 3-year schedule of conferences was decided following the success of the first conference at Cambridge University in 1993. Past venues: Edinburgh, Scotland (1996), Prague, Czech Republic (1999), Charleston, USA (2002).



The 4th International Conference  
on Urban Pests  
Charleston, South Carolina USA  
July 7-10, 2002

**Topics for this conference will include:**  
Structural Pests (ie, termites, carpenter ants, wood-destroying beetles, etc.)  
Household Pests (ie, cockroaches, ants, etc.)  
Vertebrate Pests (ie, mice, rats & pest birds)  
Vector control and its implications on urban development  
Building, construction and landscaping influences on pests  
Education Technology (ie, long distance learning, internet, etc.)



The 5th International Conference  
on Urban Pests  
Singapore  
July 10-13, 2005

ICUP 2005 Conference Secretariat  
c/o Pacific World Singapore  
73 Bukit Timah Road  
#03 - 01, Rex House, Singapore 229832  
Tel : (65) 6330 6800 Fax : (65) 6336 2123  
Email : info@icup2005.com.sg

### THEME & SCIENTIFIC TOPICS

These are the tentative sessions for this conference :-  
Vector mosquitoes in the urban environment  
Structural and household pests such as termites, ants and cockroaches  
Medical and veterinary important pests  
Pest management initiatives and case studies  
Future developments in pest management  
Regulatory discussions on urban pests and pesticides  
New active ingredients and formulations in the control of vector pests  
Molecular biology applications in urban entomology  
In addition, several workshops on timely topics in urban pest control will also be held during the conference.



### **National Pest Management Association (NPMA)**

- ★ NPMA was founded in 1933. Since then the mission as an Association of Pest Management firms has been to communicate the role of American industry as protectors of food, health, property, and the environment, and affect the success of our members through education and advocacy.
- ★ For over 65 years, the NPMA has represented the interests of its members and the structural pest control industry. Through the efforts of NPMA, the pest control industry is stronger, more professional, and more unified.
- ★ The values which the founders in 1933 shared are still embraced today as membership of NPMA exceeds 4,600 companies from the United States and other countries.

### **Entomological Society of America (ESA)**

- ★ ESA was founded in 1889.
- ★ ESA is the world largest organization serving the professional and scientific needs of entomologists and people in related disciplines.
- ★ ESA today has more than 6,000 members (educators, extension personnel, consultants, students, researchers, and scientist).

### **★International Research Groups and Conferences:**

**IUSSI IRG ICE ICUP**

### **★Asia Pacific Regional Research Groups/Associations:**

**PRTRG FAOPMA**

### **★National Research Groups/Associations:**

**NPMA ESA**

### **Conclusions**

- ★ PRTRG is a unique research group originated from Asia-Pacific region.
- ★ PRTRG is expected to expand its activities due to the high potential of termite management business in the future.
- ★ We must work hard !! to do -----(?)

# Pyruvate and Acetate Metabolism in the Termite

by

Shuji Itakura and Akio Enoki  
Kinki University, Nara, 631-8505, Japan

## Abstract

Using an oxygen electrode, oxygen consumption by the mitochondria from *Nasutitermes walkeri* and *Coptotermes formosanus* during the oxidation of various respiratory substrates was determined. Pyruvate and acetate were the major respiratory substrates in both species. The total activity of the pyruvate dehydrogenase complex (PDHc) in the mitochondria from *N. walkeri* and *C. formosanus* was determined to be  $72.8 \pm 9.0$  and  $8.3 \pm 0.4$  nmol/termite/h, respectively. Mitochondria isolated in the presence of inhibitors of PDHc interconversion were used to determine that about 60% of the PDHc was maintained in the active form in both *N. walkeri* and *C. formosanus*. The sufficient PDHc activity and high rate of pyruvate oxidation in mitochondria from *N. walkeri* suggest that pyruvate is rapidly metabolized. The low mitochondrial PDHc activity of *C. formosanus* suggests that in this species more pyruvate is produced than can be oxidized in the termite tissue.

\*This paper is a condensation of previous study (Itakura et al., 2003).

**Key words:** Pyruvate dehydrogenase complex, Acetate metabolism, Mitochondria

## Introduction

It is now well established that termites produce their own cellulases (Watanabe et al., 1998, Tokuda et al., 1999, Nakashima et al., 2002), whether or not they have cellulolytic protozoa in their hindgut. Cellulolytic protozoa also make cellulases, which are distinct from those produced by the termite (Ohtoko et al., 2000, Watanabe et al., 2002). There is no evidence that glucose produced by the termite or the protozoa is available for the bacteria in the hindgut. There is also no evidence for significant bacterial cellulase activity in any termite (Slaytor, 2000). What do bacteria use for growth and energy? A hypothesis is that pyruvate produced by the termite could be transported into the hindgut for bacterial metabolism (Slaytor, 2000). This explanation of the symbiosis between the termite and its hindgut bacteria had been supported by the absence of activity of the pyruvate dehydrogenase complex (PDHc) (EC 1.2.4.1) in several species (Breznak, 1994, Slaytor et al., 1997), and its low activity in *C. formosanus* (Itakura et al., 1999). It also supported by the high rate of pyruvate oxidation in the ruptured hindgut contents of *N. walkeri* (Slaytor et al., 1997), which suggested that pyruvate was normally available to the hindgut bacteria.

In this study, we have attempted to obtain an overview of energy metabolism in mitochondria of the termites *N. walkeri* and *C. formosanus* by defining the ability of the mitochondria to oxidise a number of substrates including pyruvate and acetate. This has been done by measuring oxygen consumption by mitochondria isolated both from the higher termite *N. walkeri* and the lower termite *C. formosanus* with oxygen electrodes, and by assessing the activity of the PDHc in its active or inactive forms in isolated mitochondria.

## Materials and methods

**Termites:** *Nasutitermes walkeri* (Hill) individuals were collected from mature nests in Greenwich, New South Wales, Australia. *Coptotermes formosanus* Shiraki individuals were collected from a nest that has been maintained in our laboratory for 5 years. Worker caste termites were used for all experiments.

**Preparation of mitochondria:** All operations were carried out at 0-4°C. One hundred termites were immobilized on ice, decapitated and the entire gut removed from each using fine-tipped forceps. The degutted bodies were combined with the heads in 10 ml of isolation buffer (IB: 1 mM EGTA, 5 mM

KH<sub>2</sub>PO<sub>4</sub>, 5 mM MOPS, 300 mM sucrose, and 0.1% BSA, pH 7.4) and homogenised in a loosely fitting Potter-Elvehjem homogeniser, to strip away the exoskeleton. After rinsing the homogeniser with a further 5 ml IB, the extract was centrifuged at 10,000 g for 10 min. Under these conditions, exoskeletal fragments remained in suspension. The pellet was retained and resuspended in 10 ml IB, and homogenized in a Ten Broeck homogeniser prior to differential centrifugation. The tissue homogenate was centrifuged at 800 g for 10 min; the resulting supernatant was centrifuged at 10,000 g for 10 min to generate the mitochondrial pellet. Mitochondria were resuspended by gently swirling in IB at a concentration of approximately 0.6 mg protein/ml for *C. formosanus* or 2 mg for *N. walkeri*.

The citrate synthase activity of the tissue homogenate and the mitochondrial homogenate was used to calculate the recovery of mitochondria for each preparation.

**Measurement of mitochondrial respiration:** All experiments were performed at 30°C. *N. walkeri*; Mitochondrial respiration was measured using Clark-type oxygen electrode (Rank Brothers, Bottisham, Cambridge, UK), with a Perspex incubation chamber and a sample volume of approximately 2.5 ml. This was modified, for low volume experiments, to accept a Perspex sleeve and mini plunger, reducing the minimum sample capacity to 0.8 ml. *C. formosanus*; Mitochondrial respiration was measured using a dissolved oxygen monitoring system with a polarographic electrode (DT-650 DO Meter, Toko Chemical Laboratories, Tokyo, Japan) and a sample volume of 1.0 ml.

Immediately after preparation, 0.1 ml mitochondrial suspensions ( $0.191 \pm 0.006$  mg protein) from *N. walkeri* were transferred to the oxygen electrode containing, 0.2 ml IB, 0.1 ml substrate, and 0.4 ml reaction medium (pH 7.4; 1.5 mM EDTA, 25 mM KCl, 8.5 mM KH<sub>2</sub>PO<sub>4</sub>, 3.0 mM MgCl<sub>2</sub>, 40 mM MOPS). The substrates acetate, acetoacetate, acetylcarnitine, acetyl-CoA plus carnitine, glutamate,  $\alpha$ -glycerophosphate, 3-hydroxybutyrate, isocitrate, malate, palmitoylcarnitine, proline, pyruvate, and succinate were added to a final concentration of 5 mM. Malate was also added after the addition of each substrate except for succinate to a final concentration of 5 mM. After an initial rate was established, representing state 4 respiration, 15  $\mu$ l ADP (as a 10 mM solution) were injected into the cell and the state 3 rate measured. Immediately after preparation, 0.125 ml of mitochondrial suspension ( $0.073 \pm 0.005$  mg protein) from *C. formosanus* was transferred to the oxygen electrode cell containing 0.25 ml IB, 0.125 ml substrates, and 0.5 ml reaction medium. After an initial rate was established, 5  $\mu$ l ADP (as a 5 mM solution) were injected into the cell and the state 3 rate measured.

All rates were presented both as  $\mu$ g-atom O<sub>2</sub>/termite/h for oxygen consumption and as nmol substrate/termite/h for substrate oxidation calculated from the stoichiometry of the oxidation reaction  $\text{CH}_3\text{COCOO}^- + \text{H}^+ + 2.5\text{O}_2 \rightarrow 3\text{CO}_2 + 2\text{H}_2\text{O}$ . Assay of palmitoylcarnitine was done at a final concentration of 0.05 mM due to its detergent-like property resulting in bursting of mitochondria from *N. walkeri* and *C. formosanus* at higher concentration.

**Preparation of enzyme extract:** Tissue homogenate and mitochondria were prepared by the method outlined above. Mitochondrial suspensions in 0.5 ml of IB without BSA were extracted for the assay of enzymes by alternately freezing in liquid nitrogen, and thawing at 30°C, three times.

**Enzyme assays:** All assays were carried out at 30°C. The activity of the PDHc in mitochondrial extracts and tissue homogenate from *N. walkeri* and *C. formosanus* was assayed by following acetyl-CoA production from pyruvate and CoA (Kerbey et al., 1976). Acetate kinase (EC 2.7.2.1), and phosphotransacetylase (EC 2.3.1.8) activities were determined on the mitochondrial extracts and tissue homogenate from *C. formosanus* as described previously (Jones and Lipmann, 1955, Kreuzberg et al., 1985). One unit of PDHc activity is defined as the amount of enzyme that produces 1  $\mu$ mol of acetyl-CoA per min. Citrate synthase (EC 4.1.3.7) activity both of mitochondrial extracts and of tissue homogenate was determined using the method of Srere (1969). Acetyl-CoA synthetase (EC 6.2.1.1) activity was assayed using both crude extracts of the termite tissues and mitochondrial extracts from *N. walkeri* (Szutowicz et al., 1981) or from *C. formosanus* (Rose, 1955).

**Estimation of activity of the PDHc in active form:** Mitochondria used in assessment of PDHc activity were prepared in IB, which contained in addition 0.1 mM dichloroacetophenone (DCAP) and 0.1 mM fluphenazine to inhibit PDH kinase and PDH phosphatase. The mitochondrial preparation was divided into three aliquots: one was assayed for PDHc activity at the time of sacrifice, or active PDH; the other aliquots were twice washed free of inhibitors by dilution in 10 ml IB followed by centrifugation (10,000 g, 10 min). These mitochondria were incubated at 30°C for 15 min in KCl medium (2 mM EDTA, 120 mM KCl, 20 mM Tris-HCl, 5 mM potassium phosphate, pH 7.4) containing either 10 µM carbonylcyanide *m*-chlorophenylhydrazone (*m*CCCP), to determine the maximum or total PDHc activity or otherwise 5 mM succinate, to determine the minimum PDHc activity.

**Reactivation of PDHc:** Mitochondria prepared in inhibitor-free media were incubated, for 10 min at 30°C, in the KCl medium containing 0.5 mM L-malate and 5 mM 2-oxoglutarate to decrease the initial activity of PDHc. Rapid activation of PDHc by endogenous PDH phosphatase was initiated by addition of 10 µM *m*CCCP. Time-course samples were rapidly centrifuged at 16,000 g for 30 s at 4°C and the sedimented mitochondria were frozen in liquid nitrogen. The PDHc activity was assayed in extracts of the frozen mitochondria as described above. Control samples contained 0.1 mM DCAP and 0.1 mM fluphenazine.

**Deactivation of PDHc:** Mitochondria used in these experiments were incubated in the KCl medium for 10 min at 30°C. Rapid phosphorylation of PDHc by endogenous PDH kinase was attained by incubation of termite tissue mitochondria in 3.0 ml KCl medium containing 0.5 mM L-malate and 5 mM 2-oxoglutarate to increase mitochondrial ATP concentration (Kerbey et al., 1976). The inactivation of PDHc was followed as described for reactivation. Control experiments contained 0.1 mM DCAP and 0.1 mM fluphenazine.

## Results and discussion

The rates of oxidation for the key energy substrates are presented in Table 1. Rates are expressed both as oxygen consumption and as substrate oxidation, with the conversion calculated on the basis of the stoichiometry for the complete oxidation of the substrate. Most significant result is the oxidation of pyruvate. Significant PDHc activity has not previously been demonstrated in termite tissue. It is clear from these results that pyruvate and acetyl groups transported as acetylcarnitine are the major respiratory substrates, with both exhibiting a substantial rate of oxidation and good respiratory control. Of the other substrates tested, those which have both low rates of oxidation and poor RCI (respiratory control index) values (<2.0), such as acetoacetate, glutamate, and 3-hydroxybutyrate, suggest that mitochondrial transport is rate-limiting effect with little increase in oxidation on the addition of ADP (data not shown). These substrates may not have specific transporters or could be serviced by low affinity transporters. Proline and  $\alpha$ -glycerophosphate, by contrast, have very high rates of oxidation combined with poor RCI values, with the exception of proline oxidation in *C. formosanus* (data not shown). Unlike the other substrates, with the exception of succinate in both termites, neither requires a functioning tricarboxylic acid cycle for oxidation to occur. The high rate of oxidation in the absence of ADP, and therefore uncoupled from ATP synthesis, illustrated by these results is most likely caused by mitochondrial fragments in the preparation. This apparently futile oxidation, in preparations which gave excellent respiratory control with pyruvate and acetylcarnitine, is the result of the enzymes in these fragments having high turnover numbers and therefore high rates in the presence of ample substrate and a discontinuous membrane. Finally, the oxidation of a long chain fatty acid derivative, palmitoylcarnitine, was investigated. The poor utilization of this substrate is not surprising, as the worker caste termites used in these experiments are foragers, which are collected always in a post-prandial condition. Their core metabolism depends on carbohydrate derived from cellulose and hemicellulose.

Table 1. Substrate utilization by the termite tissue mitochondria of *N. walkeri* and *C. formosanus*.

Substrate	Respiratory control index	ADP/O ratio	Substrate oxidation (nmol/termite/h)	Oxygen consumption ( $\mu\text{g-atomO}_2$ /termite/h)
<i>N. walkeri</i>				
Acetylcarnitine	6.3 $\pm$ 1.5	2.1 $\pm$ 0.1	94.1 $\pm$ 8.2	6.0 $\pm$ 0.5
Acetyl-CoA + carnitine	7.8 $\pm$ 3.7	2.6 $\pm$ 0.4	48.9 $\pm$ 4.1	3.1 $\pm$ 0.3
Isocitrate	3.3 $\pm$ 1.1	2.3 $\pm$ 0.3	9.4 $\pm$ 2.3	1.4 $\pm$ 0.3
Pyruvate	4.2 $\pm$ 0.6	2.5 $\pm$ 0.2	48.8 $\pm$ 5.8	3.9 $\pm$ 0.5
<i>C. formosanus</i>				
Acetylcarnitine	3.2 $\pm$ 0.6	2.3 $\pm$ 0.1	29.7 $\pm$ 5.4	1.9 $\pm$ 0.3
Acetyl-CoA + carnitine	2.6 $\pm$ 0.2	3.3 $\pm$ 0.4	24.3 $\pm$ 2.6	1.6 $\pm$ 0.2
Palmitoylcarnitine	2.7 $\pm$ 0.3	3.2 $\pm$ 1.1	2.8 $\pm$ 0.5	1.5 $\pm$ 0.3
Pyruvate	2.7 $\pm$ 0.4	2.1 $\pm$ 0.2	15.8 $\pm$ 2.4	1.3 $\pm$ 0.2

The successful demonstration of pyruvate oxidation by isolated mitochondria was supported by the first demonstration of significant PDHc activity in termite mitochondria extracts. The extracts from *N. walkeri* and *C. formosanus* contained PDHc activities of 72.9  $\pm$  9.0 nmol/termite/h and 8.3  $\pm$  0.4 nmol/termite/h, respectively, after correcting for loss of mitochondria based on citrate synthase activities in the mitochondrial suspension and tissue homogenate. These were much higher activities than those previously reported (Itakura et al., 1999). In both species, the PDHc activity is maintained at about 60% of its maximal value. A successful assay in the whole tissue homogenates, however, remains elusive. Isolation of intact mitochondria followed by extensive washing to remove endogenous protease activity before homogenization could be a key factor in protecting PDHc activity, because  $\alpha$  subunit of PDH ( $E_1$ ) and the subunit of dihydrolipoyl transacetylase ( $E_2$ ) are sensitive to proteolysis by endogenous protease (Pettit and Reed, 1982). The poor PDHc activity in the whole tissue homogenate also suggested that the PDHc may be activated during mitochondrial isolation by substrate depletion, even at low temperature. This necessitated the assessment of inhibitors of PDH kinase and PDH phosphatase to freeze PDHc activity during mitochondrial isolation and allow assessment of the activity in freshly collected termites.

A normal worker caste termite of *N. walkeri* produces 88.4  $\pm$  2.3 nmol/termite/h of CO<sub>2</sub> indicating that glucose is being produced at about 15 nmol/termite/h that from this pyruvate is being produced at about 30 nmol/termite/h (Slaytor et al., 1997). The present study shows that there is sufficient PDHc activity to oxidize about 44 nmol/termite/h; the low concentration of pyruvate in the haemolymph (0.06 nmol/termite/h) suggests that pyruvate is rapidly metabolized. The situation is different in *C. formosanus*, which has cellulolytic protozoa in its hindgut. At 25°C, worker caste termites produce 39.4  $\pm$  1.9 nmol/termite/h of CO<sub>2</sub> (Shelton and Appel, 2001), indicating a maximum rate of pyruvate of about 13 nmol/termite/h. The low activity of the PDHc in the termite tissue, 4.8  $\pm$  0.3 nmol/termite/h, suggests that about 60% of the pyruvate could be produced by the symbionts.

Table 2. Activity of pyruvate dehydrogenase complex (PDHc) in termite tissue mitochondria of *N. walkeri* and *C. formosanus*.

	Active PDHc	Total PDHc (nmol/termite/h)	Minimum PDHc
<i>N. walkeri</i>	43.7 $\pm$ 4.1	72.9 $\pm$ 9.0	25.9 $\pm$ 1.8
<i>C. formosanus</i>	4.8 $\pm$ 0.3	8.3 $\pm$ 0.4	0.2 $\pm$ 0.1

The effectiveness of inhibitors in preventing change in PDHc activity was examined in termite tissue mitochondria of *N. walkeri* under conditions designed to stimulate either the rapid phosphorylation or dephosphorylation of PDHc. The PDHc of freshly prepared mitochondria, inactivated by preincubation with high substrate (5 mM 2-oxoglutarate/0.5 mM malate or 5mM succinate), was reactivated by energy depletion with the uncoupler *m*CCCP. Incubation of mitochondria with *m*CCCP depletes mitochondrial ATP concentration and allows complete conversion of inactive PDHc to active PDHc by endogenous

PDH phosphatase (Denyer et al., 1991). Conversely, the PDHc in energy depleted mitochondria, washed of substrate and preincubated in the absence of substrate, was deactivated by the addition of high levels of substrate (5 mM 2-oxoglutarate /0.5 mM malate). Substrate oxidation leads to an increase in mitochondrial ATP concentration and promotes conversion of active PDHc to inactive PDHc by endogenous PDH kinase (Kerbey et al., 1976). Fluphenazine strongly inhibited the reactivation of PDHc by PDH phosphatase at 0.1 mM with no increase of effectiveness at 1 and 10 mM. DCAP was only partially effective in preventing the inactivation of PDHc by PDH kinase at 0.01 mM but successfully inhibited at a concentration of 0.1 mM. Higher concentrations, 1 and 10 mM, caused significant inhibition of PDHc activity, which was reduced to 10% of normal at 10 mM. In both PDHc reactivation and inactivation experiments baseline activity was, in consequence, maintained in controls through the use of an inhibitor cocktail containing 0.1 mM DCAP and 0.1 mM fluphenazine. The time-course for activation and deactivation of PDHc in the termite tissue mitochondria of *N. walkeri* plainly indicate the effectiveness of the inhibitors and allow the determination of the range of PDHc activity in termites. Thus, termite mitochondria prepared in the presence of these inhibitors allowed the maintenance of initial activity during the preparation and thus the determination of the level of activity in termite tissues, the active PDH, at the time of collection (Table 2). In addition, exposure of these mitochondria to either activating or deactivating conditions allowed the measurement of both the total and minimum PDH activities (Table 2).

The activity of acetyl-CoA synthetase was  $37.7 \pm 8.3$  nmol/termite/h in the termite tissue homogenate of *N. walkeri*. Activity was not detected in the mitochondria, which is unusual as this is typically a mitochondrial enzyme. The rapid oxidation of externally provided acetyl-CoA and carnitine by isolated mitochondria of the termite suggests a route of uptake for acetate derived from gut symbionts. Acetate must be activated in the cytosol and transported into the mitochondria as acetylcarnitine. On the contrary, the whole activity of acetyl-CoA synthetase ( $183.3 \pm 36.7$  nmol/termite/h) was detected in the mitochondria, whereas no activity was found in the termite tissue homogenate of *C. formosanus*.

The activity of acetate kinase in the termite tissue homogenate of *C. formosanus* was  $4.4 \pm 0.3$  nmol/termite/h, while this activity was not detected in the mitochondria. No activity of phosphotransacetylase, which is capable of converting acetyl phosphate to acetyl-CoA, was detected in either the termite tissue or the mitochondria of *C. formosanus*. Thus, acetyl-CoA could not be produced from acetate via acetyl phosphate by the combined action of acetate kinase and phosphotransacetylase in cytosol of the termite tissue of *C. formosanus*.

In both species, there is a high concentration of acetate, 14.7 nmol/termite in the hindgut of *N. walkeri* (Slaytor et al., 1997) and 8.6 nmol/termite in *C. formosanus* (Itakura et al., 1997). There are clear differences in the way acetate is metabolised in the two species. In *N. walkeri*, the high activity of acetyl-CoA synthetase in the termite tissue and the high rate of oxidation of acetylcarnitine (or acetate plus carnitine) by isolated mitochondria indicates that this is how acetate is oxidised in this termite (Fig. 1a). The absence of both acetyl-CoA synthetase and phosphotransacetylase activities in tissue homogenate of *C. formosanus* means that acetate cannot be converted into acetyl-CoA in the cytosol either by acetyl-CoA synthetase or by acetate kinase and phosphotransacetylase. The mechanism for cytosolic acetyl-CoA production in *C. formosanus* is unknown, but the high activity of mitochondrial acetyl-CoA synthetase ( $183.3 \pm 36.7$  nmol/termite/h) in *C. formosanus* suggests that termite mitochondria have the potential to oxidise acetate at a high rate, once it has been transported into the mitochondria by a high affinity carrier (Fig. 1b), as well as the possibility of acetate transport by the monocarboxylate carrier, needs to be examined.

The potential mitochondrial activity of pyruvate and acetate metabolism in termite tissues in *N. walkeri* does not support a mechanism for sparing pyruvate for bacterial use in the hindgut. Clearly the high activities of pyruvate and lactate metabolizing enzymes in the hindgut contents are consistent with pyruvate being available, but the mechanism by which pyruvate could enter the hindgut is not known. In *C. formosanus*, the low mitochondrial activity of the PDHc could be a pyruvate sparing mechanism for providing bacteria with pyruvate. If the bacteria in this species use pyruvate or lactate, it is not likely to be provided by the protozoa which in *Zootemopsis* sp. metabolise cellulose to acetate, hydrogen, and

carbon dioxide (Yamin, 1981). There are clear differences in mitochondrial metabolism between two species studied, but it is not yet possible to explain the symbiosis between the termite and its microorganisms in terms of the differences in pyruvate and acetate metabolism.

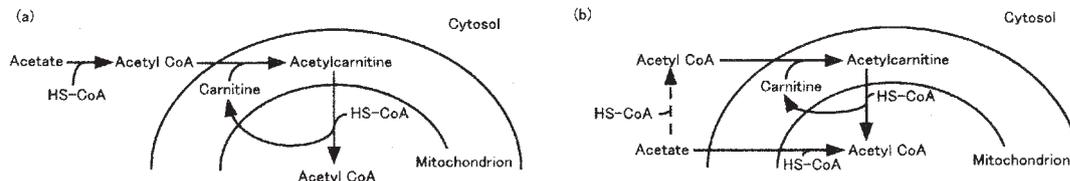


Fig. 1. Utilisation of acetate by *N. walkeri* (a) and *C. formosanus* (b).

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# Culture-Independent Approaches for Exploration of Termite-Microorganisms Symbiotic Systems

by

Tetsushi Inoue<sup>1,2</sup>, Shigeharu Moriya<sup>2,3</sup>, Moriya Ohkuma<sup>1,2</sup>, and Toshiaki Kudo<sup>2,3</sup>

<sup>1</sup>JST PRESTO <sup>2</sup>Environmental Molecular Biology Lab., RIKEN, Wako, Saitama 351-0198, Japan

<sup>3</sup>Graduate School of Yokohama City University, Yokohama 230-0045, Japan

## Abstract

The relationship between xylophagous termites and their intestinal protists is one of the most famous examples of symbiosis. Progress in understanding the symbiosis in termites has been greatly accelerated by culture-independent approaches using the molecular biological techniques during the last decade. It was demonstrated that symbiotic protists possess diverse cellulase genes, which belong to glycosyl hydrolase family (GHFs) 5, 7, and 45, suggesting that symbiotic protists of termites are rich reservoirs of novel cellulase genes. A close relationship among bacterial cellulases, animal cellulases, and the protistan cellulase was found in the phylogenetic tree of GHF5, suggesting that the symbiotic protist acquired the cellulase gene by horizontal transfer from a prokaryote. This hypothesis might open a new way for understanding the origin of symbiosis or evolutionary process of symbiotic relationships.

**Key words:** protist, symbiosis, glycosyl hydrolase, cellulase, cDNA library, in situ hybridization

## Introduction

Termites are super-abundant soil animals and play an important role in the process of litter decomposition in tropical terrestrial ecosystems. The relationship between xylophagous termites and their intestinal protists is one of the most famous examples of symbiosis. Symbiosis found in termite guts has fascinated biologists for a long time, particularly following the discovery of the cellulolytic properties of the flagellated protists in lower termites. Despite the critical role of symbiotic flagellates for the survival of lower termites on a diet of sound wood or filter paper, it has proved difficult to define the role of protists in the symbiosis. This is because pure protist cultures are very difficult to establish and only a few species have been axenically cultured (reviewed in Inoue et al., 2000). Symbiotic flagellates found in the hindgut of the lower termites belong to the orders Trichomonadida, Hypermastigida, and Oxymonadida (Yamin, 1979). Trichomonadida and Hypermastigida are classified into the class Parabasalea or into the phylum Parabasalia.

During the last decade, culture-independent approaches using the molecular biological techniques, such as PCR, in situ hybridization, have provided a remarkable advance in exploring symbiotic systems. Recently we constructed a cDNA library from the mixed population of protists in the hindgut of the subterranean termite, *Coptotermes formosanus*, to elucidate the diversity of cellulase genes of symbiotic protist origin using a culture-independent approach (Inoue et al., 2005).

Here we summarize the progress in exploration of diverse cellulase genes in symbiotic systems using the culture-independent approaches.

## Culture-independent approaches

Analysis of a cDNA library allows exploring functional genes expressed by symbiotic protists without pure culture. The cDNA library is constructed using mRNA extracted from the mixed population of protists in the hindgut of termites. To isolate functional genes, the cDNA library is screened by hybridization with a specific probe for the target gene or enzyme activities.

The organismal source of the specific gene can be identified by PCR with gene-specific primers using isolated protists as a template. Whole-cell in situ hybridization with the specific probe allows confirming the origin of the gene.

Moreover, the isolated gene can be heterologously expressed in *Escherichia coli* and the properties of the recombinant protein can be determined.

The schematic strategy, which we employed for isolation and characterization of a cellulase gene from a symbiotic protist of *C. formosanus*, is shown in Fig 1.

### ***Cellulase genes found in symbiotic systems***

Glycosyl hydrolases, including cellulases, show great multiplicity of resulting from the extensive variety of carbohydrate structures. In contrast to the conventional classification of enzymes based on the type of reactions catalyzed and substrate-specificity, a classification of glycosyl hydrolases into families based on amino acid sequence similarities was introduced to integrate both structural and mechanistic features of these enzymes (Henrissat, 1991). Over the years, the number of glycosyl hydrolase families (GHFs) has grown steadily and currently there are 85 families (Bourne and Henrissat, 2001). This classification scheme is considered to take into account evolutionary events such as divergence or convergence (Henrissat, 1991).

Despite the critical role of symbiotic flagellates in cellulose digestion by lower termites, the first sequenced cellulase gene was of termite origin (Watanabe et al., 1998). The presence of two separate cellulolytic systems in the lower termite is now well established (Nakashima et al., 2002a,b) and this dual system seems to result in the high assimilation rate (greater than 90%) of wood glucan by termites (Breznak and Brune, 1994). The endogenous cellulases of termites were classified into GHF9 and their homologues were also found in cockroaches and decapods (Byrne et al., 1999; Lo et al. 2000).

On the other hand, diverse genes encoding cellulase homologues belonging to GHF45 were identified from the symbiotic parabasal protists of the termite, *Reticulitermes speratus* (Ohtoko et al., 2000) and *Mastotermes darwiniensis* (Li et al., 2003). Furthermore, cellulase components were isolated from the hindgut of the termites, *Coptotermes* spp., and the isolated sequences showing similarity to catalytic domains of GHF7 members were obtained from the symbiotic protists, *Pseudotriconympha grassii* and *Holomastigotoides mirabile* (Nakashima et al., 2002a; Watanabe et al., 2002). Recently we isolated the cellulase gene, which showed high sequence similarity with endoglucanase genes belonging to GHF5, from the symbiotic parabasal protist, *Spirotrichonympha leidyi* in the hindgut of *C. formosanus* (Inoue et al. 2005).

In addition to the GHF45 and GHF7 cellulase genes, the presence of the GHF5 cellulase gene in the symbiotic parabasal protist suggests that symbiotic protists of termites are rich reservoirs of novel cellulase genes. Considering the substantial activity of xylanase that was found in the hindgut of the lower termite (Inoue et al., 1997), much more diverse glycosyl hydrolases are expected to exist in symbiotic protists. A 'clan' of GH families is defined as having a common ancestry and significant similarities in tertiary structure, which is better conserved than is sequence similarity (Henrissat and Bairoch, 1996). GHF5 and GHF7 are classified into clans GH-A and GH-B, respectively. So far, GHF45 is not classified into any clan. The diversity of cellulases isolated from parabasal protists was recognized from the viewpoint of clan classification.

The general architecture deduced for cellulase features two independent globular modules: a cellulase catalytic domain responsible for the hydrolysis reaction itself and a cellulose-binding module (CBM) which is devoid of catalytic activity but promotes adsorption of the enzyme onto insoluble crystalline cellulose. It is worth noting that no cellulases isolated from parabasal protists possess CBM regardless of GHF classification. In the hindgut of termites, wood components are endocytosed by symbiotic protists and degraded within food vacuoles (Yoshimura et al., 1996). Selective endocytosis of cellulose from mixtures containing other solid materials was demonstrated by the observation of *T. agilis* in *R. speratus* (Yamaoka, 1979). One of the roles of CBM is considered to be enhancement of enzyme activity by increasing the concentration of the enzyme at the surface of the polysaccharide. High enzyme concentrations occurring around the substrate within the food vacuoles as a result of selective endocytosis of protists may compensate for the lack of CBM in protistan cellulases.

### ***Evolutionary origin of the GHF5 protistan cellulase***

Ancient horizontal gene transfers are suggested to be responsible for the acquisition of cellulase and other glycosyl hydrolase genes. Evidence for the interspecific transfer of cellulase genes belonging to GHF8 between bacteria has been reported (Guiseppe et al., 1991). A *Ruminococcus*-like xylanase gene belonging to GHF11 was found in a rumen fungus, suggesting a bacteria-to-eukaryotic gene transfer (Gilbert et al., 1992). In the case of GHF5, the gene transfer of the endoglucanase from the rumen bacteria *Fibrobacter succinogenes* to the rumen fungi *Orpinomyces joyonii* was strongly suggested by combination analyses of G+C content and patterns

of codon usage for the construction of phylogenetic trees (Garcia-Vallvé et al., 2000). Furthermore, GHF5 cellulase genes from plant-parasitic nematodes show strong sequence similarity to prokaryotic cellulases and no significant sequence similarity with nematode cellulases observed in *Caenorhabditis elegans* at either the nucleic acid or amino acid levels (Yan et al., 1998). Thus, the acquisition of cellulase genes by a horizontal transfer was implied to have occurred before the divergence of these plant-parasitic nematodes.

There is a widespread perception that horizontal gene transfers between genomes of different organisms are exceptional and idiosyncratic phenomena. Recent accumulation of microbial genome data provides an exiting view in an evolutionary process. There is growing evidence that horizontal gene transfer is a very important mechanism in genome evolution, particularly among prokaryotes and mechanisms of horizontal gene transfer between prokaryotes are relatively well understood (Lawrence, 1999). Although the process of transfer between prokaryotes and eukaryotes is not well characterized, some hypotheses have been proposed.

Unicellular eukaryotes use prokaryotes as food and are therefore constantly exposed to prokaryotic DNA (Doolittle, 1998). Indeed, *Trichomonas vaginalis*, a parabasal protist, undoubtedly acquired its *N*-acetylneuraminidase lyase gene from bacteria in its environment (de Koning et al., 2000). The pathogenic protist, *Entamoeba histolytica* possesses fermentation enzymes that must have been acquired from different anaerobic prokaryotes and *Giardia lamblia* encodes an enzyme that is unquestionably of bacterial origin (Field et al., 2000; Boucher and Doolittle, 2000).

Strong sequence similarity of the GHF5 protistan cellulase to prokaryotic cellulases and the characters of unicellular eukaryotes lead us to hypothesize that the symbiotic protist acquired the cellulase gene by horizontal transfer from a prokaryote. A close relationship among bacterial cellulases, animal cellulases, and the protistan cellulase was also found in the phylogenetic tree of GHF5 subfamily 2, though the available data is currently insufficient to make a firm statement about the likelihood of horizontal gene transfer or monophyly of cellulase genes of eukaryote origin (Inoue et al., 2005).

## Conclusions

Symbiotic protists in the lower termites proved to possess diverse cellulase genes, which belong to GHF 5, 7, and 45 by culture-independent approaches, suggesting that symbiotic protists of termites are rich reservoirs of novel cellulase genes. Phylogenetic analysis of the protistan cellulase belonging to GHF5 and the characters of unicellular eukaryotes strongly suggested that the symbiotic protist acquired the cellulase gene by horizontal transfer from a prokaryote, which may give insights into the origin of the symbiosis between termites and their gut protists.

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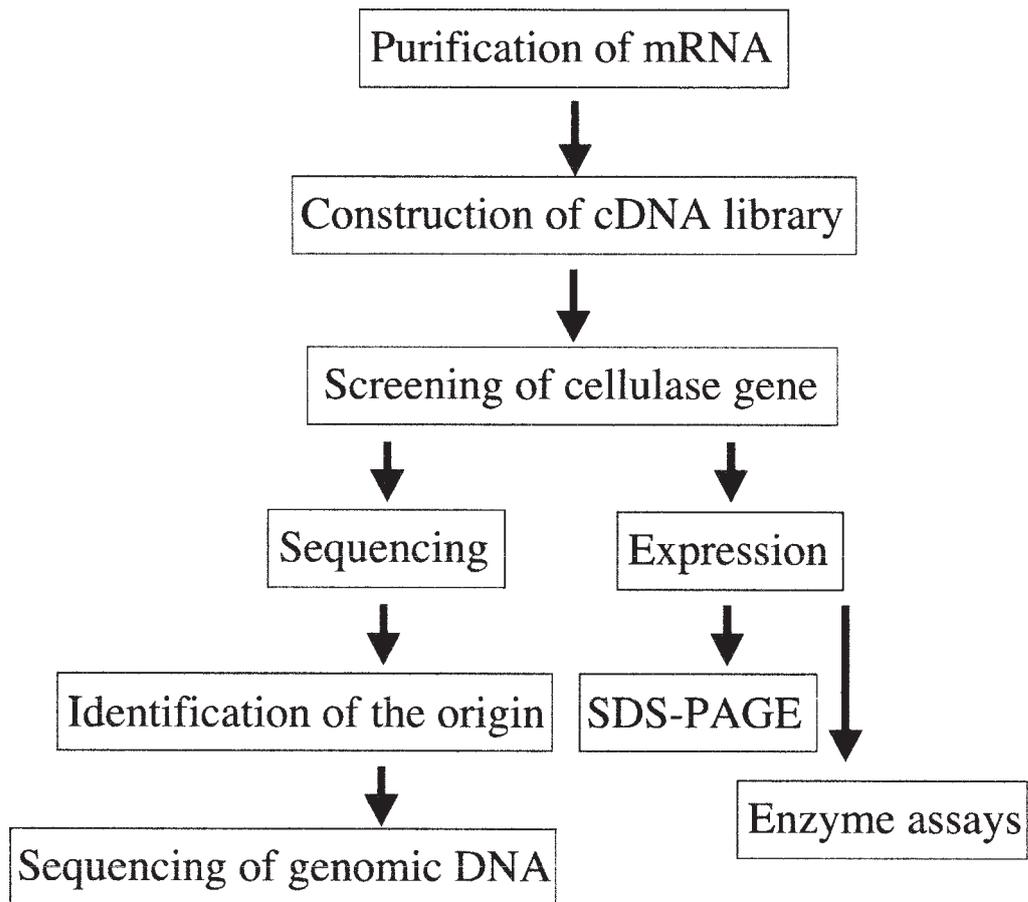


Fig.1. Culture-independent strategy for isolation and characterization of a cellulase gene from a symbiotic protist of a lower termite.

# Cuticular Hydrocarbons of Japanese *Reticulitermes* as Taxonomic and Distributional Indicators of Species

by

Yoko Takematsu and Hiroki Yasumoto  
Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8515, Japan

## Abstract

The cuticular hydrocarbons of *Reticulitermes* from the Ryukyu Archipelago and Japanese mainland were analyzed, identified and quantified. Four groups were recognized from the clustering analysis of cuticular hydrocarbon components, and these groups were corresponded with the morphological and distributional data. Furthermore the same four groups were also recognized from the clustering analysis using 15 selected components. It appears that there are several key cuticular hydrocarbons for grouping these taxa.

**Key words** : cuticular hydrocarbons, chemotaxonomy, *Reticulitermes*, GC-MS, clustering analysis

## Introduction

Classification of termites has been conventionally made by morphological and morphometrical methods. However, these methods have some drawbacks, because a broad variation often exists in a given key morphological characters. Many researchers have reported that cuticular hydrocarbons act as cues for species and colony recognition in termites and ants, and applied cuticular hydrocarbons for taxonomic characters (such as Howard 1993; Haverty *et al.* 1996, and Takematsu & Yamaoka 1999). Recent progress of the analytic technique made it possible to detect a very small amount of substances and therefore we have to be attentive in understanding the results; to understand whether each substance has a real meaning or mere “noise”.

The genus *Reticulitermes* (Holmgren) is one of the serious pests of wood and widely distributed in the northern temperate zone, and in Japan it occurs from Hokkaido to the Ryukyus. Takematsu & Yamaoka (1999) analyzed cuticular hydrocarbons of termites in Japan and recognized seven species. Although they clearly distinguished *R. speratus* of Japanese mainland from other species of the Ryukyu Archipelago, the detailed analyses of morphology and cuticular hydrocarbons of *Reticulitermes* from of the Ryukyu Archipelago have not been achieved yet.

In this paper, we made a detailed analysis of all the cuticular hydrocarbons of *Reticulitermes* from seven islands of the Ryukyu Archipelago, and tried to classify these samples and decide the controlling substances for the grouping of these samples.

## Materials and Methods

### *Termites*

*Reticulitermes* colonies were collected from 7 islands of the Ryukyu Archipelago in 2003-2004 (Fig. 1). *R. speratus* was sampled from Yamaguchi Prefecture, Japan.

### *Morphological identification*

In the present study, identification of species was done on the basis of morphological criteria according to Takematsu (1999). Observations were made in 80% ethanol.

### *Chemical methods*

Fifteen samples from *Reticulitermes* of 7 islands and *R. speratus* were analyzed. Cuticular hydrocarbons were extracted by immersing 100 workers in 2 ml of *n*-hexane for 5 min. After evaporation of the hexane, the extracts were redissolved in 1 to 10  $\mu$ l of *n*-hexane for gas chromatography-mass spectrometry (GC-MS). It was performed on a Shimadzu QP5000 mass

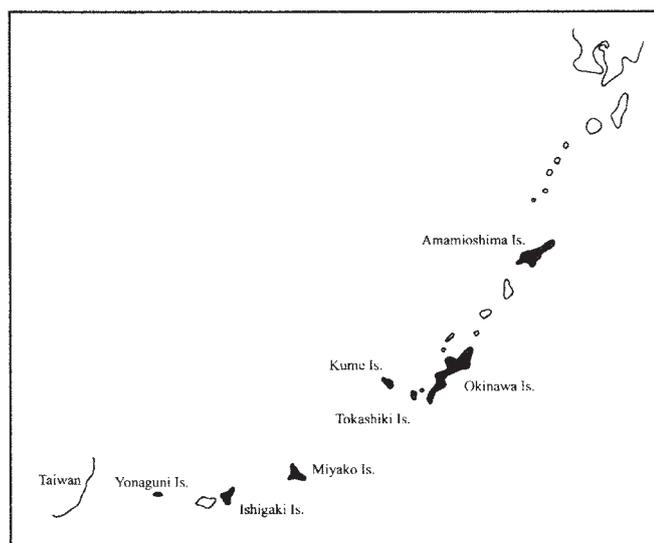


Fig.1 Sampling points of *Reticulitermes* from the Ryukyu Archipelago

spectrometer. The ion-source temperature was 280°C, and the ionization energy was 70 eV. The injection temperature was 280°C. Samples in 1 µl of hexane were injected with a split less injector. The oven temperature was kept at 60°C for the first 2 minute and then raised at 10°C/min to 280°C, and a final hold for 20 min. The even number *n*-alkanes (n-20 to n-34) were used as the standards to identify the cuticular hydrocarbons comparing with their retention times and mass spectra. The results were recorded on the CHC-matrix (cuticular hydrocarbon component/ratio table), and computed the variance of each substance.

The data of cuticular hydrocarbons of 15 samples were compared using cluster analysis. Clustering analyses with cuticular hydrocarbon component/ratio data were conducted using a “R” software (<http://www.r-project.org/>) by UPGMA (unweighted pair-group method) with Euclidean coefficient. The clustering analysis and principle component analysis were conducted with 15 substances of large variance.

## Results and discussion

### *Identification and distribution of Reticulitermes*

Sixty-six colonies were collected from 7 islands. According to Takematsu (1999), samples from Okinawa Is., Kume Is. and Tokashiki Is. were identified as *R. okinawanus*. Those of Ishigaki Is. were *R. yaeyamanus*. Those of Yonaguni Is. were *R. flaviceps*. Those of Miyako Is. did not agree with any species, *R. sp.* Those from Amamioshima Is. consisted of 2 species, *R. miyatakei* and *R. amamianus* (Fig.1).

### *Cuticular hydrocarbons of Reticulitermes in the Ryukyu Archipelago*

All substances were identified, and their relative amount of each samples and the variance of each substance were calculated. A total of 82 components were identified and 68 components of them were hydrocarbons. Chain-lengths of cuticular hydrocarbons ranged from C23 to C31. They were grouped into 3 major classes: *n*-alkanes, olefins and methylalkanes. Twenty five components were commonly found in all samples. If all the identified hydrocarbon components were taken to account (irrespective of their quantity), 15 samples were found to be different in some “micro components” of very small quantity. This is seemingly inconsistent with the previous results of Takematsu & Yamaoka (1999) that the same species from the same island had the same components. Therefore, it is important to decide whether those “micro components” have a real meaning or are mere “noise” and can be discarded.

Table 1. Cuticular hydrocarbons of Japanese *Reticulitermes* and variances of component ratio.

No.	ECL	Components	Diagnostic ions	Variance	No.	ECL	Components	Diagnostic ions	Variance
1	23.00	n-C23	324	13.22	35	27.20	C27:3(coj)	374	0.33
2	23.20	11-meC23	197	0.17	36	27.35	11-meC27	169,253	16.69
3	23.60	4-meC23	295	0.56	37	27.39	C27:2(coj)	376	0.34
4	23.70	3-meC23	309	0.07	38	27.40	7-meC27	309	0.11
5	24.00	n-C24	338	3.13	39	27.50	5-meC27	337	0.11
6	24.37	12-meC24	183,197	0.01	40	27.64	11,13,15-trimeC27	169,197,211,239	0.03
7	24.46	methyl		-	41	27.70	3-meC27	365	0.11
8	24.55	methyl		-	42	27.90	6,10,17-trimeC27	169,267,351	0.47
9	24.60	4-meC24	309	4.64	43	27.98	olefine		0.02
10	24.70	3-meC24	323	0.74	44	28.00	n-C28	394	3.34
11	24.82	C25:1	350	0.02	45	28.33	C29:3	402	0.04
12	24.87	olefine		-	46	28.45	C29:3	402	1.03
13	24.95	olefine		0.07	47	28.52	C29:2	404	3.37
14	25.00	n-C25	352	23.09	48	28.59	C29:2	404	0.38
15	25.40	13-meC25	197	1.61	49	28.71	methyl		0.03
16	25.43	methyl		0.05	50	28.78	C29:2	404	3.04
17	25.50	5-meC25	309	0.18	51	28.80	olefine		0.01
18	25.60	4-meC25	323	0.27	52	28.85	C29:1	406	41.18
19	25.70	3-meC25	337	3.75	53	29.10	C29:2(coj)	404	0.03
20	25.91	methyl		3.89	54	29.17	olefine		0.08
21	25.50	C25:3(coj)	346	0.03	55	29.20	11-meC29	169,281	8.98
22	26.00	n-C26	366	3.73	56	29.24	olefine		-
23	26.11	C25:3(coj)	346	0.53	57	29.32	methyl		0.03
24	26.16	C27:3	374	0.08	58	29.38	methyl		0.01
25	26.38	11,13-dimeC26	169,197	0.21	59	29.40	6-meC29	351	4.01
26	26.41	olefine		0.90	60	30.40	C31:1	434	0.42
27	26.50	C27:2	376	0.09	61	30.48	C31:3	430	0.67
28	26.58	C27:2	376	0.13	62	30.53	C31:2	432	14.92
29	26.60	4-meC26	337	31.02	63	30.71	olefine		0.16
30	26.69	C27:1	379	25.94	64	30.73	olefine		0.02
31	26.77	olefine		0.05	65	30.80	olefine		2.63
32	26.82	olefine		-	66	31.10	methyl		0.02
33	26.89	olefine		0.03	67	31.19	olefine		-
34	27.00	n-C27	380	1.28	68	31.30	11-meC31	169,309	0.12

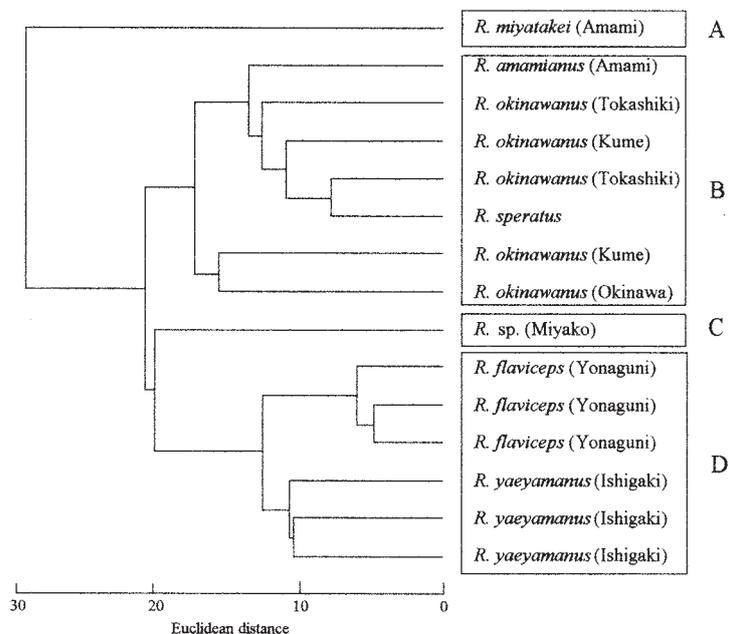


Fig.2 Clustering analysis of 15 samples based on 68 components.

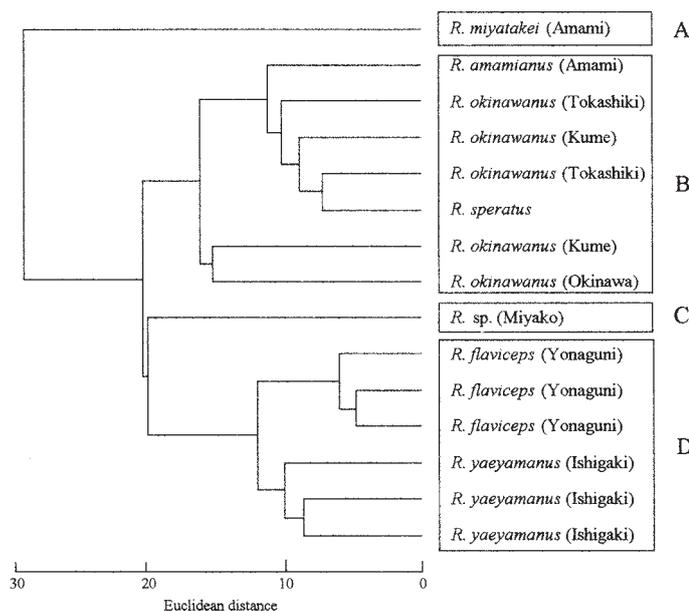


Fig.3 Clustering analysis of 15 samples based on 15 components.

The morphological and distributional results of identification corresponded with the grouping at similarity value 30. We recognized 4 groups. Group-A consists of *R. miyatakei*. *R. miyatakei* is easily distinguished from other species morphologically and this result supports the fact. Group-B consists of all *R. okinawanus* samples, *R. amamianus* and *R. speratus*. Distributional area of these three species is north of Okinawa Is. Group-C consists of *R. sp.* from Miyako Is. It is clear that the sample belongs to neither *okinawanus* nor *yaeyamanus*. Group-D consists of *R. yaeyamanus* and *R. flaviceps*, and divided into two sub-clusters. These two species distribute only Yaeyama-area, the southmost part of Ryukyu Archipelago.

#### Cluster analysis and PCA using the 15 selected hydrocarbons

We tried to select the cue components according to the largeness of variance. Then the variance of each component were calculated (Table 1) and selected 15 components; n-C23, 4-meC24, n-C25, 3-meC25, n-C26, 4-meC26, C27:1, 11-meC27, n-C28, C29:2, C29:1, 11-meC29, 6-meC29 and C31:2 (variance were 3.34 - 41.18). Fig.3 shows a dendrogram constructed based on the selected 15 hydrocarbons. It is of great interest to note that the result based on the 15 selected hydrocarbons gives the same dendrogram with 62 hydrocarbons.

Fig. 4 shows the result of principle component analysis of 15 samples using the 15 selected components. Both axes the first principal component (PC1) and the second one (PC2) expressed the value 65%. PC1 and PC2 accounted for 43.3% and 21.2% of total variance, respectively. Four groups shown by cluster analyses were easily recognized in Fig.4. Group-A showed the unique values and was defined by C29:1, which has the largest variance 41.18. Group-B was defined by n-C25. Group-D was not defined by 4-meC26 and C27:1.

Although the classification of species was ambiguous with respect to “micro components”, we here managed to recognize 4 groups on the basis of some major selected components. To ensure the present conclusions, further detailed analyses for each substance will be needed.



## Feeding Behavior of *Incisitermes minor* (Hagen)

Yuliati Indrayani<sup>1)</sup>, Tsuyoshi Yoshimura<sup>1)</sup>, Yoshiyuki Yanase<sup>2)</sup>, Yoshihisa Fujii<sup>2)</sup>,  
Hiroaki Matsuoka<sup>3)</sup>, Yuji Imamura<sup>1)</sup>

- 1) *Research Institute for Sustainable Humanosphere, Kyoto University, Gokasho, Uji, Kyoto 611-0011, Japan*
- 2) *Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan*
- 3) *Hygienic Pesticide and Wood Preservative Development Group, Development Department, Sankyo Lifetech Co.,Ltd. Tokyo 140-8710, Japan*

### Abstract

The feeding behavior of dry-wood termite *Incisitermes minor* (Hagen) was observed using a high-magnification Charge Couple Device (CCD) camera and an Acoustic Emission (AE) detector. Two species of subterranean termites, *Coptotermes formosanus* Shiraki and *Reticulitermes speratus* (Kolbe), were also subjected to the same observations for comparison. Results indicated that the feeding behavior of *I. minor* and *C. formosanus* consisted of cutting, pulling and collecting activities, while *R. speratus* showed only cutting and pulling activities. The largest peak to peak (p-p) amplitude of AE waves was noted in the pulling activity, followed by the cutting and collecting activities, respectively. Among these three species, *I. minor* appeared to have the highest p-p amplitude, due to having the largest body and mandible size of the three species studied.

**Key words:** Feeding behavior, *Incisitermes minor* (Hagen), CCD camera, Acoustic Emission (AE)

### Introduction

*Incisitermes minor* (Hagen), a dry-wood termite native to the western region of the United States (Weesner, 1970), is known as the western dry-wood termite. The infestation of the exotic dry-wood termite *I. minor* has been reported in Japan (Mori, 1976; Yasuda *et al.*, 2003; Yamano, 1998).

Since wood is the termites' food source, studying their feeding behavior is crucial for helping to develop methods of preventing termite attacks. Our research group has already reported on the feeding behavior of two Japanese subterranean termites, *Coptotermes formosanus* Shiraki and *Reticulitermes speratus* (Kolbe), and a Japanese dry-wood termite *Cryptotermes domesticus* (Haviland) (Imamura *et al.*, 1991; Fujii *et al.*, 1995; Matsuoka *et al.*, 1996). However, there has been no research on the feeding behavior of the pest dry-wood termite *I. minor*. The present study was thus conducted to observe the feeding behavior of *I. minor* using a high-magnification Charge Coupled Device (CCD) camera and an AE detector, and to compare the results with those obtained on *C. formosanus* and *R. speratus*.

### Materials and methods

#### Termites

Nymphs of *I. minor* and workers of both *C. formosanus* and *R. speratus* were used as test organisms. *I. minor* nymphs were collected from infested trees in Wakayama Prefecture. *R. speratus* workers were collected from a field colony at the Uji branch campus of Kyoto University (Uji City, Kyoto Prefecture). *C. formosanus* workers were obtained from a laboratory colony maintained at the Research Institute for Sustainable Humanosphere at Kyoto University.

#### Wood specimens

Sapwood specimens of Yezo spruce (*Picea jezoensis* (Sieb. et Zucc.) Carr.) measuring 20 (R) x 20 (T) x 10 (L) mm were used for the feeding tests. All specimens were air-dried prior to the tests. One specimen per test insect was used. A rectangular hole of about 6 (wide) x 12 (long) x 4 (deep) mm was made on the surface of each air-dried specimen to observe the feeding behavior of *I. minor*. For *C. formosanus* and *R. speratus*, several drops of water were pipetted into a rectangular hole that was about 5 (wide) x 10 (long) x 3 (deep) mm before placing a worker into the hole.

### CCD camera

A compact color CCD camera (KEYENCE, VH-6110, Osaka, Japan) with a valuable magnification lens of 25X – 175X was used for the observations. The pictures taken were transferred to a monitor and recorded with a video recorder (Fig. 1).

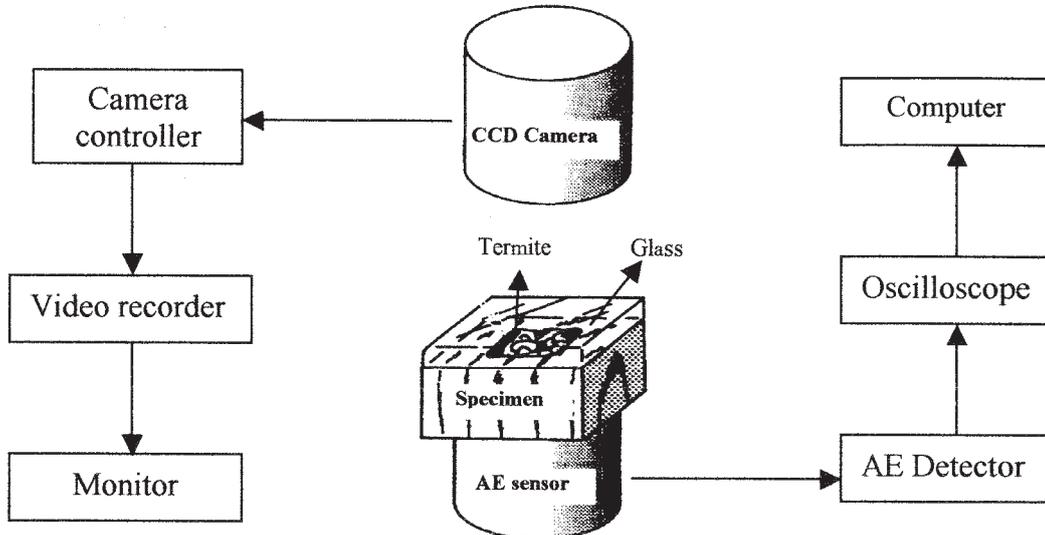


Fig. 1: Test apparatus for observing the feeding behavior of termites.

### AE monitoring

A piezoelectric AE sensor of resonant frequency 150 kHz was attached on the opposite surface to the hole of the wood specimen using hot-melt adhesive. The signal from the sensor was amplified by about 85 dB, filtered by a high-pass filter with a cut-off frequency of 50 kHz, and discriminated at a threshold voltage of 0.6 V with the AE apparatus (Maruwa Biochemical Co., Ltd., AE DETECTOR 510).

### Recording of amplitude

The peak to peak (p-p) amplitude of the AE wave generated by each type of feeding behavior was obtained using the oscilloscope. When the mandible and the maxillae were stuck into the wood surface, a buzzer beeped due to the generation of AE waves. The figures showing the p-p amplitude of the AE waves were then transferred to a personal computer.

### Observation of feeding behavior

Either a nymph or a worker termite was placed into the hole in the specimen, and the hole was then covered with a transparent glass. The specimen was then set up under the CCD camera head (Fig. 1). The feeding behavior of each termite was observed for one hour after each individual started to feed. The numbers of feeding events for each type of feeding behavior were counted. The length of time per feeding event (T/E) and numbers of mandibles that were stuck into the wood per feeding event (MS/E) for each type of feeding behavior were also calculated. The observations were conducted in a dark room maintained at  $25\pm 3^\circ\text{C}$  and  $65\pm 5\%$  relative humidity (RH), with 10 replicates for *I. minor* and 5 replicates for both *C. formosanus* and *R. speratus*.

## Results

### Types of feeding behavior

In this study, feeding behavior consisted of the three feeding activities shown bellow:

1. Cutting activity  
Cutting occurred when the maxillae were hooked into the wood, and then the mandibles cut out a wood fragment.
2. Pulling activity

At the end of the cutting activity, the majority of the termites pulled the wood using both their mandibles and maxillae, while rocking their bodies and pushing their front legs against the wood.

### 3. Collecting activity

Collecting was defined as the cleaning of the wood surface using the maxillae.

Only four out of the ten nymphs of *I. minor*, showed feeding activities during the observation. Before attacking the wood, the termites moved around inside the hole and touched the wood surface with their antennae for 15 to 27 min. Three types of feeding activity, cutting, pulling, and collecting, were recorded for the *I. minor* nymphs.

For *C. formosanus*, three of five workers showed feeding activities during the observation. The workers behaved in a similar way as the *I. minor* nymphs, moving around for 16 to 72 min before beginning to attack the wood. The same three types of feeding activity were recorded for these workers as for the *I. minor* ones.

The feeding activities of five *R. speratus* workers were recorded. Three showed feeding activities during the observation. The amount of time the three workers spent moving around ranged between 5 to 100 min. Cutting and pulling activities, but not collecting activity were observed in the *R. speratus* workers.

### Number of feeding events

Table 1 shows the average number of feeding events for each type of feeding activity in all species.

Table 1. Numbers of feeding event on each type of feeding behavior in *I. minor*, *C. formosanus* and *R. speratus*<sup>1)</sup>

Termite species	Number of event			Total number of event
	Cutting	Pulling	Collecting	
<i>I. minor</i> <sup>2)</sup>	19.00 <sup>a</sup>	19.25 <sup>a</sup>	3.25 <sup>a</sup>	41.5
<i>C. formosanus</i> <sup>3)</sup>	39.67 <sup>a</sup>	41.00 <sup>a</sup>	10.67 <sup>b</sup>	91.34
<i>R. speratus</i> <sup>3)</sup>	22.67 <sup>a</sup>	30.33 <sup>a</sup>	-	53.00

1) Feeding behavior was observed for 60 minutes.

2) Values are means of four replicates.

3) Values are means of three replicates.

4) Values in column with different letters are significantly different by the Tukey's test ( $p < 0.05$ ).

### Length of time and number of mandibles stuck into the wood

Table 2 shows the average length of time per feeding event (T/E) and average number of mandibles stuck into the wood per feeding event (MS/E) for each type of feeding activity in all species.

Table 2. The length of time per feeding event and numbers of mandibles sticking into wood per feeding event on each type of feeding behavior in *I. minor*, *C. formosanus* and *R. speratus*<sup>1)</sup>

Termite species	Cutting		Pulling		Collecting
	T/E <sup>2)</sup>	MS/E <sup>3)</sup>	T/E <sup>2)</sup>	MS/E <sup>3)</sup>	T/E <sup>2)</sup>
<i>I. minor</i>	21.06 <sup>a</sup>	17.21 <sup>a</sup>	<1	1.29 <sup>a</sup>	8.60 <sup>a</sup>
<i>C. formosanus</i>	38.50 <sup>b</sup>	31.33 <sup>b</sup>	<1	1.15 <sup>a</sup>	6.09 <sup>a</sup>
<i>R. speratus</i>	19.13 <sup>a</sup>	13.36 <sup>a</sup>	<1	1.08 <sup>a</sup>	-

1) For legends see Table 1.

2) T/E : The length of time per feeding event (s).

3) MS/E : Numbers of mandibles sticking into wood per feeding event.

4) Values in column with different letters are significantly different by the Tukey's test ( $p < 0.05$ ).

### **Relationship between feeding behavior and the AE's peak to peak (p-p) amplitude**

The relationship between the type of feeding activity of all termite species and the p-p amplitude of the AE waves is shown in Table 3. Table 4 shows the mean p-p amplitude of the AE wave for each type of feeding activity from all termite species (without any categorization by termite species).

Table 3. The p-p amplitude (mV) of the AE for each type of feeding activity of *I. minor*, *C. formosanus*, and *R. speratus*<sup>1)</sup>

Termite species	Cutting	Pulling	Collecting
<i>I. minor</i>	1012.79 <sup>a</sup>	1408.54 <sup>a</sup>	693.95 <sup>a</sup>
<i>C. formosanus</i>	611.55 <sup>b</sup>	1375.63 <sup>a</sup>	544.59 <sup>a</sup>
<i>R. speratus</i>	484.27 <sup>b</sup>	1363.74 <sup>a</sup>	-

1) For legends see Table 1.

2) Values in column with different letters are significantly different by the Tukey's test ( $p < 0.05$ ).

Table 4. The p-p amplitude of the AE waves during the pulling, cutting, and collecting activity<sup>1)</sup>

Feeding behavior	p-p amplitude (mV)
Cutting	702.87 <sup>b</sup>
Pulling	1382.64 <sup>a</sup>
Collecting	619.27 <sup>b</sup>

1) For legends see Table 1.

2) Values in column with different letters are significantly different by the Tukey's test ( $p < 0.05$ ).

### **Discussion**

Three types of feeding activities i.e., cutting, pulling and collecting were recorded for both *I. minor* and *C. formosanus*, whereas two types of feeding activities, cutting and pulling, were observed for *R. speratus*. The reason why *R. speratus* showed no collecting activity might reflect the fact that this termite nests at their feeding sites, and frequently immigrates along with their entire colonies.

*C. formosanus* showed higher numbers of feeding events than *I. minor* during the collecting activity (Table 1). As Shelton and Appel (2001a; 2001b) stated, *I. minor* had a lower basal metabolic rate than those subterranean termites, which was indicated by the release of carbon dioxide (CO<sub>2</sub>). *I. minor* exhibited  $0.250 \pm 0.012$  ml h<sup>-1</sup> CO<sub>2</sub> release on average, while the release by *C. formosanus* was  $0.373 \pm 0.018$  ml h<sup>-1</sup> (Shelton and Appel, 2001a; 2001b). The lower metabolic rate might be the reason why *I. minor* performed fewer numbers of the collecting activity. Likewise, in the pulling and cutting activities, although no significant difference was observed, *C. formosanus* had the largest number of feeding events among the three species (Fig. 3). These results clearly indicated that the *C. formosanus* workers were the most active among the three species.

The average T/E and MS/E values for the cutting activity of *I. minor* were significantly lower than those of *C. formosanus* (Tukey's test:  $p < 0.05$ ). This might be due to the lower basal metabolic rate of *I. minor* than that of the subterranean termites, as mentioned above (Shelton and Appel, 2001a; 2001b). The fact that *R. speratus* had a lower average T/E and MS/E during the cutting activity than *C. formosanus* might be partly explained by the results of Nakayama *et al.* (2004), who described lower wood consumption rates in *R. speratus* workers than in *C. formosanus* workers. Similar results were reported by Imamura *et al.* (1991), who detected a higher AE event rate in the *C. formosanus* workers than in the *R. speratus* ones.

The collecting activity of *I. minor* and *C. formosanus* had the same average T/E values (Table 2). This activity was defined as the cleaning of the wood surface, resulting in shorter T/E values than those in the cutting activity.

In the cutting activity (Table 3), the p-p amplitude of the AE wave of *I. minor* was significantly the largest among the three species (Tukey's test:  $p < 0.05$ ). In addition, in the pulling and collecting activities, although no significant difference was observed, *I. minor* had the largest p-p amplitude of the AE waves, followed by *C. formosanus* and *R. speratus* (Table 3). These results might be related to the fact that *I. minor* had the largest mandibles, and consequently released the greatest

energy to the wood fragment, resulting in a larger p-p amplitude. The size of mandibles of *I. minor* were 482  $\mu\text{m}$  (left side of the right mandible (LR)) and 400  $\mu\text{m}$  (right side of the left mandible (RL)) on average, whereas *C. formosanus* and *R. speratus* had 455  $\mu\text{m}$  and 402  $\mu\text{m}$  (LR), and 378  $\mu\text{m}$  and 310  $\mu\text{m}$  (RL), respectively (Indrayani, unpublished data). In addition, the body size of the termites might also be correlated with the p-p amplitude of the AE waves of the termites. *I. minor*, with the largest p-p amplitude of the AE wave, had a 686  $\mu\text{m}$  body size on average, whereas *C. formosanus* and *R. speratus* were 371  $\mu\text{m}$  and 322  $\mu\text{m}$ , respectively (Indrayani, unpublished data).

Matsuoka *et al.* (1996) reported that the pulling activity showed the largest p-p amplitude of AE waves than the cutting and collecting activities. Similarly, the largest p-p amplitude of AE wave (1382.64 mV on average) was recorded in the pulling activity in the present study (Tables 3 and 4). Both the mandible and maxillae release the greatest energy to the wood fragment, and as the preceding sentence stresses, the pulling activity seemed to generate a larger p-p amplitude. The lower p-p amplitude values of the AE waves ( 619.27 mV on average) generated by the collecting activity might have been due to the fact that the termites used only their maxillae in this activity.

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# Ecosystem-Scale Studies on the Role of Termites in Decomposition Processes of a Dry Evergreen Forest, Northeast Thailand

by

Akinori Yamada<sup>1</sup>, Decha Wawatwitaya<sup>2</sup> and Tetsushi Inoue<sup>3</sup>

<sup>1</sup>Faculty of Medicine, Kyoto University, Yoshida, Kyoto 606-8501, Japan

<sup>2</sup>Faculty of Forestry, Kasetsart University, Bangkok 10900, Thailand

<sup>3</sup>JST PRESTO, Kawaguchi, Saitama 332-0012, Japan/RIKEN, Wako, Saitama 351-0198, Japan

## Abstract

The importance of termites in decomposition processes is widely recognized, and is frequently emphasized especially in savannas. In tropical forests, there have been only a few studies that quantitatively demonstrated the roles of termites as a decomposer. We quantified the importance of termites in terms of carbon mineralization and nitrogen fixation on the basis of biomass data in a dry evergreen forest, northeast Thailand. By using observed respiration rates from termite individuals and fungus-combs with their biomasses, they were estimated to mineralize 11.2% of carbon (C) in the annual aboveground litterfall (AAL). Of these, fungus-combs were responsible for a major part (7.2% of the AAL) of the C mineralization mediated by termites. Based on the obtained and previously reported data, termites in dry tropical forests were estimated to mineralize about 10% of C in the AAL by respiration from their populations and fungus-combs, and their ecological impact is comparable to that in savannas in this aspect. A significant negative correlation between fraction of AAL and annual rainfall demonstrates that the importance of termites in decomposition processes is greater in dry tropical forests than in moist tropical forests. Considering that fungus-combs contributed significantly to the AAL mineralization in most of the tropical forests and savannas, fungus-growers are a much more influential group than previously expected in tropical ecosystems. We measured nitrogen (N) fixation rates of termites and asymbiotic (free-living) bacteria in litter, dead wood and soil, and estimated the total N inputs from fixation to decomposing plant material on the forest floor. Annual mounts of N fixed by termites and asymbiotic bacteria were calculated to be 0.28 and 3.95 kg ha<sup>-1</sup>, respectively, showing that termites are responsible for 6.6% of the total. Considering ecology and behavior of termites, they are suggested to contribute to decomposition processes as N fixers more significantly than expected from the value (6.6%).

**Key words:** annual aboveground litterfall, carbon mineralization, dry evergreen forest, fungus-combs, nitrogen fixation, respiration

## Introduction

Termites are a major decomposer in tropical and subtropical regions (Lee & Wood 1971; Wood & Sands 1978), where net primary production is equivalent to 50-60% of that of all terrestrial ecosystems. Termites are the most appropriate animals considered to be "soil ecosystem engineers", which are defined as large invertebrates ingesting a mixture of organic material and mineral debris, forming stable, long-lived faeces which are organo-mineral complexes (Lavelle et al. 1997). Examples of the role as soil ecosystem engineers are given by the well-known studies in savannas, which have demonstrated the ecosystem-scale impact of termites, especially fungus-growers (Lee & Wood 1971; Wood & Sands 1978; Buxton 1981; Collins 1981; Schaefer 1990; Lavelle et al. 1997). At the same time, it is increasingly recognized that termites have potentially significant contributions to decomposition processes as mediators of carbon (C) mineralization and nitrogen (N) fixation (Holt 1987; Bignell et al. 1997; Eggleton et al. 1999; Nardi et al. 2002; Konaté et al. 2003). Here, we present ecosystem-scale studies on C mineralization and N fixation carried out in the dry evergreen forest (DEF) at Sakaerat Environmental Research Station, northeast Thailand, where we previously observed the biomass of termites (Inoue et al. 2001; Yamada et al. 2003).

### *Carbon mineralization mediated by termites*

Respiration rates of termite individuals have been employed to quantify the contribution of termites to decomposition processes mainly in tropical forests (Matsumoto 1976; Martius 1994;

Bignell et al. 1997; Eggleton et al. 1999; Konaté et al. 2003), whereas studies conducting measurements of food consumption rates of termites have clearly demonstrated that the dominant fungus-growers consume the major part, up to 30% (Wood & Sands 1978), of annual aboveground litterfall (AAL) in savannas (Wood & Sands 1978; Buxton 1981; Collins 1981). Considering a wide range of foods utilized by termites in tropical forests, there are obvious difficulties in measuring consumption rates of them, especially soil-feeders and wood-feeders (Abe & Matsumoto 1979; Eggleton et al. 1996; Eggleton et al. 1999; Yamada et al. 2003). Most of the studies using respiration rates have shown that termites in tropical forests mineralize about 1% of C in AAL by respiration from their populations. A single study has demonstrated that a fungus-grower consume 22-32% of leaf litter (= 10-15% of the AAL) in a tropical forest (Matsumoto & Abe 1979). However, compared to the considerable consumption of leaf-litter by the termites, respiration from the termites has contributed to C mineralization on an unexpectedly small scale (Matsumoto 1976). This apparent anomaly has been mentioned by several authors (Bignell & Eggleton 2000; Sugimoto et al. 2000). Here, we give a possible explanation about it by observing respiration rates of termite individuals as well as fungus-combs.

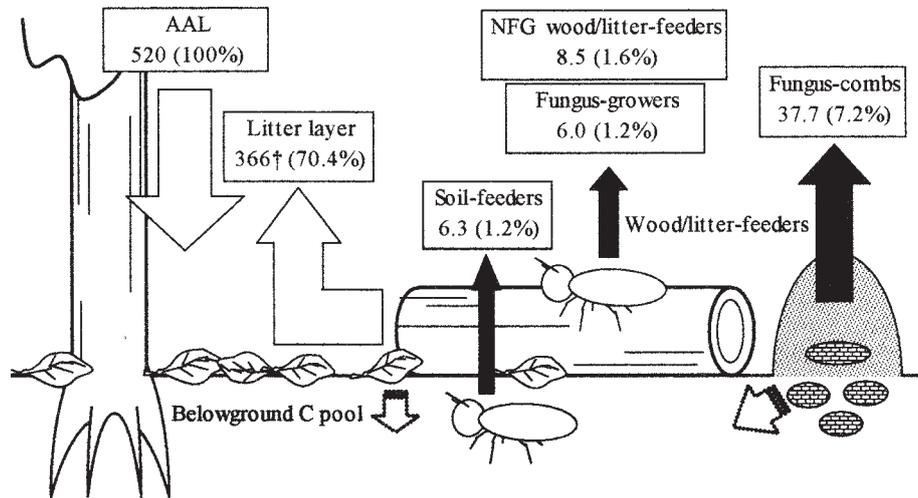
We estimated an amount of respiratory C from termite population and fungus-combs to be  $20.8 \text{ g C m}^{-2} \text{ y}^{-1}$ , which is equivalent to 11.2% of C in the AAL in the DEF ( $520 \text{ g C m}^{-2} \text{ y}^{-1}$ ; Wachirinrat & Takeda 2003; Vogt et al. 1986). This value is much larger than those previously estimated in tropical forests without considering fungus-combs (Matsumoto 1976; Martius 1994; Bignell et al. 1997; Eggleton et al. 1999). The large amount of C mineralized by fungus-combs would give an explanation to the above-mentioned disagreement between litter consumption by termites in tropical forests and respiration from them. In the DEF, against 11.2% of the AAL mineralized by termite population and fungus-combs, termites are estimated to consume 21-64%, though 30-40% would be conceivable, of the AAL by referring previous studies on their consumption rates (Wood & Sands 1978; Martius 1994).

An aspect of the fate of the AAL in the DEF is that a majority of the AAL is mineralized above the ground by respiration from wood/litter-feeders (2.8%), fungus-combs (7.2%) and microorganisms on the litter layer (70.4%), leaving a relatively small part of the AAL that subsequently enters the soil layer (belowground C pool) and might get partly mineralized by soil-feeders (1.2%) as illustrated in Fig. 1. If termites consume 30-40% of the AAL as discussed above, the AAL appears to be completely removed from the forest floor before it enters the soil layer on the place. It is suggested that there are competitive relationships between termites and microorganisms in litter layers over the AAL.

The uncertainty of the impact of termites in tropical forests comes not only from lack of consideration of fungus-combs in the previous studies, but also from variation in termite abundance and biomass among ecosystems, which is expected to reflect the impact of termites to some extents. In fact, we revealed remarkable variation in fraction of AAL mineralized by termite population and fungus-combs among tropical forests, which were calculated by estimating biomasses of fungus-combs based on termite biomass data for the well-studied sites at Pasoh (7.5%, Abe & Matsumoto 1979), Mbalmayo (8.3%, Eggleton et al. 1996), Sabah (1.1%, Eggleton et al. 1999), Sarawak (0.6%, Collins 1983) and Manaus (1.3%, Martius 1994). A significant negative correlation was found between fraction of the AAL and annual rainfall (Spearman's rank correlation coefficient,  $r_s = -1.00$ ,  $P < 0.05$ ,  $n = 6$ ), meaning that fractions of C mineralized by termite populations and fungus-combs are larger in "dry" tropical forests (e.g. tropical seasonal forests) than in "moist" tropical forests (e.g. tropical rainforests). The uncertainty of the impact of termites in tropical forests could be attributed to heterogeneity of "tropical forest". A possible explanation for this correlation could be found in a positive correlation between respiration rate of litter and its moisture content (Chambers et al. 2001) and in much lower respiration rate of litter layer during dry season than during wet season in the DEF (Yoda & Nishioka 1982). As increased respiration rate of litter imply more AAL mineralization, competition for AAL between termites and microorganisms in the litter layer could result in decreased resources for termites. In addition to annual rainfall, temperature probably affects respiration rates of litter layer (Tate et al. 1993). Currently, available data are insufficient to make a firm statement.

Fractions of AAL mineralized by termite populations and fungus-combs in the three savannas were also estimated to be 5.3% (Fete Ole), 10.2% (Lamto) and 38.7% (Mokwa), by using the termite

biomass data in Wood & Sands (1978). The dry tropical forests with the larger fractions of 7.5-11.2% are comparable to the savannas (5.3-10.2%) in the importance of termites in decomposition processes, with the exception of Mokwa, where the fraction would have been overestimated because it is given that consumption by termites is about 35% of the AAL (Wood & Sands 1978). This extraordinarily large contribution of termite at Mokwa (38.7% of the AAL) might be attributed to an overestimation of the termite abundance and biomass. In fact, even the consumption rate of termites in Mokwa has been 35% of the AAL, which is comparable to the estimated value for termites in the DEF (30-40% of the AAL).



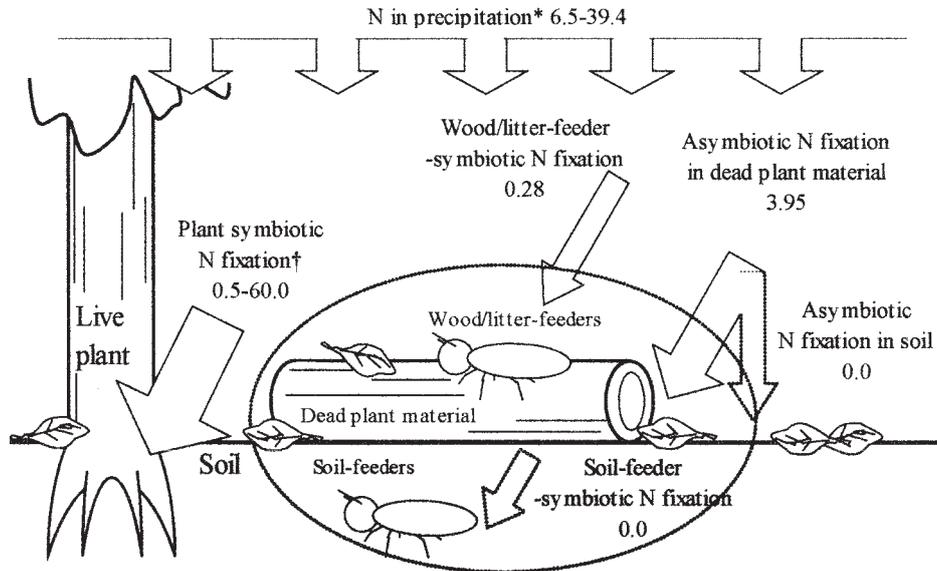
**Fig.1.** Fate of C in the annual aboveground litterfall (AAL; 520 g C m<sup>-2</sup> y<sup>-1</sup>; Wachirinrat & Takeda 2003; Vogt et al. 1986) in the dry evergreen forest at Sakaerat Environmental Research Station. The figures indicate the annual amounts of respiratory C (g C m<sup>-2</sup> y<sup>-1</sup>) with the percentages to the AAL in parenthesis. NFG: non-fungus-growing; †A season-weighted mean of respiration rates of the litter layer was calculated by using those measured in the DEF during dry and rainy seasons (Yoda & Nishioka 1982), with the assumption that the ratio of dry and rainy seasons is 5:7.

### ***Nitrogen fixation by termites***

N is one of the elements affecting decomposition of dead plant material (Vitousek et al 2002), as well as primary production (Vitousek & Howarth 1991; Vitousek et al. 2002). Biological N fixation is the primary process of N input to ecosystems, and generally occurs in bacterial symbionts in plant root nodules, asymbiotic (free-living) bacteria in surface soil layer, on the surfaces of dead plant material, and bacterial symbionts in xylophagous arthropod guts (Breznak 1975; Nardi et al 2002). N fixed in plant root nodules increases the plant growth (Vitousek & Howarth 1991), while asymbiotic bacteria and termites supply N to decomposing plant material. Many species in non-fungus-growing (NFG) wood/litter-feeders have been shown to fix N in order to compensate the N deficiency (Benemann 1973; Breznak et al. 1973; Prestwich & Bentley 1981; Tayasu et al. 1994). Although N fixation by termites has been studied by many researchers using acetylene reduction assay, only a few studies have quantified N fixed by termites on the scales covering their populations in the ecosystems (Schaefer & Whitford 1981; Pandey et al. 1992). Here, we present the first study that estimate N fixed by termites on the ecosystem scale and that evaluate the N amount in the context of N supply to decomposing plant material by considering asymbiotic N fixation.

We preliminarily assayed a diverse group of termite species by acetylene reduction method, and only the wood/litter-feeding termites were found to fix N at significant levels. More intensive samplings of two abundant species, *Microcerotermes crassus* and *Globitermes sulphureus*, were done in the DEF across several seasons. Acetylene reduction method was modified, and the assays were carried out under field conditions in order to minimize an artifactual error. An N fixation rate of 0.28 kg N ha<sup>-1</sup> y<sup>-1</sup> was obtained by using mean rates of the two species and their biomasses (Yamada et al. 2003). An asymbiotic N fixation rate was estimated to be 3.95 kg ha<sup>-1</sup> y<sup>-1</sup>, by using observed fixation rates of litter and déad wood and the biomass (Yamada et al. 2003).

Termites is calculated to be responsible for 6.6% of N inputs from fixation to decomposing plant material in the DEF. N from precipitation (Jordan 1985; Edwards 1982; Strigel et al. 1994) and



**Fig. 2.** Schematic figure on N inputs ( $\text{kg N ha}^{-1} \text{y}^{-1}$ ) to the dry evergreen forest at Sakaerat Environmental Research Station. Only non-fungus-growing wood/litter-feeders of termites fixed N, and asymbiotic N fixation activity was detected from litter and dead wood but not from the soil. \* data for tropical forests in Jordan (1985), Edwards (1982), and Strigel et al. (1994); † data for tropical evergreen and deciduous forests in Cleveland et al. (1999).

immobilization by fungal hyphae (Frey et al. 2000) is also given as potential sources of additional N. Taking these potential N sources into consideration, the amounts of N fixed by termites might be of minor importance in decomposition processes. However, from the spatial and temporal point of view, N fixed by termites is expected to contribute significantly to decomposition processes in different ways from the other sources of additional N. Many species of NFG wood/litter-feeding termites, for example *M. crassus*, attack and break more or less freshly fallen branches and trunks, and feed on woody material in the centers of the woods (Abe 1980) by utilizing fixed N in their guts. In contrast, additional N from asymbiotic fixation, precipitation, and fungus-mediated immobilization is expected to be supplied mainly to surfaces of fallen branches and trunks. Here, we suggest that N fixed by termites plays its role in the early decomposition processes and in the different part of dead wood from the part where the other additional N is supplied. With regard to the fate of N fixed by termites, it would be excreted as their feces out of their bodies. A stable isotope analysis has shown that feces of the termite *Neotermes koshunensis* contain atmospheric N (I. Tayasu, personal communication). It is most likely that at least a portion of feces containing fixed N is put into the centers of woods where termites are feeding and/or nesting. Therefore, the feces containing N fixed by termites would be an exclusive source of additional N to the centers of freshly dead wood, promoting further decomposition.

## Conclusions

The role of termites in decomposition processes was quantitatively elucidated in a dry evergreen forest, northeast Thailand, in terms of C mineralization and N fixation. Termites mineralized 11.2% of C in an annual aboveground litterfall by respiration from their population (4.0%) and fungus-combs (7.2%) (Fig. 1). Termites in dry tropical forests mineralize about 10% of C in the AAL by respiration from their populations and fungus-combs, and the ecological impact of termites is comparable to that in savannas in this aspect. The significant negative correlation between fraction of AAL and annual rainfall demonstrates that the importance of termites in decomposition processes is greater in dry tropical forests than in moist tropical forests. Fungus-growers are a much more influential group than previously expected in tropical ecosystems because of their fungus-combs. In

relation to N fixation, an amount of N fixed by termites, especially non-fungus-growing termites, was estimated to be 0.28 kg N ha<sup>-1</sup> y<sup>-1</sup> and represented 6.6% of N inputs from fixation to decomposing plant material in the DEF (Fig. 2). Considering ecology and behavior of termites, they are suggested to contribute to decomposition processes as N fixers more significantly than expected from the value (6.6%).

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# **Baits of Molybdenum and Tungsten Salts for Termite Control**

## **Part-1. Fundamental Tests**

by

Yoshio Katsuda and Koji Nakayama

Dainihon Jochugiku Co., Ltd., Toyonaka, Osaka 561-0827, Japan

### **Abstract**

Since around the middle of 1980s, we had been engaged in research works on baits containing molybdenum and tungsten salts against termites, and reported their results on academic journals and at academic meetings (ex. International Congress of Pesticide Chemistry). This time, outlines of termiticidal efficacy, mode of action, formulation study and safety on the baits will be given and field efficacy tests conducted at sites such as embankments and dams in China will also be reported.

Baits containing molybdenum and tungsten salts showed a slow-acting but high termiticidal efficacy against termites. From the obtained test results on mode of action, the primary cause for the termiticidal efficacy of molybdenum and tungsten salts is considered to be the influence on the symbiotic intestinal flagellates, or the physiological depression resulted from the accumulation of their oxides in the body, especially in the fat body.

Formulation study led us to prepare baits in which sodium salts of molybdenum and tungsten were converted to water-insoluble barium salts by ion-exchange reaction. This barium salt baits showed a high termiticidal efficacy almost equal to that of sodium salt type, and their active ingredients were found not to leach out in the water and in the soil.

Furthermore we conducted field efficacy tests in China using bait stakes prepared by having base materials impregnated with molybdenum and tungsten salts and then solidifying the mixture with resin adhesive. The bait stakes were hit into the ground of test sites such as embankments and the level of termite activities of the test sites were observed periodically. After about 1 year from the start of the tests, no termite activities were found in many of satellite nests and mud tubes, indicating that our baits are useful for the termite control of embankments and others.

Field efficacy tests at rubber plantations in Thailand (Part-2) will be reported by Dr. Charunee Vongkaluang.

**Key words:** molybdenum, tungsten, bait, termite control

### **Fundamental tests in Japan**

#### ***Laboratory efficacy tests of sodium salts of molybdenum and tungsten (Yoshimura et al. 1989)***

Termiticidal efficacy of sodium salts of molybdenum and tungsten on *Coptotermes formosanus* SHIRAKI was investigated by exposing termites to filter paper or wood block containing molybdenum or tungsten at a concentration of 0.05 -5.0 % as metal. As a result, molybdenum and tungsten compounds acted more slowly than other inorganic compounds such as CCA (chromium,

copper, arsenic), boric acid and sodium tetraborate, but they were confirmed to be practically applicable as termiticidal ingredients in the bait-block technique from the following findings obtained.

(1) In the test where filter paper was fed continuously, as shown in Fig.1, filter papers containing sodium molybdate at a concentration of 0.05 % or more as metal showed 100% mortality within 5 weeks. On the other hand, the mortality for 0.05 % sodium tungstate was about 80 % at the time after 5 weeks (Fig. 2).

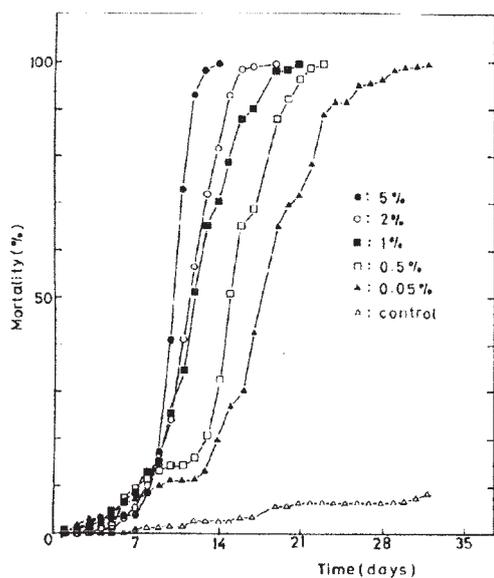


Fig. 1 Conc. of Na-Mo and mortality.

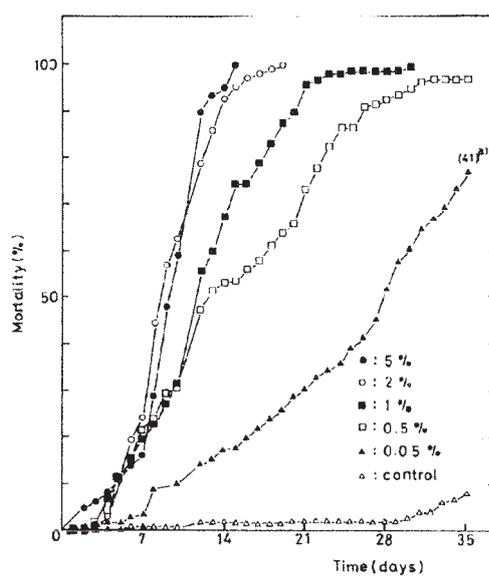


Fig. 2 Conc. of Na-W and mortality.

(2) Using filter papers containing mixtures of molybdenum and tungsten compounds at the following concentrations (%; as Mo or W), the mortality of *Coptotermes formosanus* SHIRAKI was examined for each formulation at the time after 2 weeks. As shown in Fig.3, formulations of II ~IV gave higher mortalities than I and V, suggesting a synergistic effect in mixtures of both compounds.

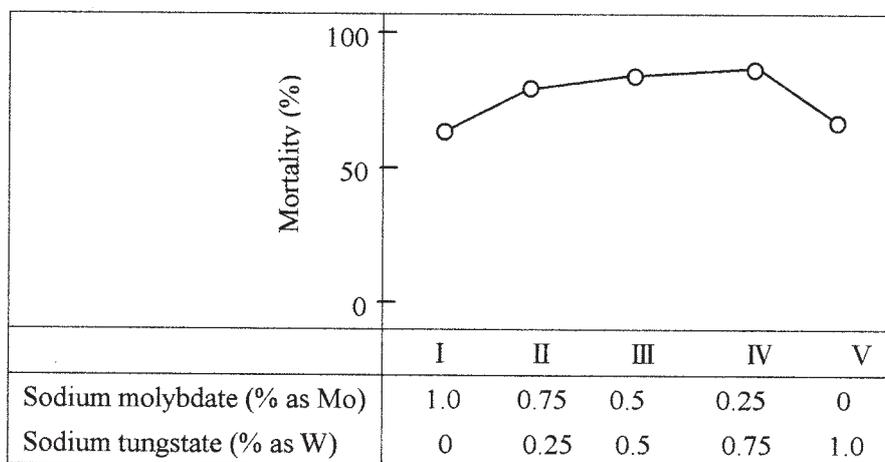


Fig.3 Synergistic effect in mixtures of both compounds.

(3) When pine tree blocks were used instead of filter paper, the efficacy of the blocks containing sodium molybdate or sodium tungstate at 5.0 % (as Mo or W ) almost corresponded to that of 0.05 % treated filter paper in each case.

(4) After exposing termites to filter paper containing sodium molybdate or sodium tungstate at 5.0 % (as Mo or W ) for 1-7 days, the termites were transferred to another container to observe the mortality. The test results showed that only one day feeding of the treated filter paper could cause almost 100% mortality of *Coptotermes formosanus* SHIRAKI eventually.

#### ***Study on mode of action (Yoshimura et al. 1989)***

Termiticidal mechanism of molybdenum and tungsten compounds was examined through the experiments on the effect on nitrogen source, the influence on the symbiotic intestinal flagellates, and the distribution of the compounds in the termite body after feeding. The test results strongly indicated that the termiticidal efficacy of molybdenum and tungsten salts might be much more associated with the influence on the symbiotic intestinal flagellates, or the physiological depression resulted from the accumulation of their oxides in the body, especially in the fat body, rather than with the nitrogen-fixing inhibition which was the initial aimed point.

#### ***Laboratory efficacy tests of barium salts of molybdenum and tungsten (Kanzaki et al. 1994)***

Baits of water-soluble sodium salts have the fear of their active ingredients being leached out in field conditions. Therefore the bait containing water-insoluble barium salts of molybdenum and tungsten was prepared by having base materials with an aqueous solution of sodium salts of molybdenum and tungsten, followed by the addition of an aqueous barium chloride solution through ion-exchange reaction.

This barium salt baits showed a high termiticidal efficacy almost equal to that of sodium salt type against *Coptotermes formosanus* SHIRAKI, and their active ingredients were found not to leach out in the water and in the soil.

#### ***Semifield efficacy tests (Umeda et al. 1997)***

Semifield tests of bait formulations containing barium salts of molybdenum and tungsten (about 5% and 3% as metal respectively, excipient;  $\alpha$ -starch) were done using a colony of *Coptotermes formosanus* SHIRAKI, whose nest was translocated from a field to our laboratory. During the test period, levels of bait feeding by termites and changes in population of the colony were examined by filter paper setting method and Acoustic Emission (AE) monitoring method.

After 23 weeks from the start, no termites were observed around the nest, and then it was dismembered for close examination of the inside. Many dead soldier termites were found inside the nest, confirming that this colony was eventually eradicated by the baits.

#### ***Formulation study (Nakayama et al. 1998)***

Barium salt baits for practical use were prepared as follows:

- 1) having base materials impregnated with an aqueous solution of sodium salts of molybdenum and tungsten,
- 2) converting to water-insoluble barium salts by addition of an aqueous barium chloride solution,
- 3) after drying, solidifying the mixture using resin adhesive such as phenolic, vinylacetate and isocyanate types under pressure,
- 4) cutting the molding to give bait stakes.

This barium salt baits were found to be practically applicable to field conditions, as they were tolerable in the soil without the collapse and leaching of active ingredients.

### **Field efficacy tests in China**

In China, damages by termites that eat woods or destroy embankments become social concern, and in particular, the damage of embankments by *Odontotermes formosanus*, *Odontotermes hainanensis*, *Globitermes*, or *Macrotermes* is serious. These kinds of termites make nests under embankments and dams. When the river has risen in the rainy season, the water sinks into the mud tubes and is known to cause a threat of destroying embankments and dams, making the working up of measures an important task. To preserve the embankment, eradication of termite colonies is indispensable, and conventionally bait formulations such as mirex were employed for this purpose. However the use of organic chlorine compounds was banned because of residual toxicity and environmental pollution, and the development of new alternatives has been desired.

Based on the research achievements on molybdenum and tungsten compounds, various tests were conducted in China from around the middle of 1990s. In the field tests, bait stakes containing salts of molybdenum and tungsten (about 5% and 3% as metal respectively) were prepared by solidifying with resin adhesive, and then the stakes were hit into the ground of test sites such as embankments of Baifen Dam in Guangzhou City of Guangdong Province, and Huinu Dam in Quanzhou City of Fujian Province. After that, periodical observations were made with the test sites.

As a result, no termite activities were observed in many emasculated satellite nests and mud tubes after one year from the start, indicating that our baits are useful for the termite control at places such as embankments and dams.

### **Conclusions**

Molybdenum and tungsten compounds are metal elements distributed on the earth, and show a slow-acting but high termiticidal efficacy against termites without causing problems of residual toxicity as organic chlorine-based termiticides. Especially barium salt baits are promising as a new termiticide, as barium salts do not leach out from the baits into the soil and are free from fear of causing the environmental pollution.

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# **Baits of Molybdenum and Tungsten Salts for Termite Control**

## **Part-2. Field Efficacy Tests at Rubber Plantations in Thailand**

by

Charunee Vongkaluang<sup>1</sup>, Yoshio Katsuda<sup>2</sup> and Koji Nakayama<sup>2</sup>

1) Royal Forest Department, Bangkok 10900, Thailand

2) Dainihon Jochugiku Co., Ltd., Toyonaka, Osaka 561-0827, Japan

### **Abstract**

Rubber trees are planted mainly in Thailand, Malaysia, Indonesia and Sri Lanka, and are important as a source of supply for natural rubber whose demand is still on the increase. At rubber plantations of these hot and humid countries, there live many kinds of termites including *Macrotermes* and *Odontotermes* which cause damages especially by attacking roots of young trees (until 7-8 years old).

We have conducted field efficacy tests of baits at Rubber Experiment Station of Phuket in Thailand, as *Macrotermes* and *Odontotermes* are difficult to be reared artificially and die within around one week in the laboratory. Bait stakes were prepared by having rubber tree chips impregnated with barium salts of molybdenum and tungsten compounds and then solidifying the mixture with phenolic-type or vinylacetate-type resin adhesive. The bait stakes were hit into the ground around each termite mound and the level of termite activities of the test sites were observed periodically. After one to two years from the start of the test, it was confirmed that our bait formulations eventually eradicated termite colonies of *Macrotermes* and *Odontotermes*. We reported the test results at the 35<sup>th</sup> annual meeting of IRG held in Slovenia last year. This time the outline of the field efficacy tests will be reported including some findings newly obtained later.

**Key words:** molybdenum, tungsten, bait, rubber plantation, termite control

### **Introduction**

Molybdenum and tungsten compounds are slow acting to termites and show a high termiticidal efficacy. Regarding their mode of action and practical studies on bait formulations for the termite control, some papers (Yoshimura *et al.* 1989; Kanzaki *et al.* 1994; Umeda *et al.* 1997; Nakayama *et al.* 1998) have been reported.

Rubber trees are planted mainly in Thailand, Malaysia, Indonesia and Sri Lanka, and are important as a source of supply for natural rubber whose demand is still on the increase. At rubber plantations of these hot and humid countries, there live many kinds of termites including *Macrotermes* and *Odontotermes* which cause damages especially by attacking roots of young rubber trees (until 7-8 years old). In case that rubber trees die all at once before the harvesting season of natural rubber resin, the damages become awfully serious for the exporting nations as well as producing farmers. Measures taken for the damages in the past are only physical ones such as removing surface soil (about 40 cm in depth). In consideration of these circumstances, we have

conducted field tests of bait formulations containing molybdenum and tungsten compounds for the termite control at rubber plantations in Thailand since July of 1999. We reported the test results and high practicability of the bait formulations at the 35<sup>th</sup> annual meeting of IRG held in Slovenia last year (Katsuda *et al.* 2004). This time the outline of the field efficacy tests will be reported including some findings newly obtained later.

## Materials and methods

### *Test materials*

The bait formulation served for field tests is shown in Table 1. An aqueous solution of sodium salts of molybdic acid and tungstic acid was impregnated uniformly in rubber tree chips, followed by the addition of an aqueous solution of barium chloride to cause formation and precipitation of water insoluble barium salts of molybdic acid and tungstic acid. After drying, the mixture was solidified using phenolic-type or vinylacetate-type resin adhesive under low pressure and then the molding was cut to give phenolic-type or vinylacetate-type bait stakes respectively (15 x 40 x 250 mm).

Table 1 Bait formulation for field tests

Materials	Contents (%)
Na <sub>2</sub> MoO <sub>4</sub> · 2H <sub>2</sub> O (MW:242)	13.0 (5.2 as Mo)
Na <sub>2</sub> WO <sub>4</sub> · 2H <sub>2</sub> O (MW:330)	5.0 (2.8 as W)
BaCl <sub>2</sub> · 2H <sub>2</sub> O (MW:244)	15
Rubber tree chips	57~61
Phenolic-type or vinylacetate-type resin adhesive	6~10
Total	100

### *Test methods*

Ten and more termite mounds at Phuket Rubber Experiment Station were selected as test sites. Phenolic-type bait stakes were installed on April of 2002, and the installation of both phenolic-type and vinylacetate-type bait stakes was made on December of 2003. In the tests, 4-6 test bait stakes were hit into the ground around each mound, and stakes of rubber wood (40 x 40 x 300 mm) were set on the top of each mound and around it if necessary. Then the level of termite activities at each mound were observed at intervals.

## Results and discussion

### *Outline of observation results (installation of baits: April of 2002)*

At most of test sites where phenolic-type bait stakes were installed, as reported at the 35<sup>th</sup> annual meeting of IRG, termite mounds having a height of about 30 cm at the beginning became flat after one and a half years. Thus it was confirmed that our bait formulations eventually eradicated termite colonies of *Macrotermes* and *Odontotermes* which attack rubber trees.

After the eradication of *Macrotermes* and *Odontotermes* colonies, activities of *Hypotermes* were observed. This species is known to be beneficial, as *Hypotermes* termites cause no damage to rubber trees, eat fallen leaves of rubber trees to make organic manure, and soften the soil physically.

One of test baits after a lapse of one year and 8 months in the soil was subjected to the analysis of active ingredients. As a result, concentrations of molybdenum and tungsten per the total weight of the bait were almost unchanged from the initial values, suggesting that barium salts of molybdenum and tungsten have no fear of leaching out from the bait in the soil.

**Outline of observation results (installation of baits: December of 2003)**

To examine the effect of resin adhesives on bait feeding by termites, both phenolic-type bait stakes (A) and vinylacetate-type bait stakes (B) were installed newly. Results of typical test sites are summarized as Table 2.

Table 2 Observation results

Test site	Date of installation 2003, 12	Date of observation 2004, 12
III	<ul style="list-style-type: none"> <li>• Five stakes of each (A) and (B) were hit into the ground around a termite mound.</li> <li>• <i>Macrotermes</i> were highly active.</li> </ul>	<ul style="list-style-type: none"> <li>• Damage was found on the stakes of both (A) and (B).</li> <li>• Activities of <i>Macrotermes</i> dropped greatly in the mound.</li> <li>• It was presumed that eradication of the nest would be confirmative at the next time observation scheduled for March of 2005.</li> </ul>
IV	<ul style="list-style-type: none"> <li>• Six stakes of (A) and 4 stakes of (B) were hit into the ground around a termite mound where <i>Odontotermes</i> were active.</li> </ul>	<ul style="list-style-type: none"> <li>• Damage was found on the stakes of (B).</li> <li>• Reduction in activities of <i>Odontotermes</i> was clearly observed.</li> </ul>

As a result, it was found that bait feeding by termites tend to be greater in case of vinylacetate-type bait stakes rather than phenolic-type ones. Physical properties of the baits are considered to be one of causes, as vinylacetate-type bait stakes were more porous or more tender compared with phenolic-type ones. These findings will be confirmed by re-observation due on March of 2005.

**Conclusion**

Molybdenum and tungsten compounds are metal elements distributed on the earth, and show a slow-acting but high termiticidal efficacy against termites without causing problems of residual

toxicity as organic chlorine-based termiticides, proving promising as a new termiticide. Especially, applications of the baits to rubber plantations suffering severe damages do a lot for the social welfare and their early practical use is expected.

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# The Application of Entomopathogenic Fungi as Biocontrol for Subterranean Termites

by :

Sulaeman Yusuf<sup>1)</sup>, Desyanti<sup>2)</sup>, Teguh Santoso<sup>3)</sup>, Yusuf Sudo Hadi<sup>3)</sup>

<sup>1)</sup> R & D Unit for Biomaterials LIPI, Indonesia, <sup>2)</sup> Bogor Agricultural University, Indonesia

## Abstract

The isolation and bioassay of fungus from cabbage and soybean pest was conducted. The isolate was multiply identification to its pathogenicity to subterranean termite of *Coptotermes* sp. The species of fungi were found from the agricultural pest are *Beauveria bassiana*, *Metharhizium anisopliae*, *Paecilomyces* sp, *Myrothesium* sp, *Cladosporium* sp. *Verticillium* sp. and *Aspergillus* sp. From the result of bioassay were indicates mostly fungi are found from agricultural pest could attack the termites of *Coptotermes* sp. for 6 days.

**Key words:** Entomopathogen fungi, subterranean termite, biocontrol.

## Introduction

Indonesia has the biggest biodiversity in the world, because of the conditions especially high humidity and adequate temperatures. The first microorganisms found to cause diseases in insects were fungi because of their conspicuous macroscopic growth on the surfaces of their hosts. Some entomopathogenic fungi, however, form no superficial growth or produce sparse, inconspicuous or minute external structures that are difficult to detect by the inexperienced investigator. Most entomopathogenic/entomogenous fungi are obligate or facultative pathogens and some are symbiotic. Their growth and development are limited mainly by the external environmental conditions, in particular, high humidity or moisture and adequate temperatures for sporulation and spore germination. The diseases caused by fungi are termed mycoses.

Fungi may be associated with insects as ectoparasites and endoparasites. The ectoparasitic forms are mostly Laboulbeniales and a few genera within the Deuteromycotina. Insects are usually infected by spores or conidia (Zygomycotina), conidia (Deuteromycotina), Zoospores (Mastigomycotina), and planonts or ascospores (Ascomycotina). Other types of infective propagules are also found, such as sclerotia or sporodochia (Prior and Perry 1980 in Butt). The fungi gain entrance into the insect mainly through the integument and in some cases through natural body opening.

Fungi infect individuals in all orders of insect, in some insect orders; the immature (nymphal or larval) stages are more often infected than the mature or adult stage. The pupal stage is infrequently attacked and fungi rarely infect the egg stage.

There is no termite control in Indonesia by the use of microorganisms (biocontrol). The chemical treatments are still mainly used for the termite control. Carruthers and Hurar, 1990 in Oliveira *et al.* (2003) mention that entomopathogenus fungi are the important microorganisms to control any kind of insects.

In this experiment, we would like to use the infected agricultural pest to control the termite activity as the alternative of termite control measure in Indonesia. The infected cabbage and soybean pests were used in this experiment to apply them to the control of termite, *Coptotermes* sp in the laboratory.

## Materials and Methods

### Materials

Entomopathogenic fungus was isolated from fungus infected of cabbage pest (*Crocidolomia* sp., *Spodoptera* sp.), Soybean pest (*Reptortus liniaris*, *Leptocorisa acuta*).

### Isolation procedure

Isolate source of entomopathogen fungi were introduced into sterilized aquadest containing 0.025 % triton 100 X and stirred for 15 min at room temperature. Then the spore suspension (0.5 ml) was aseptically diluted with distilled water (4.5 ml) in a test tube. The diluted suspension (0.1 ml) was inoculated in to potato dextrose agar (PDA) plates containing 0.1 ml tetracycline hydrochloride to prevent bacterial contamination. The PDA plates were incubated at 24 °C and 95 RH for 4 days. Well-enlarged colonies of a different shape and color were picked up and inoculated independently into fresh PDA medium. A pure culture was obtained by successive inoculation of an individual colony in PDA medium

### Identification

Identification procedure was done by visual and microscopies according to „Slide Culture Methods“ (Barneet and Hunter,1972).

### Preparation of Conidia Suspension

The conidia suspension was prepared by additional of 2 ml sterilized aquadest and Triton X 100 into petri dish which containing 3 weeks old of fungi. The petri dish was seek to get the conidia and dilute in the sterilized aquadest to get the dilution. The *haemocytometer* was used to count the total of conidia.

### The Bioassay

One each of wet filter paper were placed in a petry dish together with eighty workers and eight soldiers of termites, *Coptotermes* sp. and among of them, twenty workers and two soldiers of termites were sprayed with 0.5 ml of conidia suspension with concentration of  $10^8$  conidia/ml . The petri dish was placed in a plastic container and keep in the dark room for 6 days. The dead termites was evaluate every day and termites mortality was calculated.

### Microscopy observation

After the tests, the dead termites was evaluate under microscope.

## Results and discussion

### Identification

Identification results indicates each two species of cabbage pest and soybean pest namely *Crocidolomia* sp., *Spodoptura* sp. (Cabbage pest) and *Riptortus liniaris*, *Leptocorisa acuta* (Soybean pest). In this experiment, the samples fungi are collected from the fungi infected of cabbage and soybean pests. The results indicate that four species of fungi were found in agricultural pest and species of *Beauveria bassiana* are mainly fungi founded on agricultural pest (Table 1).

Table 1. The Fungi were founded from the infected Cabbage and Soybean Pest

Isolate Source	Species of Fungi
Cabbage Pest:	
1. <i>Crocidolomia</i> sp	<i>Beauveria bassiana</i> <i>Cladosporium</i> sp. <i>Aspergilussp.</i> <i>Paecilomyces</i> sp.
2. <i>Spodoptura</i> sp.	<i>Beauveria basiana</i>
Soybean Pest:	
1. <i>Riptortus liniaris</i>	<i>Metharhizium anisopliae</i> <i>Verticilium</i> sp. <i>Paecilomyces</i> sp.
2. <i>Leptocorisa acuta</i>	<i>Beauveria bassiana</i>

**Trial of biocontrol for termites of *Coptotermes sp.***

The termite mortality after inoculated by 25% of fungi for 6 days are showed in Table 2. In this table indicates that all of used fungi in this experiment *Cladosporium sp*, *B. bassiana*, *M. anisopliae*, *Aspergillus sp*, *Paecilomyces sp* could be suppress the termites activities and the mortality of termite achieved until 100% after 6 days of experiment. Those values are very significant if compared with the control ones (13.8%). It is indicates those fungi caused pathogens to termites.

Table 2. Termite mortality and sporulation of *Coptotermes sp.* after inoculated by fungi for 6 days.

No	Species of Fungi	Isolate Source	Mortality (%)	Sporulation (%)*
1	<i>Cladosporium sp</i>	<i>Crocidolomia sp</i>	100	31.3
2	<i>Aspergillus sp.</i>		96.3	86.3
3	<i>Paecilomyces sp</i>		85.0	7.5
4	<i>B. bassiana</i>		100	62.5
5	<i>B. bassiana</i>	<i>Spodoptura sp</i>	58.8	27.5
6	<i>M. anisopliae</i>	<i>Riptortus liniaris</i>	100	10
7	<i>Verticillium</i>		85.2	0
8	<i>Paecilomyces</i>		72.5	27.5
9	<i>B. bassiana</i>	<i>Leptocorisa acuta</i>	100	76.3
10	Control		13.8	0

\* Sporulation: the mycelium grown on the surface of termite

The termite mortality evaluated every day caused by the fungi and the figures could shows in Figures 1 and 2. In this figures shows that the species fungi of *Metharhizium* is the pathogens compared with the others fungi and could be caused dead termite 100% in couple of days.

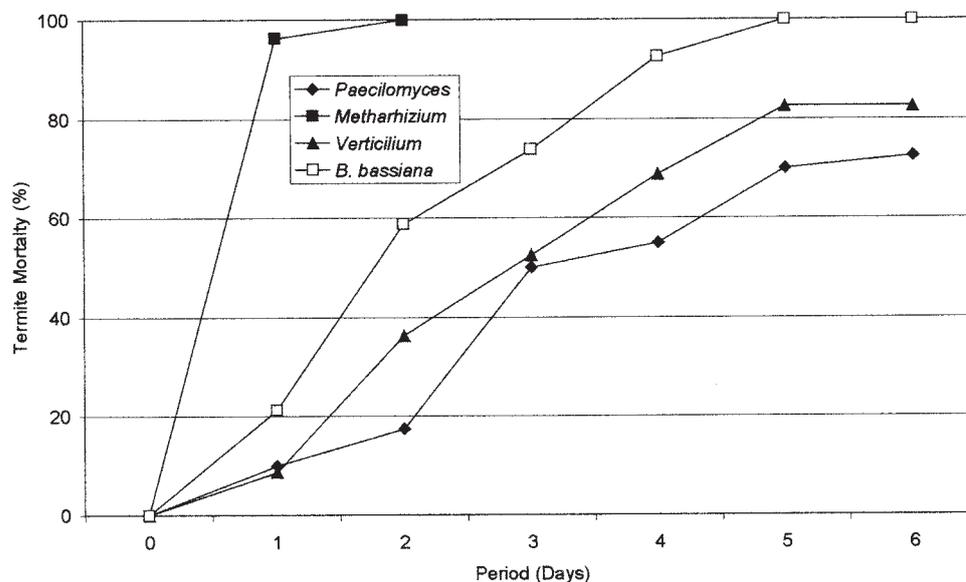


Fig. 1. The mortality of *Coptotermes sp* after infected by fungi from soybean pest (The termite mortality of control is 13.8%)

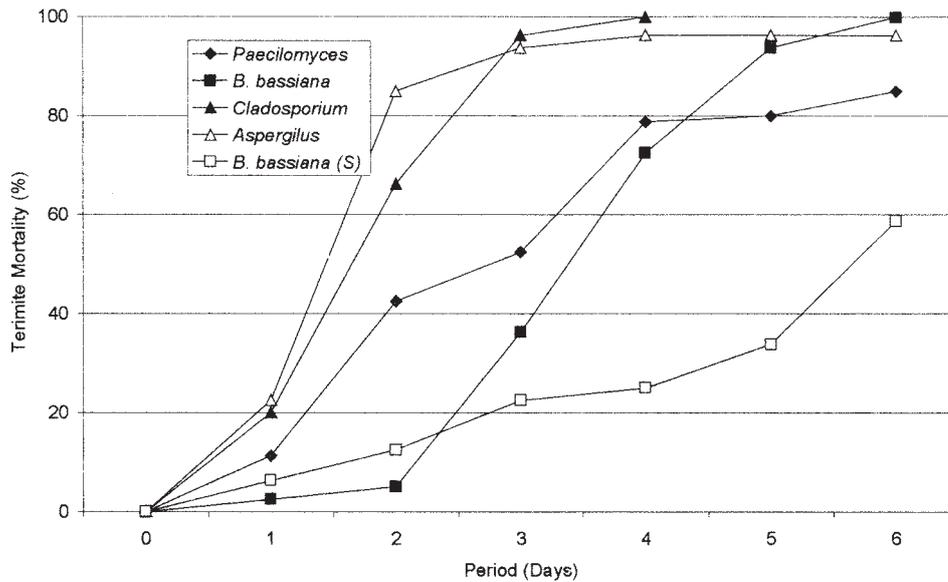


Fig. 2 The mortality of *Coptotermes* sp after infected by fungi from cabbage pest (The termite mortality of control is 13.8%)

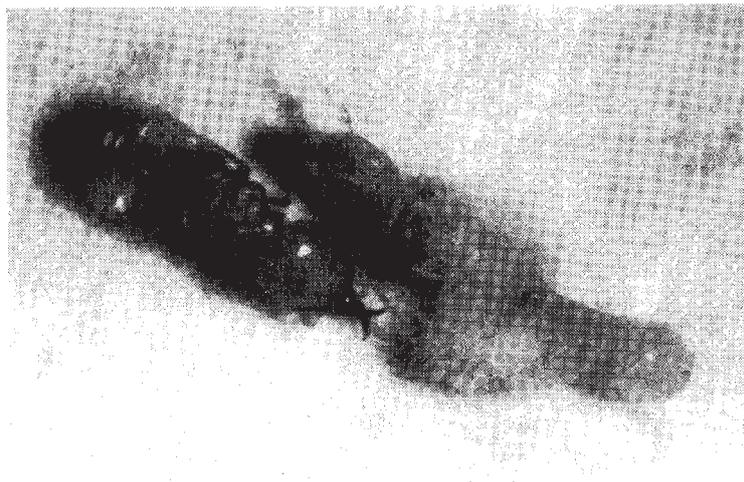


Fig. 3. The infected termites by *B. bassiana*

### Conclusion

The species of fungi were found from the agricultural pest are *Beauveria bassiana*, *Metharhizium anisopliae*, *Paecilomyces* sp, *Myrothesium* sp, *Cladosporium* sp, *Verticillium* sp and *Aspergillus* sp. From the result of bioassay were indicates mostly fungi are found from agricultural pest could attack the termites of *Coptotermes* sp. for 6 days.

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# Foraging Populations and Territories of *Coptotermes formosanus* Shiraki in Guangzhou

by  
Zhong Junhong, Xiao Weiliang and Huang Jingling  
Guangdong Entomological Institute, Guangzhou 510260, China

## Abstract

Foraging populations and territories of eight field colonies of the Formosan Subterranean termite, *Coptotermes formosanus* Shiraki, from Guangzhou, southern China, was investigated by a mark--release--recapture method in 2001—2002. Foraging populations in eight colonies ranged from 144200 to 1509600 termites. Interconnected termite galleries were found to extend from 10 to 40 m and Foraging territories ranged from 35 to 486 m<sup>2</sup> per colony during the period of survey. These foraging population sizes and territories didn't exceed those reported for *Coptotermes formosanus* Shiraki in United States.

**Key Words:** *Coptotermes formosanus* Shiraki, foraging population, foraging territory, mark-release-recapture

## Introduction

The Formosan subterranean termite, *Coptotermes formosanus* (Shiraki), was first described as a species in 1909 from specimens collected in Formosa, China (Li *et al.*, 1989) and is now generally believed that the termite is probably native to southern China. This destructive species was apparently transported to Japan prior to the 1600s and to Hawaii in the late 1800s (Su and Tamashiro 1987). By the 1950s, it was reported in South Africa and Sri Lanka. During the 1960s it was found in Texas, Louisiana, and South Carolina. *Coptotermes formosanus* are the most important economic termite in southern China and cause damage to timbers in buildings, to synthetic materials, to underground cables, to living trees and to crops. In Guangdong Province and Hainan Island, the percentage of termite-infested hours is over 90%, in other areas such as Guangxi, Hunan, Fujian, Jiangxi, about 60%--70% (Li *et al.* 1989).

Understanding the population dynamics of Formosan subterranean termite over time could give indication of treatment efficacy for strategies aimed at controlling the serious pest (Su 1994). In addition, documenting termite colony population fluctuations and correlating these with environmental conditions could allow researchers to develop models for predicting the potential for structural damage by termites (Forschler 1996). In China, Census of excavated nest of *Coptotermes formosanus* Shiraki was conducted by scientists in Guangdong Entomological Institute in 1970 (GDEI 1979). Excavation methods destroy termite nests, making it impossible to continuously monitor the population dynamics of termite colony (Su 1988) and ignoring the foraging populations and escaping populations when nests were disturbed. Mark—recapture methods was used by Lai (1977) and Su *et al.* (1984, 1988 ) for the Formosan subterranean termite and by Esenther( 1980), Forschler *et al.* (1996), Thorne *et al.*(1996) and Tsunoda *et al.* (1999) for subterranean termites. Foraging distances of The Formosan subterranean termite, *Coptotermes formosanus* (Shiraki), were estimated by tracing iodine radioisotope—labeled foragers in the their tunnels in Guangzhou, southern China ( Li *et al.* 1982). The radioisotope—labeled method is not suitable for estimating *Coptotermes formosanus* in crowded urban environments where nests and foraging activities mainly occur under concrete slabs and inside the buildings (Su 1988). Consequently little was known about the foraging populations and territories of *Coptotermes formosanus* in urban environments in china.

We conducted a survey on the foraging populations and territories of *Coptotermes formosanus* using the mark--release—recapture method on an uptown in Guangzhou in 2001—2002.

## Materials and Methods

Colonies of *Coptotermes formosanus* for estimations of foraging population and territory were located at two plots in same uptown in the suburb of Guangzhou. Termites attacked the wooden framework such as doorframes, window frames, ceilings, floors and cupboards in houses. Pine stakes pointed at one end (*Pinus massoniana*, 4 x 4 x 30 cm) were driven into the ground at ca. 3 m intervals around the houses, leaving ca. 5 cm exposed above ground for checking. Check stakes weekly. When infested stakes were checked, move the soil around the infested stake, place a plastic bucket with lid and bottom removed (25 x 25 x 30 cm), lay ca. 6 pine boards (1.8 x 20 x 25 cm) in the bucket and cover the lid. After a number of monitoring stations were set up, a mark--release--recapture program was conducted to estimate of foraging populations and territories of *Coptotermes formosanus*. In order to differentiate the colony at the same plot, two dyed filter papers were used in the study. Filter papers in a diameter of 9 cm were treated with 0.05% Nile Blue A solution (wt/wt) or 0.25 Neutral Red solution (wt/wt). Collected termites from four monitoring stations/stakes (two stations/stakes which had more termite activity and were at most intervals each plot ) were placed in petri dish (9 cm diameter) and respectively fed on filter paper containing 0.05%(wt/wt) Nile Blue A or 0.25%(wt/wt) Neutral Red for three days. During dyeing, termites were maintained in dark environmental chamber at 27±0.2 °C and 85±2% RH. Marked workers were counted and returned to the original station/stake. Foraging territories were mapped by the stakes and monitoring stations from which marked workers were recovered after the release. At least four successive collections were made from all stations in both plots. A weighted mean model was used to estimate the foraging populations (N) and associated error (SE) (Su et al. 1993; Tsunoda et al 1999):

$$N=(\sum Mi ni)/[(\sum ni)+1];$$

$$SE=N*[1/(\sum ni+1)+2/(\sum ni+1)^2+6/(\sum ni+1)^3]^{1/2}$$

Where for each *i*th, *ni* is the number captured, *mi* is the number of marked individuals among captured termites, and *Mi* is the total number of marked individuals up to the *i*th cycle.

## Results and discussion

About 120—180 pine stakes were used for each plot (start on 3 June 2001). At plot C, the ratio of infested stakes was 27.60% 10 days after (check on 13 June 2001) and the ratio of infested stakes was up to 78.75% 23 days after (check on 26 June 2001). At plot E, the ratio of infested stakes was 15.8% 10 days after (check on 13 June 2001) and the ratio of infested stakes was up to 81.34% one month after (check on 3 Jul. 2001) (Table 1). Monitoring stations were established (Fig. 1 C3--1 to 7; Fig. 2.C4--1 to 12) on 13 June 2001, termites were collected from 4 monitoring stations (Fig. 1 C3--6, C4--6, Fig. 2. E5/6--5, E1/2--6) on 26 June 2001 and marked termites were released into the monitoring stations from which they were originally collected on 30 June 2001. On 7 Jul. 2001, the foraging territories of colony I, IV, VI and VIII of *Coptotermes formosanus* were demonstrated by the 1<sup>st</sup> mark--release--recapture. The 2<sup>nd</sup> and 3<sup>rd</sup> mark--release--recapture from 7 Jul. 2001 to 9 Aug. 2001 showed that the foraging territory areas of colony I, IV, VI and VIII respectively occupied 81, 224, 122 and 386 m<sup>2</sup> and the linear foraging distances of colony I, IV, VI and VIII were up to 15, 40, 15 and 28m. Colony VIII was the mature, bestrode two house and connected 10 monitoring stations, at least 30 stakes outside house and countless infested points. Foraging territory area of colony IV was much smaller than colony VIII, but its linear foraging distance was the longest among above four colonies. From 7 Jul. 2001 to 9 Sep. 2001, the triple mark--release--recapture program was made for colony II, VII and IX, and from 30 May. 2002 to 22 Jul. 2002 for colony III, V and X. Table 3. presented the results of foraging territories of colony II, III, V, VII, IX and X. In this study we showed that *Coptotermes formosanus* colonies maintain distinct frontiers and share the same house but noninterference in each other (Figs. 1 & 2). The colony of *Reticulitermes spp* was incompatible with the colonies of *Coptotermes formosanus* and was extruded by two *Coptotermes formosanus* colonies to form narrow territory (Fig. 1).

Foraging populations of eight *Coptotermes formosanus* colonies surveyed in two residential plots

ranged from 144200 to 1509600 termites (Table 3). Foraging populations of colony III and VII were not shown in Table 2. owing to the data of these two colonies was unsound and Colony VII was *Reticulitermes*. Colony I, VIII and X were mature colonies and contained 899000 to 1509600 termites. The estimates of the foraging population showed a significant correlation between brisance and foraging populations of *Coptotermes formosanus* because three mature colonies caused heavy damages to the houses in which termites ranged from the 1<sup>st</sup> to 3<sup>rd</sup> floors and destroyed all wooden framework such as doorframes, window frames, ceilings, floors, cupboards and so on. Although Colony IV and IX presented biggish territories (224 and 206 m<sup>2</sup> in Foraging territory area, 40 and 19 m in linear foraging distance respectively), they possessed less foraging populations than the mature colonies and only infested on the 1<sup>st</sup> floor (Table 2).

Our estimates of foraging territory in Guangzhou, southern China, were distinctly smaller than those reported previously for *Coptotermes formosanus* occupying several thousand square meters and foraging across a distance of over 100m in United States. (King & Sping 1969, Lai 1977, Su et al. 1988), but similar to those reported by Li in Guangzhou, southern China (Li 1976).

The results also indicated the biomasses of foraging populations of eight *Coptotermes formosanus* colonies were between  $1.4 \times 10^5$  and  $15 \times 10^5$  individuals and most of estimated *Coptotermes formosanus* colonies contained much less  $10 \times 10^5$  foragers and the colonies of *Coptotermes formosanus* in Guangzhou were much smaller than those reported by Su et al. in United States (Su et al. 1988; Yates 1990).

As endemic species in southern China, *Coptotermes formosanus* may possess a high density of small termite colonies. With regard to the possibility of using baits to eradicate termite colonies, a small number of baits might be sufficient to distribute a toxic agent throughout a large termite foraging population (Grace 1989). By contraries, it would be more difficult for us using baiting techniques to eliminate *Coptotermes formosanus* colonies with high density and small population in Guangzhou, southern China. The studies implied that we would use more baiting stations in one house before understanding the foraging territories of *Coptotermes formosanus* in Guangzhou

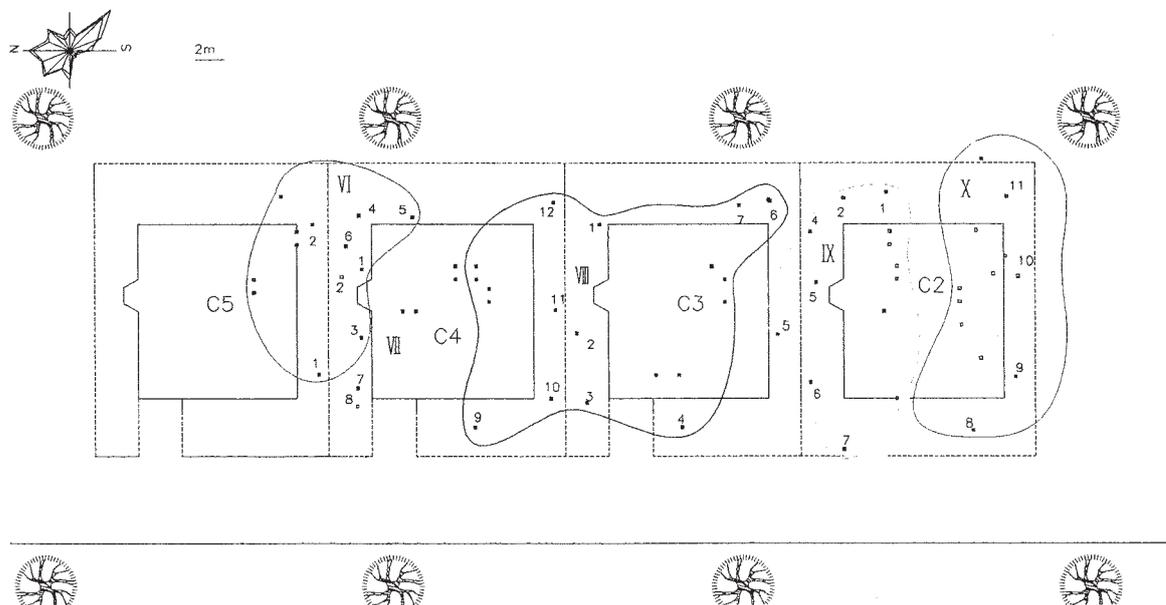


Fig 1. Foraging territories of *Coptotermes formosanus* (Colony VI, VIII, IX and X) and Foraging territory of *Reticulitermes* spp. in plot C in the suburb of Guangzhou

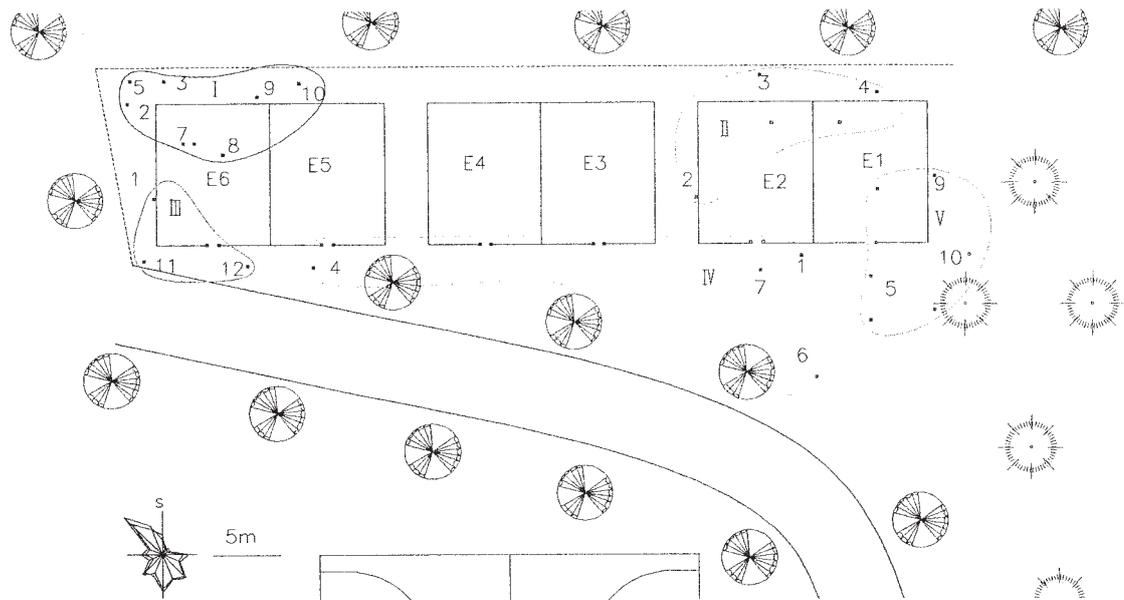


Fig 2 Foraging territories of *Coptotermes formosanus* (Colony I, II, III, IV and V) in plot E in the suburb of Guangzhou.

Table 1. No. of monitoring stations and ratio of infested stake at plot C & E

Colony	Survey period	No. of monitoring station	Ratio of infested stake
I	3 Jun.-9 Aug.2001	E5/6--2, 3,5,7,8,9,10	81.34% at plot E
II	7 Jul. - 9 Aug. 2001	E1/2--2, 3, 4	
III	30 May. -22 Jul. 2002	E5/6--1,11,12	
IV	3 Jun.-9 Aug.2001	E5/6--4; E1/2--1, 6, 7	
V	30 May.-22 Jul. 2002	E1/2--5, 9, 10	
VI	3 Jun.-9 Aug.2001	C5--2, 3; C4--1, 2, 3,4,5,6	78.75% at plot C
VII	7 Jul. - 9 Aug. 2001	C4--7, 8	
VIII	3 Jun.-9 Aug.2001	C4--9, 10, 11, 12; C3--1, 2, 3,4,6,7	
IX	7 Jul.-9 Aug. 2001	C2-1, 2, 3, 5, 6, 7; C3--5	
X	30 May.-9 Jul. 2002	C2--8, 9, 10, 11	

Table 2 Foraging territory areas and linear foraging distances of Formosan subterranean termite in residential areas

Colony	Survey period	Foraging territory(m <sup>2</sup> )	Perimeter(m)	linear Foraging distance(m)
I	3 Jun.-9 Aug.2001	81	37	15
II	7 Jul. - 9 Aug. 2001	97	43	17
III	30 May. -22 Jul. 2002	42	26	10
IV	3 Jun.-9 Aug.2001	224	90	40
V	30 May.-22 Jul. 2002	94	36	13
VI	3 Jun.-9 Aug.2001	122	43	15
VII	7 Jul. - 9 Aug. 2001	35	30	14
VIII	3 Jun.-9 Aug.2001	386	72	28
IX	7 Jul.-9 Aug. 2001	206	47	19
X	30 May.-9 Jul. 2002	229	55	21

Table 3 Number of total marked termites released ( $M_i$ ), number of termites recaptured ( $n_i$ ) and number of marked termites among those recaptured ( $m_i$ ) during a triple mark—recapture program.

Colony	$i$ th Mark—recapture	$M_i$	$n_i$	$m_i$	Foraging population (mean±SE)
I	1	3952	14650	64	899000±87423
	2	5937	3874	23	
	3	2204	6533	18	
II	1	2200	12810	128	212200±8247
	2	8934	7922	329	
	3	4629	9063	206	
III *					
IV	1	1920	3194	10	578400±34091
	2	10181	13505	231	
	3	4535	5322	48	
V	1	1705	3695	35	198900±13502
	2	2932	6650	78	
	3	3378	5258	105	
VI	1	1860	15120	237	144200±4373
	2	12468	8936	682	
	3	1855	9365	168	
VII*					
VIII	1	963	57203	39	1408400±84470
	2	5783	16985	64	
	3	9476	25439	176	
IX	1	3564	11559	102	411200±30069
	2	2793	7185	51	
	3	3837	4290	35	
X	1	5583	13537	38	1509600±137815
	2	2305	11835	16	
	3	6268	12974	67	

\* Lack of data in Colony III and colony VII.

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# Laboratory Bioassays with Termites – The Importance of Termite Biology

by

Michael Lenz

CSIRO Entomology, GPO Box 1700, Canberra ACT 2601 Australia; [Michael.Lenz@csiro.au](mailto:Michael.Lenz@csiro.au)

## Abstract

Subterranean termites are frequently used in bioassays to assess the effectiveness of insecticides or the resistance of materials. Termites which appear to be vigorous with high inherent levels of activity are often relied upon, yet at the end of the experiments survival may be very unsatisfactory, even in the favourable environment of controls. Consequently, results from such bioassays may be meaningless. Factors that can influence the vigorous performance such as: colony origin; quality of the termite supply; the physical environment; group composition and size; and the size of timber specimens, are discussed. The conclusion is that performance of a given termite source must be judged against standards and accepted levels of activity (i.e. for survival and wood consumption). Only if the experimental termites reach or exceed such minimum standards can a researcher be assured of the adequacy of the termite supply for the experiment in hand, and hence the reliability of the results of the assessment.

**Key words:** Subterranean termites, bioassays, effectiveness of termiticides, resistance of materials, performance standards

## Introduction

Many researchers choose to work with subterranean termites and use them for evaluating the effectiveness of insecticidal formulations and the resistance of materials. There are several reasons for this, quite apart from the economic significance of termites. Termites are the most active insects among wood-boring insects. Once placed in a 'test jar' subterranean termites will move around, construct tunnels and chambers throughout the soil or soil replacement substrate, build galleries along the container walls, and may cover the test specimens with a sheathing of their building material. These various indicators of termite activity can provide additional vitality measures during an experiment as well as the standard termite mortality and cellulose substrate consumption data recorded at the end of an experiment for assessing the performance of materials. Further, larger experiments can be quite readily established as it is often possible to obtain termites in significant numbers. In general, the handling of termites also appears straightforward.

However, all too often termites do not 'perform'. For example, in laboratory trials the survival of termites in untreated controls can be as poor as those in groups exposed to insecticide. This may indicate that the termites (both control and test) were not as vigorous as they were thought to be at the start of the experiment, thus preventing any valid conclusions on the performance of the experimental material. Presentations at recent IRG meetings, at other conferences and in the literature attest that this scenario is not that uncommon. In other words, termite behaviour can be quite deceptive. Any one isolated observation of termite activity may well seem to indicate that vigorous insects are used in the study, but it is only when we compare termites from different colonies, or termites freshly collected from the field with ones maintained in the laboratory for longer periods, or include reference materials with known resistance levels and accepted survival thresholds that we can see differences in termite vigour and performance. There is clearly more to termite biology than would first appear to be the case. It is worthwhile to raise the level of awareness about at least some of the factors that influence termite behaviour, and hence the reliability of results from assessments of materials and insecticides.

This presentation aims to review some of the factors that influence termite vigour and some of the measures researchers will have to take to ensure that termites perform at their best in experiments. The author and others have discussed many of the issues earlier (Becker 1962, 1970, 1976a; Lenz 1986; La Fage and Jones 1986; Lenz et al. 1993), however, all too often these issues tend to be ignored or sidelined to the detriment of the quality of data. The need to focus on these factors remains as great as ever.

## **Variables influencing termite vigour**

### ***Colony origin***

In the mid eighties, several researchers independently presented data which showed that termites of a given species taken from different colonies often do not respond uniformly to an experimental situation (Su and La Fage 1984; Creffield *et al.* 1985; Lenz 1985). Survival, wood consumption and building behaviour can vary significantly. In fact, wood consumption rates can differ as much within a species due to colony origin of the termites than between species (Lenz 1994).

The main consequence of these findings was that laboratory and field studies should be conducted with a minimum of three replicate termite sources (preferably more), in order to capture the range of possible responses from any given species. Inevitably, the size of such studies and the effort and cost involved in evaluating materials against termites would rise considerably.

The variability in termite response places question marks over any conclusions derived from assessments of an insecticide (or material) which relies on only a single source of termites, i.e. the single laboratory-held colony which an institution uses for such studies. One alternative to the latter scenario is for several laboratories (which each keep only one or two colonies of a key species for evaluations of materials) to combine their efforts and thus decrease the problem of 'termite source'. For example, this option could apply to laboratories in Europe working with *Reticulitermes santonensis*. In this context, it is interesting that the current draft of one of the European standards on wood preservative assessments with termites (prEN 117 2003) prescribes the use of three source trees of a given softwood species for the timber specimens (in recognition of variability between trees for preservative uptake etc.) but does not specify a requirement to use termites from more than one colony. The current draft standard has not changed from an earlier version despite ample evidence that 'termite source' is an important variable that has to be taken into account when designing experiments (see also Lenz *et al.* 1993).

### ***Quality of the termite supply***

Many laboratories routinely collect termites from the field at the time they require insects for a laboratory study; other institutions rely on termite supplies from laboratory-reared colonies. In general, termites freshly-collected from the field and used within a short time (up to a few months) after they were obtained tend to be most vigorous. Laboratory colonies maintained over long periods in a constant environment tend to lose some of their aggressive behaviour (to experimental materials) compared to termites just collected from the field (CSIRO unpubl. data). Likewise, termites obtained from the field and maintained under optimal conditions as groups of workers, soldiers and nymphs (i.e. without reproductives or without that reproductives develop from workers or nymphs) will become less active after six months or more of being held in the laboratory, and will hence be less suitable for critical assessments. In a comparative study using termites from different colonies but each with a widely separate collection date, any inter-colony differences may be confounded by the varying extent to which vigour in colonies may have declined as a result of the differing lengths of laboratory confinement.

In other words, termites kept as groups have a "use-by date" although even if they may appear to be vigorous insects judging just from the inherent high level of mobility in subterranean termites. This inherent level of activity remains evident in termites even when their general health has been in decline or the termites have grown old. Clearly, termite performance has to be viewed in relative terms. Performance rather than appearance is the key in judging termites. Performance of a given termite source has to be compared against standards and accepted levels of activity (i.e. for survival and wood consumption). Only if the experimental termites reach or exceed such minimum standards can a researcher be assured of the adequacy of the termite supply for the experiment in hand (see for example prEN 117 2003).

When assessing the relative vigour of a given termite source it is important to include reference situations that will place the groups under stress. Common practice is to have as controls (and indicators of termite health and hence the validity of the results), groups supplied with a piece of highly susceptible timber (for example prEN 117 2003). Survival and wood consumption or wood consumption rates (g wood consumed/g termites) have to be at or above a pre-set threshold. However, termite performance under such ideal conditions may tell little of the ability of the termite source to deal with less susceptible materials or even insecticide treatments. Indeed, under such conditions, groups may quickly collapse despite satisfactory performance in the controls. Performance against additional reference materials treated with a sublethal dose of a commonly-used insecticide or a moderately resistant timber or other material will be more telling of the vigour of a given termite source than just supplying them with a preferred food.

The value of this approach in separating active from less active sources of termites was demonstrated a long time ago by comparing five colonies of *Coptotermes formosanus* kept on either a highly susceptible or a moderately resistant timber. While all colonies had reached or were well above a pre-set survival threshold of 50% (53.2 to 77.7%) on the susceptible timber, groups from the colony with lowest survival on the susceptible timber collapsed when kept on the resistant timber, while groups from the other four survived. The overall conclusion was that performance of termite groups on one timber did not allow predictions to be made on their performance on another timber due to strong termite-source/timber-type interaction (Lenz and Dai Zi-Rong 1985).

### ***Maintenance conditions***

It becomes very important to identify the best maintenance conditions for the selected species of termite, not only as far as the obvious temperature and moisture requirements are concerned, but also preferred food timber, suitable matrix (soil, sand, nest carton, vermiculite, urethan foam etc.), use of containers of a size in the right proportion to the number of termites (i.e. termite behaviour and vigour are affected by the number of individuals per volume = 'density', see overview by DeSouza and Miramontes 2004). Even container shape and the material from which the containers are made can affect termite performance. For example, termites will perform better (survival and wood consumption) in containers with a circular base and made of glass compared to ones with a rectangular base or produced from certain plastics (see review in Lenz *et al.* 1993). There is no doubt, that defining the optimal maintenance conditions for a given termite species can be a rather tedious, time-consuming task (Lenz *et al.* 1987), and for that reason it is often not, or only incompletely carried out.

One common pitfall is to use 'vermiculite' (laminar aluminium-iron-magnesium silicate) as a soil replacement substrate. However, there are so many different types of vermiculite available. While all are non-nutritious and have a high water-holding capacity, thus making them ideal for longer-term keeping of termite groups or colonies, some contain other ingredients that render them unsuitable, even lethal to the insects. Hence, operators have to be sure to obtain a supply that has no such side effects.

All too often experimental conditions are chosen for the convenience of the operator rather than for meeting termite requirements for optimal performance. While practical considerations often may be paramount, this is acceptable only if the impact and limitations on termite performance are recognised and acknowledged when drawing conclusions from laboratory studies, and when trying to extrapolate from laboratory-derived data to a field situation. Operators should nevertheless be guided by the rule of making conditions for termites as field-like and as optimal as possible.

Defining optimal maintenance conditions may go beyond the obvious components listed above. Various less apparent physical factors such as magnetic and electric fields can strongly influence insect behaviour and well-being (Becker 1976b; Becker and Gerisch 1973; Starrick *et al.* 2005). Daily fluctuations of physical fields, largely outside our control, can impact on daily wood consumption patterns. The impact of such changing physical fields (on e.g. the rates of attack on materials) can be compensated for by running experiments for at least four weeks, preferably eight weeks (Becker 1976a). Specific physical fields inside different brands of environmental cabinets, around air conditioning and other equipment in a room can favour or hinder termite activity as well (Becker 1979). One implication can be that when running choice trials termites may avoid the side of an experimental arrangement close to e.g. an air conditioner, or groups in such a position may

have lower activity levels compared to groups some distance apart from the equipment. Thus placement of the experimental containers within a room may be critical for the outcome of a study. Randomising the position of experimental groups within a room and as far away from potentially disturbing equipment may assist in alleviating such influences. Further, if effects of temperature are evaluated using different types of environmental cabinets (or combinations of climate-controlled rooms and cabinets), it may first be necessary to compare all facilities at one temperature to ensure uniform termite response before temperature effects are evaluated in an experiment.

### ***Group composition and size***

Termites are social insects and function best in populous groups with a mix of castes such as workers and soldiers. Their performance is critically linked to the nest which houses the reproductives, brood, younger worker stages and nymphs and individuals of all castes at the time of moulting. Foragers (workers) tend to collect food in amounts that meet their own requirements and an excess for provisioning of dependents in the nest. If groups of termites are kept without the link to the nest, (the most common experimental situation in material assessments) their need to feed, i.e. attack a material, is reduced to merely meeting the demands for self maintenance - one of the reasons for generally lower attack rates on timber and other materials in the laboratory compared to the field. In other words, termites, as part of a colony with all castes, especially with reproductives present, will function differently to groups of termites that are isolated from their nest, the reproductives and brood.

In this context, it is worthwhile to point out that the term “colony” should be restricted to experimental termite assemblages containing all castes – including the reproductives. Any other termite assemblages that exclude reproductives should not be termed “colonies” but simply “groups”. Unfortunately, many publications and standards use these terms interchangeably, referring to “cultures” when meaning entire laboratory-maintained colonies with all castes, and term experimental groups as “colonies” - as for example in prEN 117 (2003). There are significant implications for the behaviour of a termite forager if it is part of a full colony or is only a member of an isolated group.

In a termite colony the different castes are present in certain ratios to each other. Many experimental procedures aim to address this situation by recommending the addition of given numbers of soldiers and nymphs to groups of workers; otherwise some workers will develop via presoldiers into soldiers to restore the natural worker : soldier ratio. These individuals will be lost to the forager population.

Access to termite supplies varies between institutions. Some can collect large numbers of termites from the field; others can only use termites they are able to rear in the laboratory. Under the latter circumstances, it is understandable that experiments are often conducted with small groups of termites from 10 to a couple of hundred individuals. But it is often surprising that institutions with ready access to termites fresh from the field, especially in light of published population estimates of several million foragers in colonies (e.g. reported for *Coptotermes formosanus* in the USA (Su and Scheffrahn 1988; Grace *et al.* 1995) nevertheless carry out laboratory experiments with small groups of termites. This contrasts with the practice in Australia and China of using groups numbering several hundred to several thousand individuals in assessments of materials.

The convenience of establishing less labour-intensive bioassays, or the option of running large experiments with small termite numbers for each experimental unit, appear to be among the main motives for restricting group size. The underlying assumption is that termites, irrespective of group size, will function in the same way. Indeed, even small groups of termites will aim at ‘organizing’ their environment, i.e. they will tunnel into the substrate, commence foraging etc.. But in a termite colony at any given time, only part of the population will be active and involved in specific tasks, (such as constructing exploratory tunnels through the soil, gathering food or provisioning brood). Other colony members may be resting or preparing to moult. No doubt there is a turn-around with individuals switching back and forth between being active and resting.

The smaller the group, the more pressure is on all members to perform basic tasks (such as establishing a system of tunnels and chambers, searching and gathering food and feeding the dependent castes - soldiers, nymphs) more frequently than any individual may do under natural conditions. As a result, energy expenditure and stress levels may be higher for individuals that are

members of smaller rather than larger groups. Many years ago, Becker (1970) pointed out that reducing group size too far makes no sense since small groups will be less active, possible attack on test materials is more difficult to recognize and the reliability of results can be doubtful. The mortality rate of termites in small groups, even without exposure to termiticides, will be higher and their ability to withstand starvation will be less than in groups with more individuals (see also Santos *et al.* 2004).

Jones (1990) compared the capacity of smaller and larger groups of *Coptotermes formosanus* (100 versus 1000 individuals) to tunnel through soil treated with different termiticides. The larger populations always tunnelled deeper into the treated soil, yet mortality in the larger groups was not necessarily greater than that recorded for the smaller groups. With more potential foragers available in the larger groups each one of them may receive only limited exposure to the insecticide-treated soil, and hence receive only a smaller dose.

Another example of this phenomenon for differences in survival of groups of the Australian *C. acinaciformis* after exposure to fipronil-treated soil is given below (Fig. 1). The groups of 100

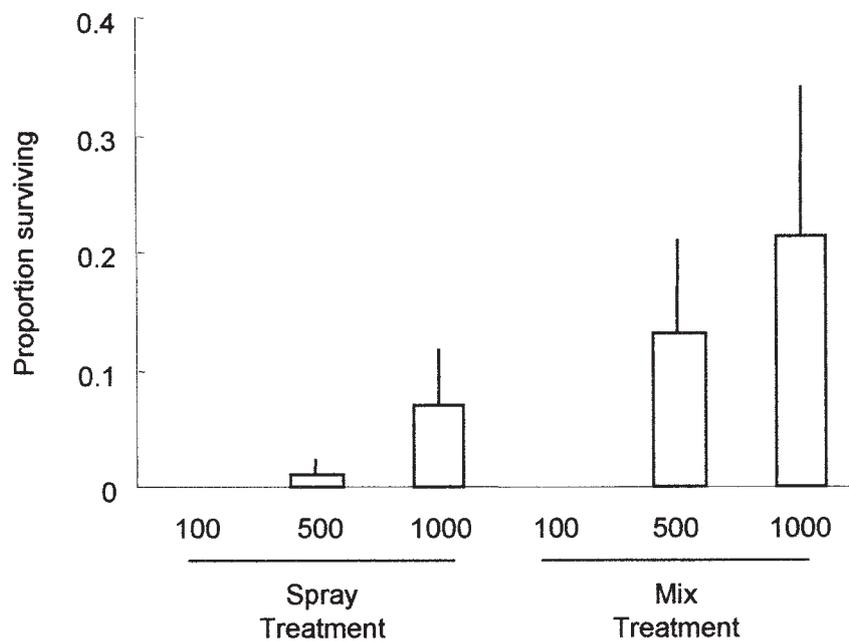


Figure 1: The proportion survival (mean  $\pm$  standard error) of *Coptotermes acinaciformis* groups of different size from Darwin (Australia) in 3.0 g a. i. m<sup>-2</sup> (spray) and 18 g a. i. m<sup>-3</sup> (mix) suspended concentrate formulation fipronil treated soil. N.b. for treatments: s = spray, m = mix applications; numbers indicate group size (CSIRO unpubl.).

termites hardly penetrated into the treated soil, (at the most a few millimetres irrespective of treatment) while groups of 500 or 1000 termites entered for 30 and 50 mm respectively in the case of the surface spray, and both larger groups for around 30 mm into soil mixed with chemical.

The survival (%) of groups at the end of the experiment differed significantly between group size: groups of 1000 termites in the soil/chemical mix had significantly higher survival than groups of 100 termites in either spray or soil/chemical mix (Fig. 1).

This means that a prediction about the potential performance of an insecticide in the field from laboratory data obtained with groups of either 100 or 1000 termites would result in very different conclusions (see also Santos *et al.* (2004) on the effect of group size on insecticide-driven stress in termites).

#### **Size of timber test samples**

In termite experiments with untreated or treated timber, the size of the blocks that are offered to termites can vary considerably between laboratories, protocols or other circumstances. As a rule, the block dimensions are such that termites have an excess of wood left by the end of the experiment

while at the same time the experimenter tries to keep the dimensions as small as possible. However, termites increase their wood consumption rate (= g wood removed/g termites) with every increase in the volume of offered timber, i.e. they adjust their feeding rates to the amount of available food. One example of such a correlation is given in Fig. 2 for three group sizes of *Reticulitermes speratus* (Lenz *et al.* 2003).

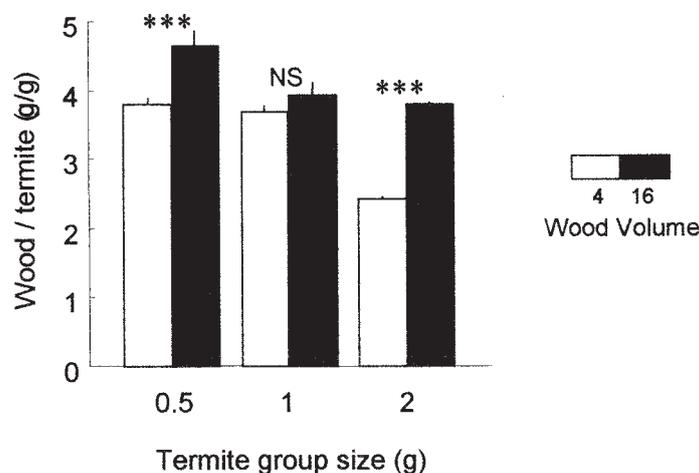


Figure 2. The amount of wood consumed per gram of termites in the initial group (mean  $\pm$  standard error) for each group size and wood volume (number of blocks). NS = not significant, \*\*\* =  $p < 0.001$  (from Lenz *et al.* 2003)

There is a further complication. What termite groups of a given size might consider a significantly large food supply tends to differ from the operator's assessment. Only quite large blocks of wood will trigger higher wood consumption rates. Confronted with small volumes of food, termites will feed at rather low, constant rates. The past and current practice of using test specimens with a volume of no more than 50 cm<sup>3</sup> (in most cases only 20 cm<sup>3</sup> or less) means that the termite hazard which wood-based materials may experience in the field is only poorly simulated in most laboratory assays with timber products (see overview Lenz 1994). The correlations described here between volume of food, termite numbers and consumption rates hold also true for experiments in the field (Lenz *et al.* 2001).

For example, this has significant implications for the design of bait stations for bait systems to manage active termite infestations. Stations that provide greater volumes of matrix have higher visitation rates (termite numbers) and matrix uptake, and in consequence greater amounts of the active ingredient will be taken up and returned to the colony within shorter periods of time (Lenz and Evans 2002; Evans 2005).

### General conclusions

Use of termites in laboratory assays is clearly not as simple and straightforward as many operators like to think. Termites display high inherent levels of activity even when aged or of declining health. This high mobility in termites can easily lead to a given termite source being classed as suitable for experiments when in reality these termites may show only poor survival even in favourable experimental controls, let alone that they could challenge in any significant way the test materials. Bioassays with termites of reduced vigour can only produce meaningless results. Termites do have "used-by" dates.

Performance of a given termite source has to be judged against standards and accepted levels of activity (i.e. for survival and wood consumption). Only if the experimental termites reach or exceed such minimum standards can a researcher be assured of the adequacy of the termite supply for the experiment in hand. Performance rather than appearance is the key in judging termites. Many factors can influence termite performance, and the range of factors discussed here is by no means exhaustive. It is up to the experimenter to address these issues and to define the conditions of a bioassay that will allow termites to be at their most vigorous.

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# Laboratory Evaluation of the Termite Resistance of Polyamide

by

Gregory Rosenblat<sup>1)</sup>, Won-Joung Hwang<sup>2)</sup> and Kunio Tsunoda<sup>2)</sup>

1) Arkema K. K., Kyoto Technical Center, SCB#3, Kyoto Research Park, 93 Chudoji Awatacho, Shimogyo-ku, Kyoto 600-8815, Japan

2) Research Institute for Sustainable Humanosphere, Kyoto University, Uji, Kyoto 611-0011, Japan

## Abstract

Termite resistance of bar and film specimens of 4 kinds of polyamide was evaluated by Japanese standard methods in the laboratory. Specimens were separately exposed to workers of *Coptotermes formosanus* for the desired period. Percent mass loss and termite mortality were measured for bar specimens together with visual ratings after test. Termite penetration was examined for film specimens. All bar materials were free from termite attack after three weeks test duration. Films were intact after 30 days test duration regardless of heat aging ( $60\pm 2^{\circ}\text{C}$  for 3 months) of the test samples.

**Key words:** plastic bars, plastic films, polyamide, JWPS-TW-S. 1, JWPA Standard No.17, *Coptotermes formosanus*

## Introduction

Termite resistance of plastic materials has not been tested extensively in the laboratory. However, Japanese standardized methods seem applicable to evaluate the termite resistance of plastic materials. Among Japan Wood Preserving Association (JWPA) standard, JWPS-TW-S. 1 (1992), which has been originally designed for determining the termiticidal efficacy of chemicals applied superficially to wood, is applicable to evaluate termite resistance of plastic bar materials. JWPA Standard No. 17 (1992), which is related to the method to evaluate the termite resistance of non-woody materials treated with termiticides, can assess the resistance of plastic films against termite penetration. In the current investigation polyamide bars and films were tested for their resistance against termite by Japanese standard methods in the laboratory.

## Materials and methods

### *Laboratory termite test with plastic bar materials*

Four kinds of test bar materials were prepared in a dimension of 2.5 x 1.0 x 0.4 cm (Table 1). Laboratory bioassay was conducted under a forced feeding condition (nothing but a test specimen as food) according to JWPS-TW-S. 1. A single specimen was placed at the center of plaster bottom of an acrylic cylindrical test container together with 150 workers and 15 soldiers of *Coptotermes formosanus* Shiraki. A plastic net was inserted between the test specimen and the plaster bottom to

avoid direct contact with each other. The test unit was placed on the water-moistened cotton pad in a larger container and incubated at  $28\pm 2^{\circ}\text{C}$  for three weeks in the dark. Following three weeks' exposure, test specimens were recovered and cleaned off surface debris with tap water for the subsequent visual inspection. Number of live worker termites was counted to determine mortality. Each recovered specimen was air-dried and reweighed for the determination of percent mass loss so that mean percent mass loss of each test material group was calculated for comparing the termite resistance among plastic specimens tested. Five replicates of each material group were tested. Five replicates of the reference material (sugi sapwood,  $2 \times 2 \times 1$  cm) were also tested for monitoring feeding activity of termites.

Table 1 Test plastic materials

Bar No.	Film code	Common name (material description)	Trade name
1	A	Amorphous polyamide	Cristamid® MS 1700
2	B	Polyamide 11 (reference)	Rilsan® BECV TL
3	C	Polyamide 12 (reference)	Rilsan® AECV black T8L
4	D	Polyamide 12 (plasticised)	Rilsan® AECV black P40 T8L

#### Laboratory termite test with plastic film materials

Termite resistance of 4 kinds of plastic film materials ( $150 \mu\text{m}$  in thickness) (Table 1) was evaluated in the laboratory according to the standard test method [JWPA Standard No. 17 (1992)].

Laboratory evaluation was conducted according to JWPA Standard No. 17 (1992). A film previously exposed to aging process ( $60\pm 2^{\circ}\text{C}$  for 3 months) was sandwiched by two containers to determine the resistance against termite penetration (Figure 1). Approximately 150 g of nest materials (20-100 mesh) was put into in the lower incubation chamber with a piece of red pine

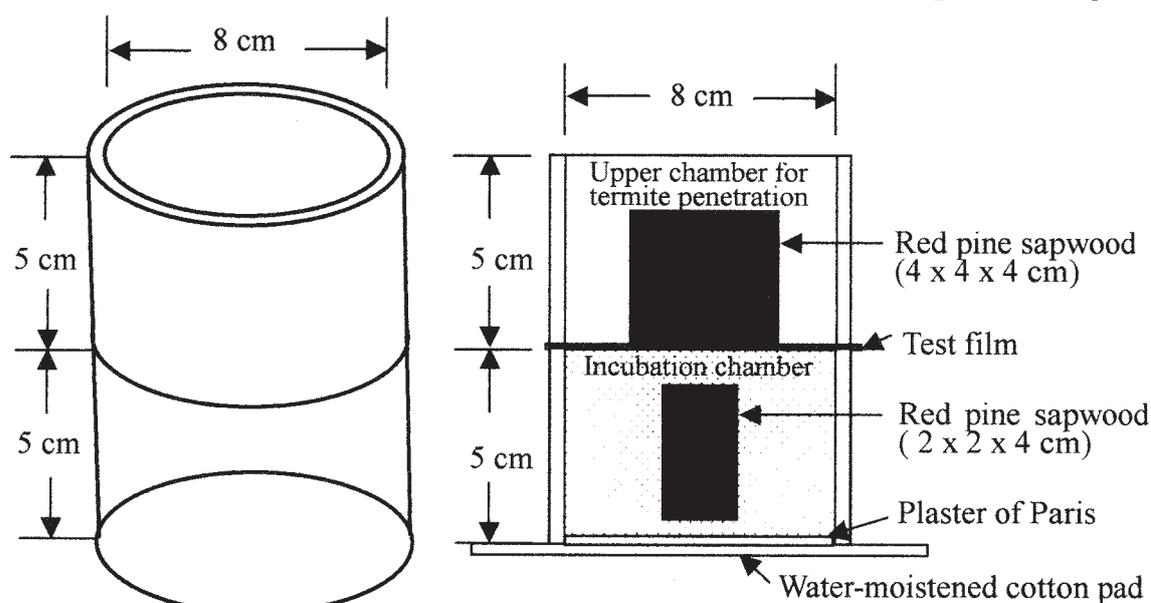


Figure 1 Experimental apparatus for determining the resistance of test materials to termite penetration

sapwood in the center, and 50 ml water was added to the medium. Three hundred workers and 30 soldiers of *Coptotermes formosanus* Shiraki were introduced into this assembled incubation chamber and the chamber was kept at  $28\pm 2^{\circ}\text{C}$  until termites show their high activity inside the chamber. A test film was then placed on the top of the incubation chamber and covered with another chamber to provide termites with an arena for penetration through the test material. After the initiation of the bioassay regular inspection was conducted every three days for 30 days. When the termite succeeded in penetrating the test material, the period required for penetration was recorded. A single replication was prepared for each test material. In the current study, materials, which were not aged, were also tested.

## Results and discussion

### *Laboratory termite test with plastic bar materials*

Results are shown in Table 2. No attack was found on any test material, although relatively higher mortality was recorded with plastic bars when compared with that of a reference material, sugi sapwood.

Table 2 Termite resistance of plastic bar materials

Bar number	Mass loss (%) $\pm$ SD	Mortality (%) $\pm$ SD	Visual rating*
1	0 $\pm$ 0	29.9 $\pm$ 4.15	10, 10, 10, 10, 10
2	0 $\pm$ 0	32.8 $\pm$ 5.57	10, 10, 10, 10, 10
3	0 $\pm$ 0	29.2 $\pm$ 5.24	10, 10, 10, 10, 10
4	0 $\pm$ 0	27.9 $\pm$ 6.74	10, 10, 10, 10, 10
Sugi sapwood (2 x 2 x 1 cm)	23.8 $\pm$ 3.30	10.3 $\pm$ 1.21	4, 4, 4, 4, 4

10: sound (no attack), 9: very slight attack (surface nibbling), 7: moderate attack, 4: heavy attack, 0: failure (destroyed, no more available for further testing)

### *Laboratory termite test with plastic film materials*

#### *(1) Test with not aged materials*

All test termites were dead within 10 days after the initiation of bioassay when film materials A and C were tested. There was no clear explanation regarding this unexpected death of termites. After 30 day test, other test materials were free from termite attack. Even nibbling scars were not found.

#### *(2) Test with aged materials:*

Even after aging, no termite penetration occurred in any test materials.

Reference Polyamide samples (bar numbers 2 and 3; film codes B and C) were not attacked by *Coptotermes formosanus* Shiraki in the current test conditions. All materials could withstand termites in the specific conditions of the test. A plasticised material, more flexible, was also free from termite attacks (bar numbers 4; film codes D).

Since subterranean termites in the tropics attacked some polyvinyl chloride and polyethylene plastics (Beal and Bultman 1978), the additional field evaluation of the current test results is needed.

Polyamide products have been investigated to replace conventional outer sheathing of cables because they are thought to be better than existing materials in terms of termite resistance and mechanical properties such as abrasion resistance, chemical resistance, weather resistance, and heat resistance.

### **Conclusions**

The current laboratory test demonstrated that polyamide products were resistant to *C. formosanus*. However, field evaluations are needed to draw a final conclusion on the termite resistance of plastic materials because field evaluation previously demonstrated that termites penetrated some other plastic materials such as polyvinyl chloride and polyethylene plastics.

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# Transfer of Fipronil from Exposed Workers of the Subterranean termite *Coptotermes formosanus* (Isoptera: Rhinotermitidae) to Unexposed Workers

by

Kunio Tsunoda<sup>1)</sup> and Masatoshi Yokoyama<sup>2)</sup>

1) Research Institute for Sustainable Humanosphere, Kyoto University, Uji, Kyoto 611-0011, Japan

2) Bayer CropScience K. K., 4-10-8 Takanawa, Minato-ku, Tokyo 108-8572, Japan

## Abstract

A non-repellent termiticide, fipronil, was tested in the laboratory for its ability to be transferred from exposed workers of *Coptotermes formosanus* Shiraki to unexposed workers. The effect of ratio of exposed and unexposed termite workers on the transfer of toxicants was also examined. When termite workers were first exposed to fipronil for the desired duration and mixed with unexposed workers at a ratio of 1:1, the mortality of fipronil-exposed workers reached over 90% after 5 days of incubation regardless of the treatment concentration (0.1~10.0 ppm) and the exposure period, while few unexposed workers died within the first week. At higher treatment concentrations (1.0 and 5.0 ppm), mortality increased with time and was approximately 60% and 90% after 21 and 28 days, respectively. At an exposed vs. unexposed mixing ratio of 1:10, the changes in the mortality of exposed termite workers were not much different from those at a mixing ratio of 1:1. The final mortality of unexposed workers ranged from 20 to 31% regardless of the concentration or duration of exposure in the first contact with fipronil-treated soil.

**Key words:** fipronil, non-repellent termiticide, transfer of toxicant, grooming, *Coptotermes formosanus*

## Introduction

Non-repellent termiticides are thought to act differently against termites than existing ordinary soil-poisoning agents. Since termites hardly detect the presence of toxicants, they should not hesitate to penetrate into soil treated with non-repellent termiticides. Toxicant that adheres to the body surface of termites will be transferred to their nestmates through grooming and/or trophallaxis.

The transfer of toxic substances from exposed termite individuals to nestmates has not yet been fully examined for any toxicant or termite species, although there is some limited information on the subject (Ferster *et al.* 2001; Thorne and Breisch 2001). In addition, the effect of termite behavior on subsequent death after exposure to toxins has not been examined. Since behavioral interactions among nestmates seem to be important for accelerating the transfer of toxins (Iwata *et al.* 1999), such transfer should be carefully tested for each termite species.

The current investigation was designed to determine the transfer of a non-repellent termiticide from previously exposed worker termites to unexposed workers of the subterranean termite *Coptotermes formosanus* Shiraki in the laboratory.

## Materials and methods:

### *Test termite*

Undifferentiated workers of *C. formosanus* were collected from a laboratory colony.

### *Initial exposure*

**Preparation of treated soil:** Sandy loam was treated with fipronil. The concentrations of active ingredient in the soil were 0.1, 0.5, 1.0, 5.0 and 10.0 ppm (m/m).

**Test containers and method of initial exposure:** Twenty workers were introduced into a test container (6 cm  $\phi$  Petri dish) with 5 g treated soil and 1.2 g distilled water, and forced to remain in contact with the treated soil for the required period of time (8 hours for 0.1 ppm and 1 hour for the remaining 4 concentrations). Approximately one-third of the duration that killed all of the test termite workers after exposure to treated soil was taken to obtain toxicant-exposed termite workers

Test containers were maintained in the dark at  $28 \pm 2^\circ\text{C}$  and  $>80\%$  relative humidity. Five replicates were prepared for each test concentration so that the required number of exposed workers was easily obtained for the subsequent exposure tests.

#### *Indirect exposure test*

**Test containers:** Plastic Petri dishes with diameters of 6 and 9cm were used for mixing ratios of 1:1 (exposed vs. unexposed) and 1:10, respectively.

**Discriminating between toxicant-exposed and from unexposed workers:** Unexposed workers were marked with Nile Blue A by a fast-marking technique. Termite workers collected from a laboratory colony were kept in a Petri dish without water until they lost 10% of their body weight. These workers were then fed filter paper that had been saturated with an aqueous solution of Nile Blue A (400 mg/l) for 24 hours to obtain dyed unexposed workers.

**Indirect exposure test at a mixing ratio of 1:1 (exposed vs. unexposed):** Exposed and unexposed termite workers (10 each) were placed in a Petri dish with a moistened filter paper at the bottom.

**Indirect exposure test at a mixing ratio of 1:10 (exposed vs. unexposed):** Ten exposed and 100 unexposed termite workers were placed in a Petri dish with a moistened filter paper at the bottom.

**Incubation:** Assembled dishes with lids on were placed in a container with moistened cotton pads at the bottom, and maintained at  $28 \pm 2^\circ\text{C}$  and  $>80\%$  relative humidity. Separate containers were used for each test termiticide.

**Number of replicates:** Three replicates were prepared for each test condition.

**Test period:** The maximum duration of incubation was four weeks, even if all of the test termites had not yet died.

**Measurement:** Numbers of dead termites were regularly counted. With a mixing ratio of 1:1 the number of live termite workers was separately recorded for exposed and unexposed termite workers, whereas the numbers of live exposed termites and dead unexposed workers were recorded with a mixing ratio of 1:10, and live workers were counted at the end of incubation.

#### *Effect of marking*

Termite workers exposed to untreated soil were regarded as untreated controls. Untreated controls were used in the tests as with treated soil samples to examine the effect of marking on termite activity.

## **Results and discussion**

### *Indirect exposure test at a mixing ratio of 1:1 (exposed vs. unexposed)*

The results with untreated controls are shown in Figure 1. No dead termites were found for the first 14 days, and a gradual increase in dead termites was observed afterwards. However, there was no any conspicuous difference in mortality between the exposed and unexposed groups. The final mortality rates for exposed and unexposed termite workers were 20% and 27%, respectively.

It is possible that exposed workers might show higher mortality because the termites may be injured when they were recovered from the soil and introduced to a Petri dish, which is thought to reduce the activity and soundness of termite workers (Thorne and Breisch 2001), however such differences in mortality were not recorded with a mixing ratio of 1:1.

Figure 2 shows the effect of fipronil on the mortality of termite workers after exposure to treated soil and the subsequent mixture with unexposed workers. The mortality of exposed workers reached over 90% after 5 days of incubation regardless of the treatment concentration and exposure period, while few unexposed workers died during the first week. After 14 days, mortality clearly increased at the higher treatment concentrations (1.0 and 1.5 ppm), and was approximately 60% and 90% after 21 and 28 days, respectively. These values were definitely higher than those in untreated controls. This suggested that some amount of fipronil adhered to the exposed workers and was transferred to unexposed workers before the exposed individuals died, since dead workers were carefully removed at every observation. Another explanation is that fipronil, which originally adhered to the exposed workers was passed indirectly to unexposed workers through the soil or test container. Since all of the termite workers were dead at 2-5 days with treatment at 10.0 ppm regardless of contact with the treated soil, these are not shown in Figure 2.

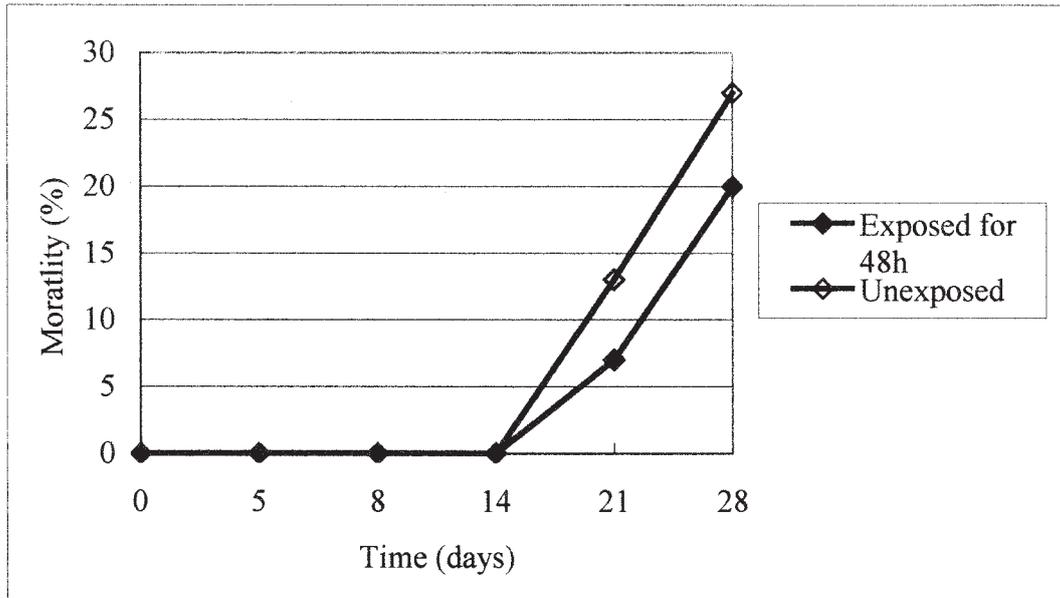


Figure 1 Mortality of exposed and unexposed termite workers after incubation on untreated soil (mixing ratio of exposed vs. unexposed = 1:1)

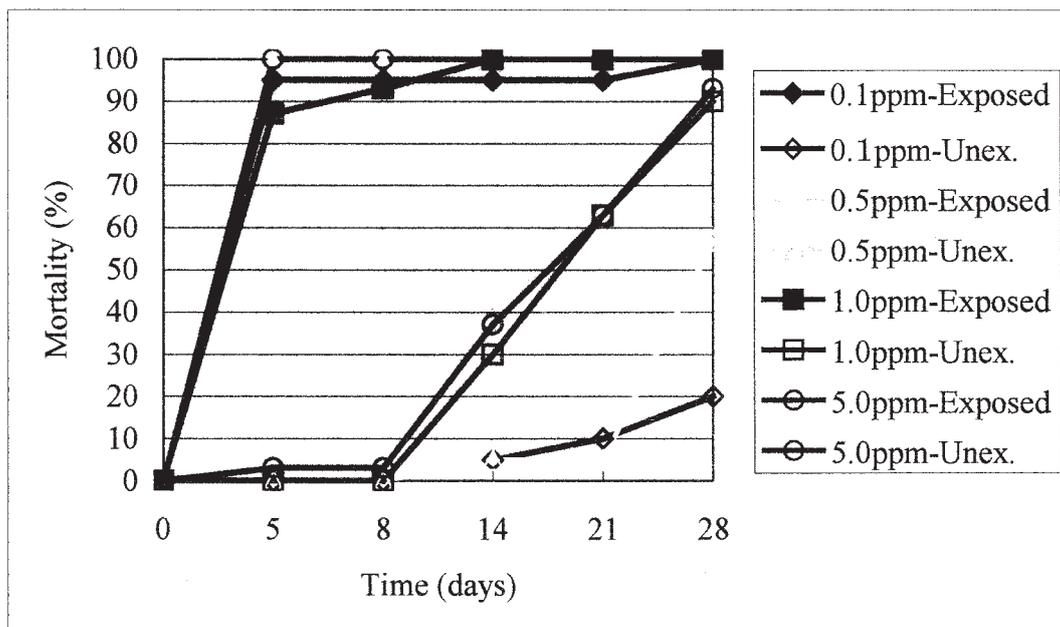


Figure 2 Effect of transfer of fipronil on the mortality of termite workers (mixing ratio of exposed vs. unexposed = 1:1)

**Indirect exposure test at a mixing ratio of 1:10 (exposed vs. unexposed)**

Figure 3 shows the results for the untreated control. The mortality of previously exposed termite workers was definitely higher than that of unexposed individuals. As suggested above, handling of exposed workers is thought to contribute to the reduction in termite activity and soundness. Cannibalistic behavior might also have reduced the number of live exposed termite workers after a certain period of time even when tested with untreated soil. These considerations can help to explain the higher mortality of >60% after four weeks.

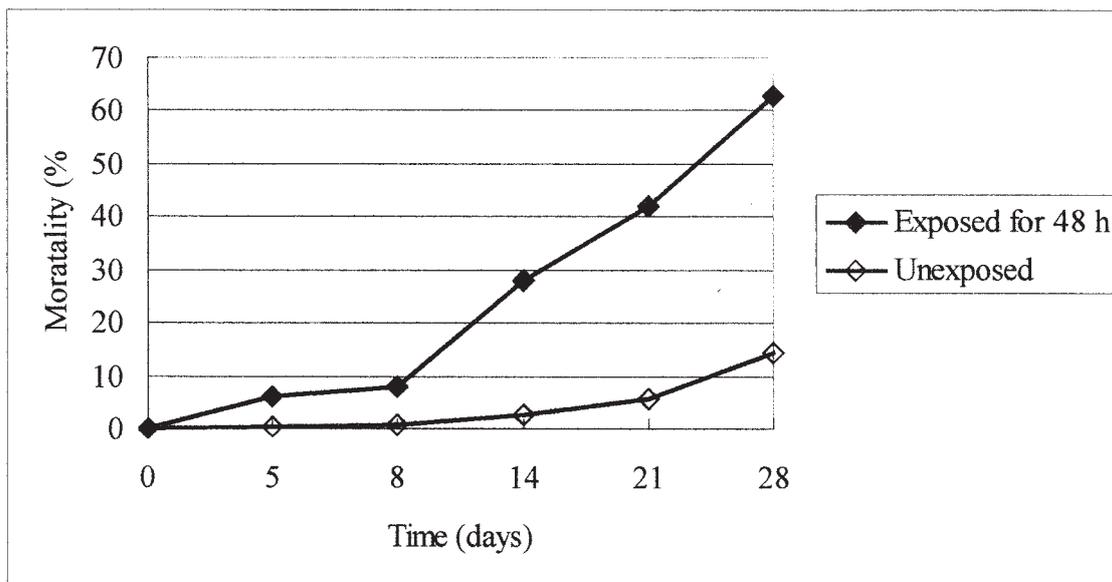


Figure 3 Mortality of exposed and unexposed termite workers after incubation on untreated soil (mixing ratio of exposed vs. unexposed = 1:10)

Figure 4 shows the results with fipronil tested at a mixing ratio of 1:10 (exposed vs. unexposed). The changes in the mortality of exposed termite workers were not much different from those at a mixing ratio of 1:1. This seemed quite natural because the direct cause of death in these exposed workers primarily depended on direct contact with test toxicants in treated soil.

The final mortality of unexposed workers ranged from 20-31% regardless of the concentration or the duration of the first contact with fipronil-treated soil at  $\leq 5.0$  ppm (Figure 4). At 10 ppm, the number of dead unexposed termite workers increased with time and reached 100% by the 14th day. Comparison of these data with those at a mixing ratio of 1:1 mixing ratio suggests that there was little dependence on the concentration except at 10 ppm due to the smaller relative amount of fipronil for each termite individual in the test dish. Although the low mortality of the unexposed workers also seemed natural, the number of dead workers was similar to that at a ratio of 1:1.

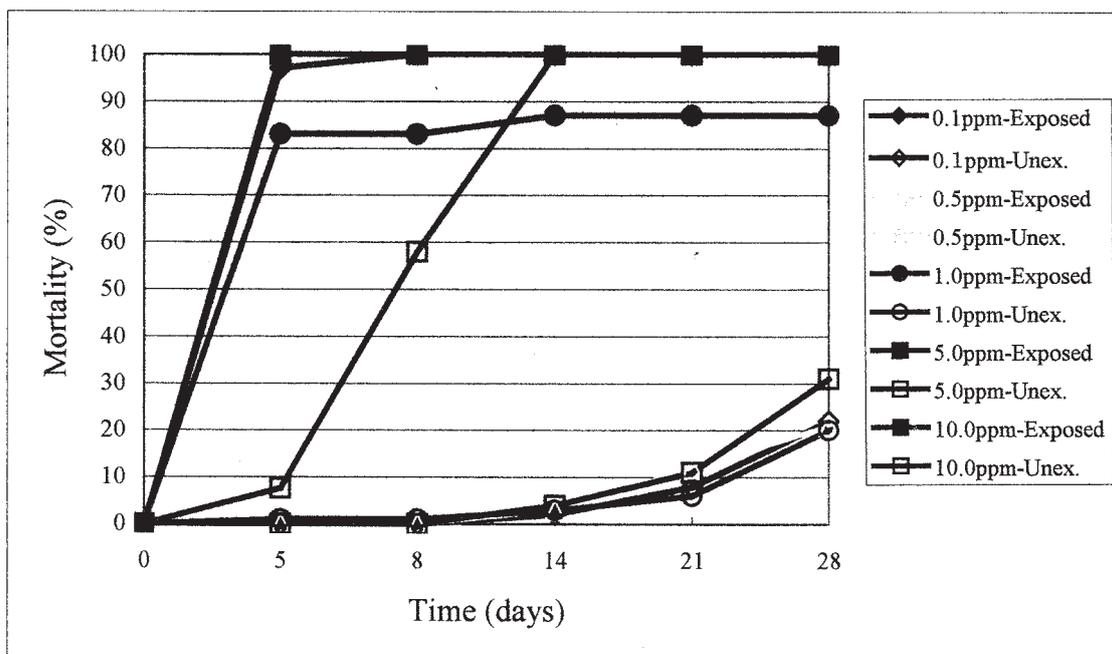


Figure 4 Effect of transfer of fipronil on the mortality of termite workers (mixing ratio of exposed vs. unexposed = 1:10)

Since dead termites were carefully removed at every inspection, the effects of indirect exposure decreased when the exposed workers died soon after the initiation of the indirect exposure test. Cannibalism of unhealthy weakened or dead termite individuals was not considered to contribute to the transfer of chemical substances from exposed termite workers to unexposed termite workers.

The possibility of repellent activity toward termites based on learning through contact with toxicant-treated soil was not confirmed in this study. Further studies are needed to examine whether termites isolate dead individuals and/or show any reluctance in feeding, since actions often indicate that termites can distinguish toxic substances from other materials.

### Conclusions

The current results strongly support the notion that fipronil was transferred from termite workers that had been previously exposed to the termiticide to other workers of *C. formosanus*, while the transfer of imidacloprid remained unclear due to the high test concentrations and the duration of initial exposure. Therefore, further studies are needed to determine the effect of first contact on termite mortality after an initial exposure for a fixed period. The characteristics of toxicant transfer may be important for termite management with the use of less termiticide.

The ability of termites to learn, which would enable them to detect chemical-treated soil after they had once passed through soil treated with the same termiticide, should be further investigated, since repellent activity through experience is considered to greatly decrease the reliable efficacy of a termiticide. In addition, the effects of various soil types with different pH levels on the effectiveness of the test termiticides should be examined, since the efficacy of termiticides against *C. formosanus* workers definitely varies with the soil type (Osbrink and Lax 2002).

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# “Silafiuofen” and Its Termiticidal Properties

by

Yoshio Katsuda, Yoshihiro Minamite and Koji Nakayama  
Dainihon Jochugiku Co., Ltd., Toyonaka, Osaka 561-0827, Japan

## Abstract

In addition to advantageous properties of conventional pyrethroids such as high insecticidal activities and low mammalian toxicity, silafiuofen is characterized by low fish toxicity which is not common to pyrethroids, and has been used as agricultural insecticides for plant protection of paddy rice, fruit trees, tea trees and turf from 1995. The demand for the silafiuofen-based insecticides is still on the increase. As termiticides, EC and oil formulations of silafiuofen have been widely used in Japan for soil treatment and timber treatment respectively since 1991 due to excellent chemical stabilities under sunlight, in the soil and under alkaline environments. Moreover anti-termitic plastic sheets prepared by impregnating silafiuofen in plastic resin have been put into practical use since 1998 and the laying of these sheets under the floor has been integrated into part of anti-termitic engineering method for newly-built houses, proving to be highly useful for the termite control.

We conducted field efficacy tests of silafiuofen EC at a rate of 0.05-0.2 % as silafiuofen at termite test sites of Kagoshima and Okinawa in Japan and Guangdong Province in China. As a result, no termite damage was found at any of the plots treated with silafiuofen at a rate of 0.1% or more after a period of 7 years whereas untreated plots gave serious termite damage, confirming high residual efficacy of silafiuofen against termites. And the chemical stability of some termiticidal ingredients under alkaline environments was examined by mixing them with mortar (pH :12.8). In contrast to imidacloprid, bifenthrin, fenobucarb and ethofenprox, silafiuofen showed a remarkably high recovery rate and was found to be characteristically stable under alkaline environments.

Silafiuofen is low toxic to mammals and fish not to speak of high termiticidal activity. Moreover in consideration of the facts that termiticidal ingredients are in some cases formulated into alkaline resin adhesive for plywood protection and termiticides often make contact with alkaline building materials such as mortar and concrete, silafiuofen which is chemically stable under alkaline environments, is considered to be one of extremely effective termiticidal ingredients.

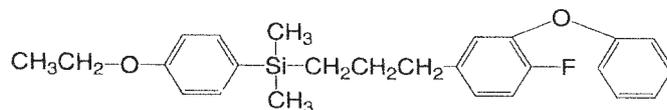
**Key words:** silafiuofen, termite control, soil treatment, anti-termitic plastic sheet

## Characteristics of silafiuofen

Silafiuofen (Katsuda *et al.* 1986) whose chemical structure is shown in Fig.1 has the following excellent characteristics. Among those, (3)-(5) are not common to pyrethroids.

- (1) high insecticidal (termiticidal) activity,
- (2) safety to mammals,

- (3) low fish toxicity,
- (4) high chemical stability ( to light, in soil, in alkaline environments, etc. ),
- (5) mode of action as a stomach poison as well as a contact poison.



4-Ethoxyphenyl [3-(4-fluoro-3-phenoxy phenyl)propyl]  
dimethyl silane

Fig.1 Chemical structure of silafluofen.

### Efficacy tests of silafluofen

#### *Fundamental efficacy tests*

The wood block test against *Coptotermes formosanus* SHIRAKI was conducted using wood blocks treated with oil formulations containing test termiticidal ingredient at a concentration of 0.1-1.0% according to the Japan Wood Preserving Association Standard 11(1). As a result, silafluofen-treated blocks showed smaller wood weight loss and higher mortality of termites than those treated with conventional pyrethroids such as permethrin and ethofenprox after a 3 week test period.

Kern *et al.* (Kern *et al.* 1990) reported that silafluofen acts as a stomach poison as well as a contact poison in contrast with pyrethroids which act only as a contact poison. This higher termiticidal activity of silafluofen than that of permethrin in the wood block test is considered to reflect silafluofen's stomach poison, taking into consideration the fact that permethrin was superior in termiticidal activity to silafluofen in the filter paper contact method. Endowed with both modes of action as a stomach poison and a contact poison, silafluofen can be said to be greatly advantageous for the termite control.

#### *Field efficacy tests of silafluofen EC*

The field efficacy tests of silafluofen EC (Minamite *et al.* 1990, Rustenburg *et al.* 1991, Nakayama *et al.* 1998) were conducted at termite test sites of Architectural Research Association in Kagoshima and Ryukyu University in Okinawa (the soil of the latter test site; weak alkaline of pH 7-8) and further at a termite-inhabiting forest site near Luxi River of Conhua City in Guangdong Province in China. In the tests, the soil was treated with a dilution of silafluofen EC at a rate of 0.05-0.2 % as silafluofen and was assessed for the preventive performance against termite penetration. As a result, no termite damage was found at any of the plots treated with silafluofen at a rate of 0.1% or more after a period of 7 years whereas untreated plots gave serious termite damage, confirming high residual efficacy of silafluofen. Due to high chemical stability in alkaline environments, silafluofen is considered to be useful as a termiticide, especially in the alkaline soil in places such as Okinawa.

### *Efficacy tests of anti-termitic plastic sheets*

According to the modified ground board field test (Fig.2), efficacy tests of anti-termitic plastic sheets were conducted using a test box (width; 45 cm, depth; 45 cm, height; 30 cm). As a result, there was a big difference in the level of termite damage on the wood bait placed on the sheet between any of silafluofen-treated sheet plots and a control of untreated sheet plot. That is, the wood bait at the silafluofen-treated sheet plots received no termite damage after a period of 5 years whereas the untreated sheet plot gave serious termite damage after one year.

Furthermore silafluofen anti-termitic plastic sheets were found to show repellency against termites in the laboratory tests.

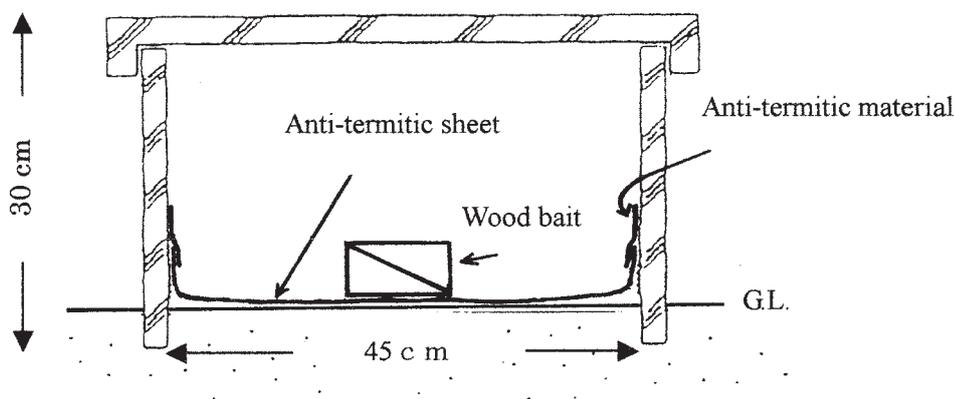


Fig.2 Field test of anti-termitic sheet (sectional plan of a test box).

### **Outlines of silafluofen-based termiticides**

Table 1 outlines silafluofen-based termiticides on the current market.

Table 1 Outlines of silafluofen-based termiticides

Items	EC for soil treatment	Oil formulation for timber treatment	Anti-termitic plastic sheet
Termiticidal ingredient	silafluofen 3.0% (w/w)	silafluofen 0.15% (w/w) (in combination with a fungicidal ingredient)	silafluofen 0.1% (w/w)
Usage	Spray 30 part dilution on the soil (conc.; 0.1%)	Spray or paint the oil on woods without diluting	Lay the sheet on the soil surface
Application rate	3L/m <sup>2</sup> (soil)	0.3L/m <sup>2</sup> (woods)	
Start of marketing (initial formulation)	1991~	1992~	1998~

## Chemical stability of termiticidal ingredients in mortar

### Termiticidal ingredients

- Silafluofen [Organosilicon (pyrethroid-like)]
- Imidacloprid (Neonicotinoid )
- Bifenthrin (pyrethroid)
- Fenobucarb (carbamate)
- Ethofenprox (pyrethroid-like)

### Test method

A dilution of EC containing a designated amount of test termiticidal ingredient was mixed with commercially available mortar (pH of water-extract; 12.8). After air-drying for one day, the test sample on the way to solidification was broken and stored at 50°C. After 1 week and 4 week storage, portions of the sample were taken and the remaining amount of test termiticidal ingredient was analyzed.

### Test results

As shown in Fig.3, silafluofen gave a remarkably high recovery rate in mortar (pH of water-extract; 12.8) in contrast to imidacloprid, bifenthrin, fenobucarb and ethofenprox, being characteristically stable under alkaline environments.

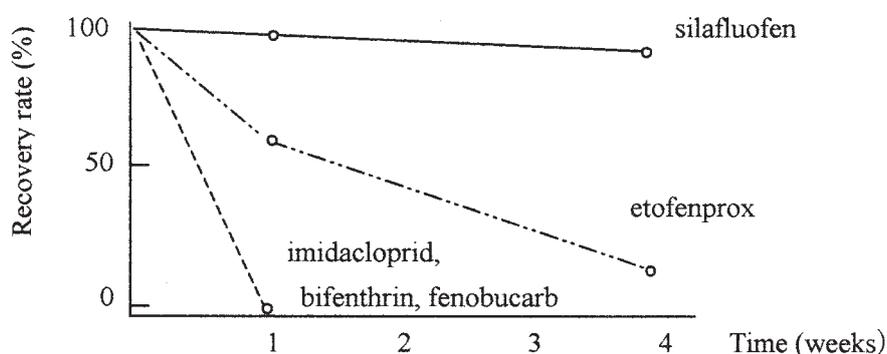


Fig.3 Stability test in mortar ( pH of water-extract; 12.8).

### Conclusion

Recently in Japan, silafluofen, imidacloprid, bifenthrin, fenobucarb and ethofenprox have been mainly used as termiticidal ingredients.

Silafluofen is an ordinary substance under the Poisons and Deleterious Substances Control Law and its fish toxicity is ranked into A rank (the least toxic) under the Agricultural Chemicals Regulation Law in Japan.

Accordingly silafluofen is low toxic to mammals and fish not to speak of high termiticidal activity. Moreover in consideration of the facts that termiticidal ingredients are in some cases formulated into alkaline resin adhesive for plywood protection and termiticides often make contact with alkaline building materials such as mortar and concrete, silafluofen which is chemically stable

under alkaline environments, is considered to be one of extremely effective termiticidal ingredients.

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# How Bait Design Affects *Coptotermes* Activity and Feeding in Baits

by

Theodore Evans

CSIRO Entomology, Canberra, ACT, 2600,

## Abstract

Bait stations should be designed to encourage termite presence and to maximize bait matrix consumption in order to expedite control in minimal time. A field experiment examined the effect of bait size (one large or four small baits of equivalent total size), compaction (tightly rolled or loosely folded paper) and composition (paper only or paper plus wood) on termite presence and on untreated bait paper removal rates over four months. All three factors were significant. The least effective baits were small, compacted (rolled) paper-only baits with monthly inspections; these had the highest abandonment rate (70%) and had the least paper removed (mean of 24g). The most effective baits were large, folded paper-plus-wood baits with inspections at two months; these had the lowest abandonment rate (20%) and had the highest paper removal (mean of 112g). The difference between these bait types demonstrates that bait efficacy can be altered considerably merely by changing bait presentation and inspection schedules without adding new ingredients to the bait.

**Key words:** Baiting, *Coptotermes*, disturbance, inspection schedule.

## Introduction

Baiting has been promoted as an environmentally sound method of termite pest control as it uses very small amounts of toxicants in a cellulose matrix. However, baiting is often slow: a successful baiting program can take up to nine months (e.g. Su 1994; Tsunoda et al. 1998; Su and Scheffrahn 2000), due in part to slow feeding. Achieving control faster is desirable because this lack of rapid control can be a deterrent to using a baiting program. Better understanding of termite foraging behaviour may increase termite feeding rates, and so speed up control in baiting programs.

Most economically-important, wood-eating termite species evolved to eat relatively large pieces of timber (which is the reason why these species are pests), including whole trees (e.g. *Coptotermes* species; Greaves 1962; Lenz 1994). Most bait systems are much smaller than natural food resources and typically do not include wood. Instead, either paper, cardboard or purified cellulose powder are offered (Su 1994; French et al. 1995; Tsunoda et al. 1998; Su and Scheffrahn 2000). It is important to remember that bait stations will be in competition with other food sources for attention from foragers (Forschler 1996; Perrott et al. 2004); it is of interest to note that bait matrices are not accepted uniformly (e.g. paper LaFage et al. 1973; French and Robinson 1980).

Baits must be inspected and replaced, typically monthly, which is a disturbance to the foraging termites compared with termites feeding in large pieces of timber. There are no published data that demonstrate how bait station design and the inspection regime affect termite foraging. Therefore the aim of this experiment was to compare three attributes of bait station design: bait size, matrix presentation, and matrix ingredients; on bait matrix removal (consumption) to determine if these factors affect termite foraging and whether bait (Lenz and Evans 2002).

## Materials and methods

The field site was a *Pinus radiata* D. Don (Pinaceae) plantation in the Brindabella Mountains of the Australian Capital Territory (35°17' S, 149°13' E, elevation c. 800m, c. 30 km W of Canberra). The endemic *Coptotermes lacteus* (Froggatt) has adapted to the pine plantation and mounds are common.

Foraging sites were created around 10 termite mounds in March 1997. Wood (*P. radiata* and *Eucalyptus regnans* F. Muell) was dug into a circular trench (10 m diameter), with 20 milk cartons (one liter, waxed paper, 25 x 6.5 x 6.5 cm) filled with wooden slats (*E. regnans*, 24 x 5 x 0.7 cm) were placed at regular intervals (ca. 1.6 m). The top of the 'carton-bases' were flush with soil surface, and covered with a transparent plastic lid and opaque plastic sheet and soil) and spaced evenly around the circular trench. By November 1997, termites were foraging in 16 - 20 milk cartons

at each mound-colony; this was seen through the transparent plastic lids.

Three different bait attributes were tested. 1. Size: small baits (36 g paper) and large baits (144 g paper - four times larger than small baits) (Versatowel; Kimberley Clark Australia). 2. Compaction: rolled tightly (to minimize volume) or folded loosely (to maximize surface area). 3. Composition: paper-only or paper-plus-wood (*E. regnans*); small baits had one small slat (12 x 5 x 0.7 cm) and large baits had two large slats (24 x 5 x 0.7 cm) (four times more wood). There were eight treatment combinations (see Table 1). Small baits were housed in half milk-cartons (12 cm long) and large baits were housed in whole milk cartons (all with holes 2 cm diameter).

Baits were placed on the termite infested 'carton-bases' (as suggested by French 1991), and so are similar to commercial "above ground bait stations" (Su et al. 1997). Two replicates of each treatment combination were placed randomly on the carton-bases around each mound on 20 January 1998; a total of 20 replicates of each treatment combination. The clear plastic covers were removed from the carton bases and the baits placed on top, and the plastic sheets were replaced over them. The small sized baits were inspected four times, approximately monthly, in a regime similar to that of commercial baiting practice (Table 1), with termite activity and the amount of paper removed from the baits recorded. Whenever small baits had been contacted by the termites another small bait was added regardless of how much paper had been removed. Once the large baits were placed they were not disturbed, except for a brief inspection at two months and for the final inspection at four months. Twenty carton-bases were left completely undisturbed to measure abandonment caused by bait placement and inspection.

### Results and discussion

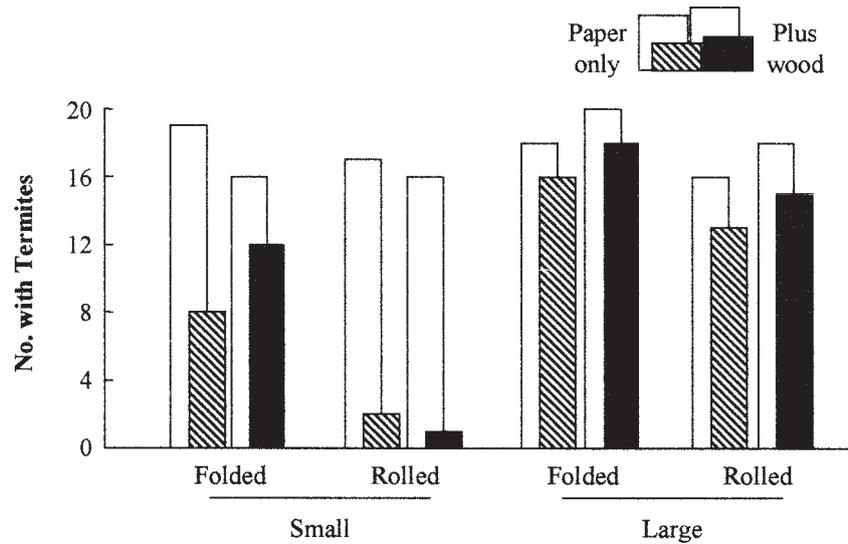
Only one of 20 undisturbed carton bases was abandoned by the final inspection (16 June); a rate of 5% after four months (Table 1), which was due to the wood being eaten completely by this time. Because termites foraged in these bases until the wood was exhausted, any abandonment of bases used for baiting could be considered a result of disturbance from baiting and inspections.

**Table 1.** The number of bases and baits with *Coptotermes lacteus* activity at each inspection. N.b. numbers are 'bases, baits'; initially there were 20 bases per bait treatment combination (placed 20 Jan); large baits were not inspected in February or April. Undisturbed bases did not receive baits, which is indicated with a dash.

Bait:		Inspection date:				
Size	Composition	Compaction	25 Feb	27 Mar	24 Apr	16 Jun
Large						
Paper only		Folded	-	20, 13	-	18, 16
		Rolled	-	17, 12	-	16, 13
Plus wood		Folded	-	19, 17	-	20, 18
		Rolled	-	18, 14	-	18, 15
Small						
Paper only		Folded	19, 12	20, 10	20, 12	19, 8
		Rolled	18, 9	18, 7	18, 3	17, 2
Plus wood		Folded	20, 14	20, 12	19, 11	16, 12
		Rolled	19, 15	19, 10	16, 7	16, 1
Undisturbed bases			20, -	20, -	20, -	19, -

Bases used for baiting were abandoned over the course of inspections: 9 of 160 bases (5.6%) had been abandoned by the second inspection (27 March), which doubled to 20 bases (12.5%) by the fourth inspection (16 June) (Table 1). Therefore, the slight disturbance of the bases to place baits caused ~7.5% abandonment of established feeding sites. There were differences, although not significant, between treatments (16 June data): more abandonment in bases with small baits than large (12 : 8) and rolled baits than folded (13 : 7); but they were the same in paper-only and plus-wood baits (10 : 10).

Baits were abandoned more frequently than bases: 65 of 160 baits (41%) had been abandoned on the second inspection (27 March), which increased to 75 abandoned baits (47%) by the fourth inspection (16 June). In general, the termites sealed off the bases from the baits, and the baits were then ignored. Yet baits could be re-attacked, and consequently the pattern differed slightly between bait-treatments from March to June. In March, significantly more small than large baits were abandoned (41 : 24,  $\chi^2_1 = 4.45$ ;  $p = 0.035$ ), and more rolled than folded baits were abandoned (37 : 28) and paper-only than plus-wood (38 : 27) though these were not significant. By June these differences had increased and were significant for small and large baits (57 : 18,  $\chi^2_1 = 20.3$ ;  $p < 0.001$ ) and rolled than folded (49 : 26,  $\chi^2_1 = 7.05$ ;  $p = 0.008$ ), but there was a smaller and non-significant difference between paper-only and plus-wood (34 : 41) (Table 1, Figure 2).



**Figure 1.** The number of bases and baits with *Coptotermes lacteus* activity after four months. Hatched columns = paper only baits; black columns = paper plus wood baits; white columns = carton bases. N.b. that termites are generally present in all bases whereas they are more likely to be absent from small, paper only and rolled baits.

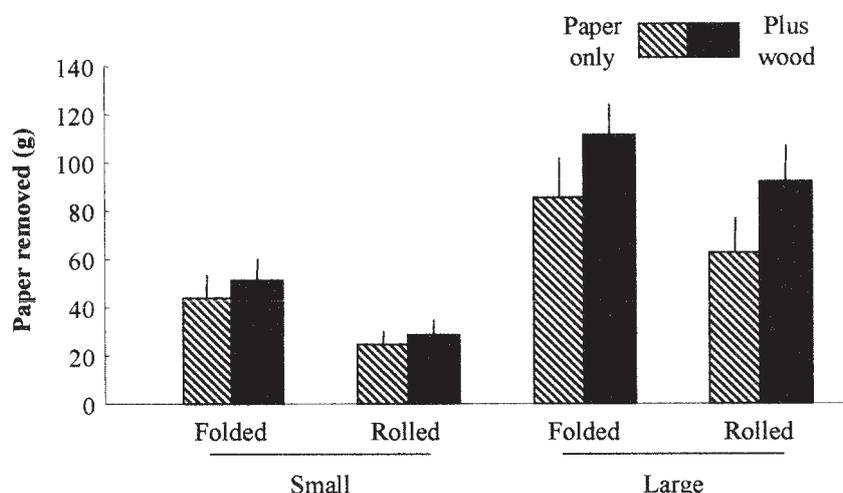
Paper removal ('consumption') was compared over time for each bait size separately (Table 2). For small baits, there was a significant effect of compaction ( $F_{1,76} = 7.67$ ;  $p = 0.007$ ) with less paper removed from rolled compared with folded baits, but no significant effect of composition (paper only cf. paper plus wood) on paper removal. The decrease in paper removal over time (repeated measure factor) was significant ( $F_{3,228} = 18.5$ ;  $p < 0.001$ ). For large baits, compaction was not significant, but composition was significant ( $F_{1,76} = 4.31$ ;  $p = 0.041$ ), with more paper removed from plus-wood compared with paper-only baits. The decrease in paper removal over time (repeated measure factor) was significant ( $F_{1,76} = 56.2$ ;  $p < 0.001$ ), as was the interaction term for time and compaction ( $F_{1,76} = 8.12$ ;  $p = 0.006$ ); thus the decrease in removal over time was different between rolled and folded. Essentially, folded baits were 'eaten' faster than rolled baits until March, but the reverse was true by June. This pattern was due to folded baits being almost completely 'eaten' in March, so little was left to be 'eaten' by June, whereas rolled baits were about 50% 'eaten' in March, leaving more to 'eat' by June.

**Table 2.** The amount of paper removed by *Coptotermes lacteus* (grams, average  $\pm$  std error) from each bait treatment combination at each inspection.

Bait Type:		Inspection date:			
Size	Composition	25 Feb	27 Mar	24Apr	16 Jun
Large					

Paper only	Folded		85.0 ± 15.9		0.6 ± 0.4
	Rolled		49.3 ± 14.5		13.0 ± 4.9
Plus wood	Folded		108.0 ± 13.7		3.6 ± 2.3
	Rolled		78.6 ± 16.1		30.0 ± 18.2
Small					
Paper only	Folded	15.7 ± 3.8	16.0 ± 4.5	7.1 ± 3.2	5.3 ± 1.9
	Rolled	8.0 ± 3.2	11.9 ± 4.4	2.2 ± 1.0	2.3 ± 1.0
Plus wood	Folded	23.8 ± 3.8	17.6 ± 4.0	6.9 ± 2.9	3.3 ± 2.0
	Rolled	10.5 ± 3.4	12.8 ± 3.8	4.2 ± 1.6	1.4 ± 1.2

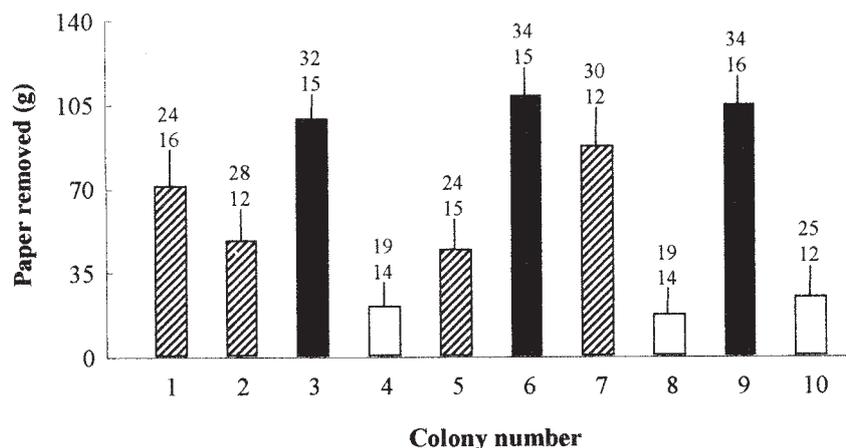
The total (cumulative) amount of paper removed ('consumed') after four months (Figure 2) was analysed with a three-way ANOVA. No interaction terms were significant, therefore the main treatments can be considered separately. All three were significant, with size being the most important (large vs small  $F_{1,152} = 38.3$ ;  $p < 0.001$ ), followed by compaction (folded vs rolled  $F_{1,152} = 6.78$ ;  $p = 0.010$ ) and then composition (paper-only vs plus-wood  $F_{1,152} = 4.26$ ;  $p = 0.041$ ). The analysis explains 25% of the variability observed ( $r^2 = 0.25$ ), therefore other factors are important.



**Figure 2.** Total paper removed from baits by *Coptotermes lacteus* (grams, average ± standard error) after four months of baiting.

Differences in paper removal between colonies explains 35% of the variability observed ( $r^2 = 0.35$ ). Colonies removed significantly different amounts of paper ( $F_{9,150} = 8.81$ ;  $p < 0.001$ ). Colonies 4, 8 and 10 removed significantly less paper (means of 20.6g, 16.6g and 24.5g respectively), explained by the reluctance of termites from these colonies to come out of the bases and attack the baits, and colonies 3, 6 and 9 removed significantly more paper (means of 98.8g, 108.5g and 104.9g respectively (Figure 4).

Of the three attributes tested in this study, bait size, compaction and composition were important in affecting bait consumption. More of the paper in the large baits was removed than that in the small baits, and faster too. Several laboratory studies have shown that termites vary food consumption rate inversely with food size, i.e. they eat small food slowly (Lenz 1994; Hedland and Henderson, 1999). Large baits allowed termites to chew the bait at their own rate; termites fed small baits could be



**Figure 3.** The total amount of paper removed (g) from the baits for each colony. Columns of the same pattern are not-significantly different. N.b. numbers above columns, top = number of baits, bottom = number of bases contacted still infested at the end of experiment (16 June) (maximum number = 20).

delayed by the monthly inspection. Furthermore, large baits were placed only once, so decreasing disturbance from placing small baits repeatedly. The results from this experiment are in agreement with earlier studies, showing that termites abandon more disturbed baits (Jones 1990, French et al. 1995). Minimizing disturbance it is likely to improve bait removal in real baiting programs.

More loosely folded paper was removed, at a faster rate, than tightly rolled paper. This was because the much higher surface area of folded paper allowed more termites to chew the paper immediately and simultaneously. The rolled paper had a small surface area so relatively few termites could chew the paper. The addition of wood had a small but discernable effect, with more paper being removed in baits with wood compared with those without. This can be explained as the holding power of wood: termites remained in the small baits after all the paper was eaten to eat the wood, and so were still present at the next inspection when another small bait was placed. Paper only small baits were abandoned baits when all the paper had been removed.

### Conclusions

The results of this study show that a change in the presentation of materials in the bait station can have large effects on bait matrix removal. No new ingredients were necessary to increase paper removal by a factor of ten after only two months, if small, paper-only, rolled baits are compared with larger, plus-wood, folded baits. Further consideration of bait station design from the perspective of the termite, especially designs that minimize disturbance, is likely to produce increases in bait matrix removal, and so further advances efficacy of bait stations.

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# Termite Management of Multi-Genera Faunas

By

Chow-Yang Lee<sup>1</sup>, Charunee Vongkaluang<sup>2</sup> & Michael Lenz<sup>3</sup>

<sup>1</sup>School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia.

<sup>2</sup>Forest Products Research Division, Royal Forest Department, Bangkok 10900, Thailand.

<sup>3</sup>CSIRO Entomology, GPO Box 1700, Canberra ACT 2601, Australia.

## Abstract

Termites are an important group of insect pests in the Asia Pacific region. Due to the high diversity of termite species in this region it is common to find several termite pest species co-existing and infesting buildings and structures. In Malaysia, 12 species of subterranean termites from 7 genera (*Coptotermes*, *Macrotermes*, *Microtermes*, *Globitermes*, *Odontotermes*, *Schedorhinotermes* and *Microcerotermes*) can be readily found in- and around buildings and structures, particularly in suburbia and rural settlements. Similar observations with species in the genera *Coptotermes*, *Microcerotermes*, *Macrotermes*, *Hypotermes* and *Odontotermes* are also recorded in urban and rural Thailand. Termites from 3 to 6 genera (*Mastotermes*, *Coptotermes*, *Schedorhinotermes*, *Heterotermes*, *Nasutitermes* and *Microcerotermes*) may be found co-existing as structural pests in Australia with the highest number of genera in the tropical north of the country. Since the introduction of baiting in Malaysia, secondary pest species are more frequently encountered. Following elimination of the principal pest species (*Coptotermes* spp.) with bait, it is not uncommon to find species from other genera such as *Macrotermes* and *Schedorhinotermes* infesting the same building or structure after several months. Most of these species, particularly those belonging to genera such as *Macrotermes*, *Globitermes* and *Odontotermes* from the higher termite (Termitidae), however, do not respond well to paper-based bait matrices. Options for managing multiple genera termite pest faunas in the tropics are discussed.

**Key words:** Termite management, multi-genera, control strategies, Malaysia, Singapore, Thailand, Australia.

## Introduction

Termites are an important group of insect pests in the urban environment (Su & Scheffrahn 2000, Lee 2002a). In the Northern Hemisphere (eg. US, Europe and Japan), termite management often focuses on a few genera of termites which belong to the family Rhinotermitidae (Pearce 1997). These rhinotermitids include the genus *Reticulitermes*, *Coptotermes* and *Heterotermes*. It is normally rare to find more than one genus infesting a structure in this region. Among the various termite management systems used, baiting has been relatively successful against the rhinotermitids.

However, around the Equator and in the Southern Hemisphere there is a wider range of pest termite genera consisting of both rhinotermitids and non-rhinotermitids. In many instances, a structure can be attacked simultaneously by more than one species or in succession by different species (Lee 2002a). Newer technologies such as baiting have limited success against non-rhinotermitids in these regions. In addition, when baiting reduces/eliminates populations of rhinotermitids such as *Coptotermes*, other species which are normally considered as secondary pest species or non-pest species, can become important and enter buildings. This paper discusses termite management issues in countries with multi-pest genera based on the situation in Malaysia, Singapore, Thailand and Australia.

### Common species and termite infestation in Malaysia and Singapore

In Malaysia and Singapore, several termite genera that can be found in buildings and structures include *Coptotermes*, *Schedorhinotermes*, *Microcerotermes*, *Macrotermes*, *Nasutitermes*, *Globitermes* (Ngee and Lee 2002, Lee et al. 2003) and *Odontotermes*. Among these genera, infestation caused by *Coptotermes* spp is most prevalent and accounts for almost 85% (Table 1) of the total. *Coptotermes gestroi* is the most common termite pest species in buildings and structures (Kirton & Brown 2003). Another species of *Coptotermes*, *C. curvignathus* may be found in premises built on ex-agricultural land or plantations, particularly rubber, oil palm and coconuts.

**Table 1: Termite pest species found in infested premises in Northern Peninsular Malaysia (n = 132).**

Species	Percentage (%)
<i>Coptotermes</i> spp.	84.1
<i>Macrotermes gilvus</i>	6.0
<i>Schedorhinotermes medioobscurus</i>	3.8
<i>Microcerotermes crassus</i>	2.3
<i>Nasutitermes javanicus</i>	2.2
<i>Odontotermes</i> sp.	0.8
<i>Globitermes sulphureus</i>	0.8

In addition, it is also common to find several species of termites infesting a building at any one time. For example, in Figure 1, a condominium block in Penang Island, Malaysia was infested by four species of subterranean termites, *C. gestroi*, *Globitermes sulphureus*, *Odontotermes* sp. and *Macrotermes gilvus*.

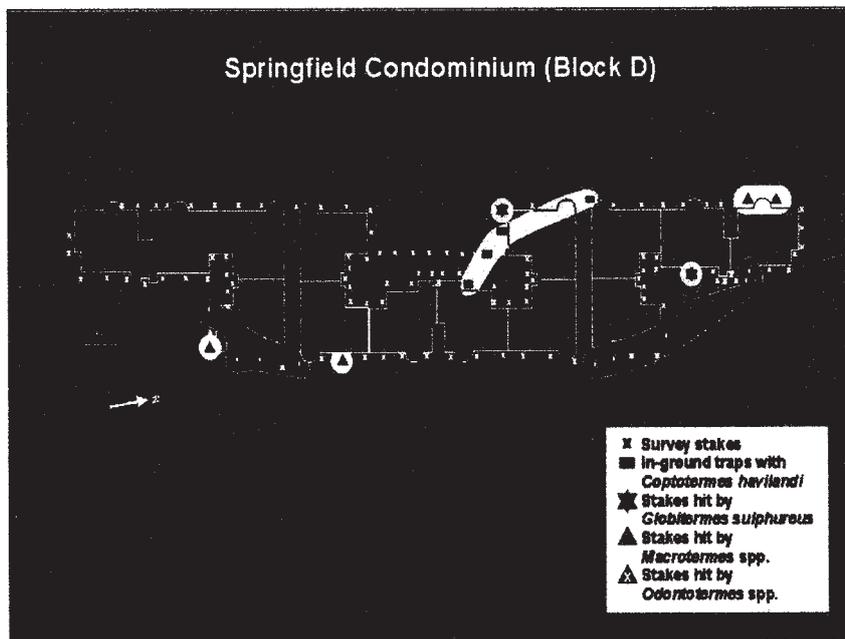


Figure 1: Infestation by multiple species of subterranean termites in an apartment block in Penang Island, Malaysia (unpublished figure provided by N.Y. Su, University of Florida).

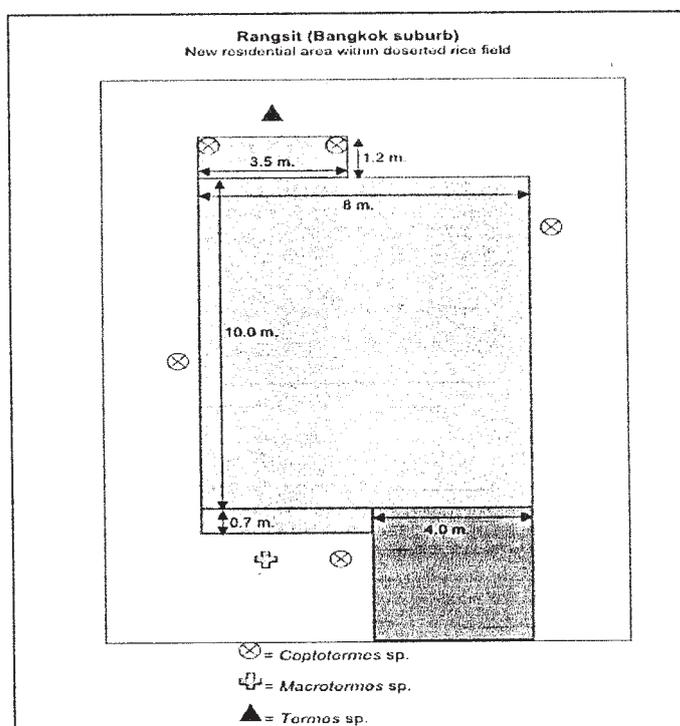
### Common species and termite infestation in Thailand

In Thailand, a similar situation was observed. Table 2 shows the termite species found in infested residential premises in rural and urban areas of Thailand. This study recorded a total of 13 species belonging to the families Rhinotermitidae (5 species) and Termitidae (8 species). The most common infestation in the urban area was caused by *C. gestroi* (Sornnuwat et al. 1996a, 1996b, 1996c), while houses in the rural area were predominantly infested by *Microcerotermes crassus* (Sornnuwat et al. 1996a). An almost similar situation as in Malaysia was also observed in Thailand: termites of several species infested a building or structure simultaneously (Figure 2 & Figure 3). The situation is more evident in rural areas with a higher diversity of termite species.

Table 2: Termite pest species found in infested houses in Thailand (n = 200) (Sornnuwat et al. 1996a).

Species	Urban	Rural
<i>Coptotermes gestroi</i>	90	22
<i>Coptotermes kalshoveni</i>	3	-
<i>Coptotermes premasmii</i>	1	1
<i>Coptotermes travians</i> [haviglandi]	4	-
<i>Schedorhinotermes medioobscurus</i>	2	1
<i>Globitermes sulphureus</i>	-	7
<i>Macrotermes gilvus</i>	-	4
<i>Microtermes pakistanicus</i>	-	2
<i>Microtermes anandi</i>	-	2
<i>Microcerotermes crassus</i>	-	42
<i>Odontotermes proformosanus</i>	-	8
<i>Odontotermes longignathus</i>	-	6
<i>Odontotermes feae</i>	-	5

Figure 2: Multi-genera termite infestation in a residential area in Rangsit, Bangkok, Thailand.



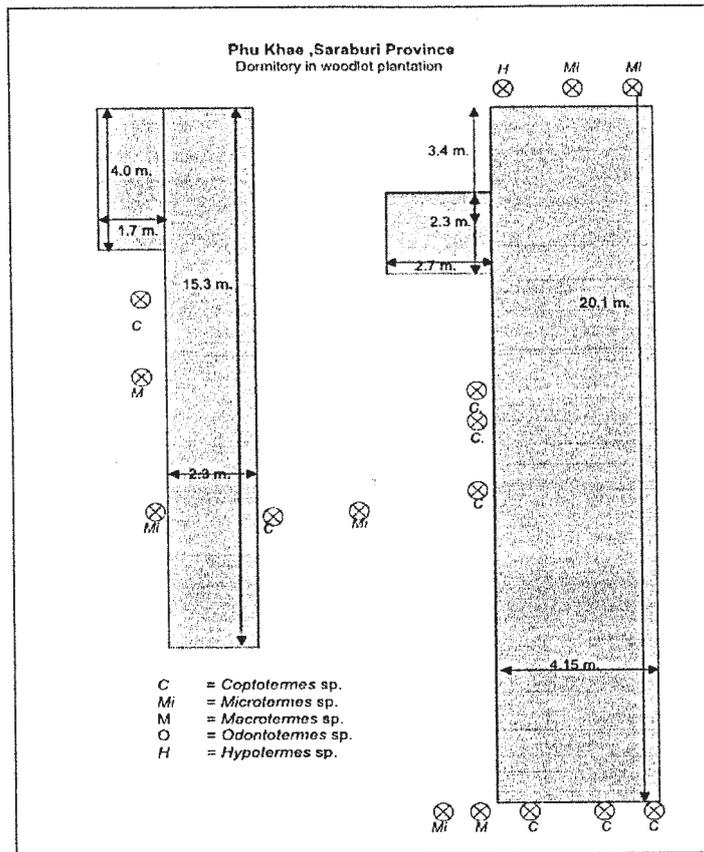


Figure 3: Multi-genera termite infestation in Saraburi, Thailand.

### Common species and termite infestation in Australia

In Australia, a total of 7 genera of termites can be found in buildings and structures (Watson & Abbey 1990, Lenz 2002, Australian Standard 1995) (Table 3). The common ones, *Coptotermes* and *Schedorhinotermes*, are distributed over much of mainland Australia, while *Mastotermes* is important in the tropical region of Australia.

Table 3: Australian termite pests in buildings and structures.

Genus (no. pest species)	Distribution
<i>Mastotermes</i> (1)	In the tropics south to the Tropic of Capricorn, large populations
<i>Coptotermes</i> (4)	Almost all of mainland Australia.
<i>Schedorhinotermes</i> (5)	All of mainland Australia (mainly east coast and tropics), except the very southern parts. On the east coast, it is becoming more common, in some areas replacing <i>Coptotermes</i> as the primary structural pest.
<i>Heterotermes</i> (10)	Widely distributed, 3 species in the tropics.
<i>Microcerotermes</i> (sev.)	Localized pest can appear in bait stations after <i>Coptotermes</i> is eliminated/ suppressed.
<i>Nasutitermes</i> (3)	2 arboreal nesters on the east coast, and 1 mound-builder across the South.
<i>Porotermes</i> (1)	Localized pest in South east, specific requirements.

One major challenge in managing multi-genera termite faunas, especially with the use of termite baits, is re-infestation by higher termite faunas after the rhinotermitids have been eliminated or suppressed. As mentioned earlier, it is not possible to manage these non-rhinotermitids effectively with baits. Earlier, Lee (2002b) reported the difficulty of managing *M. gilvus* which infested residential premises which had been previously baited for *Coptotermes* infestation. *M. gilvus* was found in the house as early as two months after the suppression or elimination of *Coptotermes*. Table 4 shows the succession of termite genera after suppression or elimination of *Coptotermes* spp by baits. Although re-infestation by *Coptotermes* is relatively high (almost 85%), pest control operators find it difficult to explain to home owners that baits were only effective against *Coptotermes* and *Schedorhinotermes*. There have been many incidences in Malaysia where pest control operators have to resort to chemical spraying to repel these non-rhinotermitids. However, it is only seen as a temporary measure, as re-occurrence of these higher termites in other locations of the premises can be observed within a very short period of time.

Table 4: Succession of termites after suppression/elimination of *Coptotermes* spp. by baits in Malaysia (2001 - 2004) (n = 82 premises)

Species	Percentage (%)
<i>Coptotermes</i>	83.0
<i>Schedorhinotermes</i>	7.3
<i>Macrotermes</i>	4.9
<i>Globitermes</i>	1.2
<i>Nasutitermes</i>	1.2
<i>Microcerotermes</i>	2.4

#### Management strategies against multi-genera termite fauna

Current termite baits are most effective against *Coptotermes* and *Schedorhinotermes*. However, in Malaysia, for example, it takes a minimum of 6 months to suppress/eliminate a colony of *Schedorhinotermes* (CY Lee, unpublished). Baits are generally not effective against non-rhinotermitids (higher termite genera) such as *Macrotermes*, *Globitermes*, *Microtermes*, etc. Because of this, Malaysian pest control operators normally sell baiting contracts only for control of *Coptotermes*. Whenever non-rhinotermitids are encountered, chemical soil treatment is carried out. In addition, where a structure is infested by higher termites following elimination of a previous *Coptotermes* infestation through baiting, pest control operators will embark on chemical soil treatment.

The most common termiticides are: chlorpyrifos, fipronil and imidacloprid (Malaysia & Singapore); fipronil, imidacloprid, fenoxycarb, fenvalerate (Thailand); bifenthrin, fipronil, imidacloprid (Australia). For the management of *Mastotermes* in Australian tropics, standard chemical soil barriers require higher rates. All preventative termite management systems in tropical Australia have to be evaluated against *Coptotermes* and *Mastotermes* to gain official approval, but only against *Coptotermes* for the rest of the country (Lenz 2002). For active infestations in tropical Australia, fipronil applications are replacing other methods, however, treatments also have to focus on surrounding areas targeting nest sites, etc. due to the diverse pest species community (eg. vegetated area, tree trunk [for *Mastotermes*] and visible nests [*Microcerotermes*]). In Malaysia, Singapore and Thailand, it is essential to excavate the mounds of the higher termite species found along the perimeter of the baited homes to reduce the chance of these species infesting the premises upon suppression or elimination of *Coptotermes* species.

On the research front there is a serious need to determine the underlying reason behind the lack of effectiveness of a chitin synthesis inhibitor used in bait matrices against the higher termites. It has been found that some of these non-rhinotermitids feed on the baits without any visible detrimental effect to the colony (Ngee et al. 2004). In addition, the lack of palatability of paper-based bait

matrices to *M. gilvus* is also a major issue to the pest control industry in SE Asia. These issues warrant urgent investigations.

### Summary & Conclusion

Multi-genera pest termite faunas are not uncommon in Malaysia, Singapore, Thailand and Australia. Suppression/ elimination of one species may quickly result in re-infestation by the same species or by a succession of different species/genera. This is particularly evident upon suppression/ elimination of *Coptotermes* using baits. Overall, managing multi-genera termite faunas in the tropical region can best be done by taking the biology of different target species into account, and by adopting several management strategies.

*Acknowledgements:* We thank Janette Lenz, Pooi-Yen Loke and Say-Piau Lim for proof-reading the manuscript draft.

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## Annex



**Minutes of the Establishment of Pacific-Rim Termite Research Group  
8 March 2004, Eastern & Oriental Hotel, Penang, Malaysia**

**Attendees:**

1. Michael Lenz (CSIRO Entomology Australia)
2. Jim Creffield (CSIRO Forestry & Forest Products, Australia)
3. Junhong Zhong (Guangdong Entomological Institute, P.R. China)
4. Tsuyoshi Yoshimura (Kyoto University, Japan)
5. Dong-heub Lee (Korean Forest Research Institute, Korea)
6. Vu van Tuyen (Centre for Treatment of Termite & Subsurface Defects, Vietnam)
7. Chow-Yang Lee (Universiti Sains Malaysia, Penang, Malaysia)
8. Magdalena Giron (Forest Products R&D Institute, Philippines)
9. Carlos Garcia (Forest Products R&D Institute, Philippines)
10. Sulaeman Yusuf (Indonesian Institute of Sciences – LIPI, Indonesia)
11. Charunee Vongkaluang (Royal Forest Department, Thailand)
12. Po-Yung Lai (National Pingtung University of Science & Technology, Taiwan)
13. Chun-Chun Tsai (Tunghai University, Taichung, Taiwan)
14. Kunio Tsunoda (Kyoto University, Japan)
15. Masatoshi Yokoyama (Bayer CropScience, Japan)
16. Izumi Fujimoto (BASF Agro, Japan)
17. Gregory Rosenblat (Atofina, Japan)
18. Kazunori Tsushima (Sumitomo Chemicals, Japan)
19. Mark Birchmore (Syngenta Crop Protection, Switzerland)
20. Atsushi Suzuki (DuPont Japan)
21. Leng-Choy Lee (Dow AgroSciences Asia)
22. Eric Loh (Kam-Han Young) (Koppers Arch Chemicals Malaysia)
23. Hiroshi Kobayashi (FMC Chemicals KK Japan)
24. Wahyudi Sasprihanto (FMC Chemicals, Indonesia)
25. Noriko Nakada (FMC Asia Pacific, Hong Kong)
26. Paul Vincent Grassick (FMC (Chemical) Pty. Ltd., Australia)
27. Ian Francis (FMC Australia)
28. Takashi Miyahara (FMC Chemicals KK Japan)
29. Salman Mujahid (FMC United (Private) Ltd., Pakistan)
30. Philip Morrow (Bayer Environmental Science, Australia)
31. Sanoto Utomo (Pt Johny Jaya Makmur Co. Ltd., Indonesia)
32. Nai-Pin Lee (Bayer Environmental Science, Malaysia)
33. Muhammad Zaini Abd Rashid (Bayer Environmental Science, Malaysia)
34. Abu Hassan Ahmad (Universiti Sains Malaysia)
35. Daniel Kidder (Syngenta Crop Protection Inc., USA)
36. Murray Hern (Syngenta Crop Protection Pty. Ltd., Australia)

- 1.0 The plenary session started at 10.00 am with welcoming remarks from Kunio Tsunoda (KT) as the Organizing Chairman of the Pacific-Rim Termite Research Group (TRG) meeting. KT stated that there is an urgent need to establish a group on termite research in the Pacific region to consolidate the existing research activities and to promote collaborative research among Asian countries and scientists.

The draft statutes of the TRG (see Appendices III of the proceedings) was then discussed by the participants. Po-Yung Lai's suggestion to have Article 4 moved to Article 1 was not supported. One of industrial participants asked whether it is possible for industrial people to apply for ordinary members, but KT explained that he wished to clearly separate ordinary members from sponsor (industrial) members. The draft was finally accepted and approved by all participants after the following corrections and modification were made.

Before	After
<u>4.1</u>	<u>5.1</u>
<u>4.2</u>	<u>5.2</u>
5.2 Companies with a <u>global</u>	Companies with a <u>commercial</u>

- 2.0 Election of the President was subsequently called into order. Michael Lenz proposed Kunio Tsunoda as the President, and his proposal was seconded by Phillip Morrow. The nomination was then closed, and Kunio Tsunoda was officially elected as the Founding President of TRG. KT later spoke that his vision and expectation of having the TRG, and announced that he will disclose the names of his executive councils in the afternoon. KT then presented a proposal from China on the protection of historical/cultural buildings and structures from attack by termites, namely the Whampoa (Huangpu) Military Academy building. This proposal was provided by Dr Junhong Zhong. Members were asked to read the proposal for discussion in the afternoon.
- 3.0 The meeting was then adjourned for lunch at 12.00 pm.
- 4.0 The plenary session reopened at 4.00 pm after excursion to the Butterfly Farm. KT announced to the members that he has chosen Tsuyoshi Yoshimura as Secretary-General, Michael Lenz and Masatoshi Yokoyama as executive council (EC) members.
- 5.0 Thailand was nominated as the venue for the second TRG annual meeting. Charunee Vongkaluang agreed to take charge of the local organizer for the meeting in 2005. Members suggested the meeting to be held in either Phuket, Bangkok or Chiang Mai.
- 6.0 As there were no other issues to be discussed, the meeting was adjourned at 5.00 pm.
- 7.0 The first EC meeting fixed the dates of the second meeting: 28 February (Monday) – 1 March (Tuesday) 2005.
- 8.0 Following oral presentations on the second day, KT proposed that copy right of the proceedings should belong to the TRG and that the submitted materials are translated into Japanese for the journal, "Shiroari (Termite)" quarterly published by Japan Termite Control Association. The participants favorably accepted these two proposals.
- 9.0 Lastly, two proposals were suggested for the future activities. First from KT: Sponsor members are also supposed to give their presentations on R & D (not for

advertising their products). The second one by Michael Lenz: Ordinary members (country representatives from 10 countries at this stage) are kindly requested to send a list of the first 2 priority areas/needs for their countries in relation to termite management and research, to the President for the EC to consider future collaborative projects within the TRG. The participants favorably accepted these two proposals.

10.0As there were no other issues to be discussed, the meeting was adjourned at 5.30 pm with word of thanks from KT to the local organizer for his arrangement and efforts for the first meeting in Penang, Malaysia.

**Chow-Yang Lee**

Local organizer, First Pacific-Rim Termite Research Group Meeting 2004

## Ordinary Membership Application Form for the Pacific Rim Termite Research Group

Please fill out the form and e-mail, fax or mail it to Dr. Kunio Tsunoda not later than 31 December, 2004

Dr. Kunio Tsunoda

Research Institute for Sustainable Humansphere, Kyoto University

Uji, Kyoto 611-0011, Japan

Fax: 81-774-38-3664 Tel: 81-774-38-3661

e-mail: [tsunoda@rish.kyoto-u.ac.jp](mailto:tsunoda@rish.kyoto-u.ac.jp)

### Application form for the ordinary membership

Name	
Title Tick the appropriate box.	<input type="checkbox"/> Mr. <input type="checkbox"/> Ms <input type="checkbox"/> Dr.
Position	
Affiliation	
Address	
Telephone number (country code + area code + number)	
Fax number (country code+ area code +number)	
e-mail address	

Please list best 5 papers related to termite research [author(s), article title, journal name, volume (number), page, year].

1)

2)

3)

4)

5)

## Sponsor Membership Application Form for the Pacific Rim Termite Research Group

Please fill out the form and e-mail, fax or mail it to Dr. Kunio Tsunoda not later than 31 December, 2004

Dr. Kunio Tsunoda

Research Institute for Sustainable Humanosphere, Kyoto University

Uji, Kyoto 611-0011, Japan

Fax: 81-774-38-3664 Tel: 81-774-38-3661

e-mail: [tsunoda@rish.kyoto-u.ac.jp](mailto:tsunoda@rish.kyoto-u.ac.jp)

### Application form for the sponsor membership

Name of company	
Contact person	
Title Tick the appropriate box.	<input type="checkbox"/> Mr. <input type="checkbox"/> Ms <input type="checkbox"/> Dr.
Position	
Address	
Telephone number (country code + area code + number)	
Fax number (country code + area code + number)	
e-mail address	

# Conference Announcement and Call for Papers



## TRG 2, 2005

Pacific Rim Termite Research Group  
Rama Gardens Hotel, Bangkok, Thailand  
28 February & 1 March 2005

Date: 28 February & 1 March 2005

Venue: Rama Gardens Hotel, Bangkok, Thailand

The objective of TRG 2 is to provide a forum for oral presentations and exchange of scientific and technical information among participants for two days.

TRG Executive Committee (EC) would be very pleased to receive papers on the following topics:

- ★ Termite management technology
- ★ Termite-resistant materials
- ★ New termiticides
- ★ Problems with termites
- ★ Termite biology
- ★ Termite ecology
- ★ Termite physiology

Proposals are also welcome to facilitate collaborative research projects among termite researchers in the Pacific Rim region.

Those who would like to present papers are kindly requested to submit a 4-6 page full paper by **20 January 2005** to the secretary general (Dr. Tsuyoshi Yoshimura) electronically at [tsuyoshi@rish.kyoto-u.ac.jp](mailto:tsuyoshi@rish.kyoto-u.ac.jp). The printed materials should be mailed to him as well (Dr. Tsuyoshi Yoshimura, Research Institute for Sustainable Humanosphere, Kyoto University, Uji, Kyoto 611-0011, Japan). Earlier submission is greatly encouraged.

A paper should contain title, authors and their affiliation, abstract, introduction, materials and methods, results and discussion/conclusions and references. "Times New Roman" (Microsoft Word) is the format required for inclusion of your paper in the conference proceedings. A lay-out example of a paper is shown below. **Submitted papers are reformatted in pdf files to produce CD proceedings when over 25 papers are submitted.**

Inquiry: Please contact the Secretary General of the TRG, Dr. Tsuyoshi Yoshimura (Tel: 81-774-38-3662, Fax: 81-774-38-3664, e-mail: [tsuyoshi@rish.kyoto-u.ac.jp](mailto:tsuyoshi@rish.kyoto-u.ac.jp)) or the President of the TRG, Dr. Kunio Tsunoda (Tel: 81-774-38-3661, Fax: 81-774-38-3664, e-mail: [tsunoda@rish.kyoto-u.ac.jp](mailto:tsunoda@rish.kyoto-u.ac.jp)).

## Guidelines for preparing a paper for TRG 2

**Margins: 30 mm each (top, bottom, left and right)**

### **Termite Resistance of New Building Materials (14p bold)**

by

Kunio Tsunoda and Tsuyoshi Yoshimura  
Kyoto University, Uji, Kyoto 611-0011, Japan

#### **Abstract (12p, bold)**

Of 21 termite species recorded in Japan, two subterranean termites,---. (text in 10.5 p)

**Key words (4-6 keywords 10.5p bold):** soil treatment , wood treatment, baiting program, physical barrier

#### **Introduction (12p, bold) (text in 10.5 p)**

Termites are serious pests -----development somewhat improved the crawl space environment to prevent an invasion of termites into houses (IBEC\* 1998, 1999a, 1999b).

#### **Materials and methods (12p, bold)**

Wood samples were randomly taken from the freshly-sawn sapwood.----- (text in 10.5 p)

#### **Results and discussion (12p, bold)(text in 10.5 p)**

#### **Conclusions (12p, bold) (text in 10.5 p)**

#### **References (12p, bold author(s) in alphabetical order) (Citations at appropriate places in the text )**

(text in 10.5 p)

IBEC 1998 Report on the project to spread foundation insulation method in the fiscal year 1997. 128 pp. IBEC (Tokyo) (in Japanese).

Tsunoda, K. 2003 Economic importance of Formosan termite and control practices in Japan. *Sociobiology* 41(1), 27-36.

Yamauchi, K., H. Ishikura, J. Otake, I. Maeda, A. Kashihara and K. Uechi 1997 Field evaluation of the Sentricon system – confirmation of colony elimination. *Shiroari* (Termite) 110, 2-7. (in Japanese).

**NO PAGE TYPED**

# Preparation for Oral Presentation

Every paper will be given 15-30 min for an oral presentation when TRG-EC decides to accept the paper.

1. PowerPoint presentations are strongly recommended, although OHP is also available. If you like to use OHP instead of PowerPoint, please advise our local organizer Dr. Charunee Vongkaluang at [wpcv1@yahoo.com](mailto:wpcv1@yahoo.com).
2. You are kindly requested to bring your presentations in a CD-ROM or to send your PowerPoint file electronically to Dr. Charunee Vongkaluang by Wednesday 23 February 2005. When you electronically send your file, do not forget to bring CD-ROM, just in case.
3. The CD-ROM for your presentation should be passed to Dr. Charunee Vongkaluang at the registration desk during registration on Monday 28 February 2005.

## Registration Form for the Second Conference of the Pacific Rim Termite Research Group

**Venue:** Rama Gardens Hotel in Bangkok, Thailand; approximately 25-30 min from Bangkok Airport by taxi; address: 9/9 Vibhavadi Rangsit Road, Laksi, Bangkok 10210, Thailand; Tel: 66-2-561-0022; Fax: 66-2-561-1025; e-mail: [rama@ramagardenshotel.com](mailto:rama@ramagardenshotel.com); home page: <http://www.ramagardenshotel.com>. Major credit cards are accepted for payment at the hotel.

**Date:** 28 February and 1 March 2005

**Registration fee:** JPY20,000 or USD200 per person for sponsor members and JPY4,500 or USD40 for ordinary members. The registration fee covers conference proceedings, lunch, morning and afternoon coffee/tea and conference banquet during the two-day meeting. There is no charge for an accompanying person.

**Payment method:** Registration fee has to be paid by wire transfer no later than 10 February 2005. You are kindly requested to pay for the handling charge of wire transfer.

Name of bank: Mizuho Bank, Fushimi-chuo Branch

Name: PRTRG TSUNODA KUNIO

Account number: 1666268

Please fill out the form and e-mail or fax it to the Secretary General, Dr. Tsuyoshi Yoshimura (Fax: 81-774-38-3664; E-mail: [tsuyoshi@rish.kyoto-u.ac.jp](mailto:tsuyoshi@rish.kyoto-u.ac.jp)).

### *Delegate Details*

Membership category	Sponsor	Ordinary
Surname		
Given name		
Affiliation (where you work)		
Mailing address		
Telephone number		
Fax number		
E-mail address		
Accompanying person's name, if any		
Total amount of fees		
Date of payment		

**Accommodation Booking Form for the Second Conference of the Pacific Rim Termite Research Group**  
**Rama Gardens Hotel in Bangkok**  
**28<sup>th</sup> February -1<sup>st</sup> March 2005**

A special rate is arranged for the delegates, THB1,600-2,600 (USD40-64/JPY5,100-8,300) per night inclusive of tax and breakfast. You are kindly requested to clear your bill at departure.

**Room categories**

Superior room in Rama wing -

Single room: THB1,600 (USD40/JPY5,100); Twin room: THG1,800 (USD44/JPY5,800)

Executive deluxe room in Grand Courtyard wing-

Single room: THB2,400 (USD59/JPY7,700); Twin room: THG2,600 (USD64/JPY8,300)

Please fill out the form and e-mail (wpcv1@yahoo.com) or fax (66-2-561-4872) it to Dr. Charunee Vongkaluang no later than 10 February 2005.

**Delegate Details**

Room category request	Superior room	Single or Twin
	Executive deluxe room	Single or Twin
Surname		
Given name		
Passport number		
Affiliation (where you work)		
Mailing address		
Telephone number		
Fax number		
E-mail address		
Accompanying person's name (1)		
Accompanying person's passport number (1)		
Accompanying person's name (2)		
Accompanying person's passport number (2)		
Arrival date (day/month)		
Arrival flight		
Estimated time of arrival at the Bangkok airport		
Departure date (day/month)		
Special requests, if any		

