

Proceedings of the 9th Pacific-Rim Termite Research Group Conference

***Melia Hotel, Hanoi, Vietnam
27 – 28 February 2012***

*Dedicated to the Memory
of*

Dr Kunio Tsunoda (1948 – 2011)
The Founding President of Pacific-Rim Termite Research Group



Notice

This publication contains the papers that were presented at the 9th Pacific-Rim Termite Research Group Conference (TRG 9) that was held in Hanoi, Vietnam from 27 – 28 February 2012. All received papers were formatted for the proceedings, not edited and did not undergo any peer-review process. Kindly contact the respective author(s) directly should there be any queries, inaccuracy and issues pertaining to the papers.

***Organizing Committee of TRG 9, and
Executive Committee of the Pacific-Rim Termite Research Group***

Table of Contents	Page
Notice	2
Table of Contents	3
<u>Plenary Session - Kunio Tsunoda Memorial Lectures</u>	
Innovations in termite control technology - Mark A. Coffelt	7
Cellulose digestion by termites and their symbionts - Gaku Tokuda	10
Merging traditional taxonomy and molecular techniques to identify termite species - a blueprint for proper pest control in emerging Asian economies - Brian T. Forschler, Su Yee Lim, Beng Keok Yeap and Chow Yang Lee	16
Entomopathogenic fungi as a termite control agent - Aya Yanagawa	25
Invasive termites - Theodore Evans	31
<u>Session 1 - Evaluation of termite management products, compounds and resistant materials</u>	
Are results on the termite resistance of plastic materials comparable between countries? - Summary report of a field study with <i>Coptotermes</i> , <i>Mastotermes</i> and <i>Reticulitermes</i> (Isoptera) - Michael Lenz, Brad Kard, James W. Creffield, Theodore A. Evans, Kenneth S. Brown, Edward D. Freytag, Jun-Hong Zhong, Chow-Yang Lee, Boon-Hoi Yeoh, Tsuyoshi Yoshimura, Kunio Tsunoda, Charunee Vongkaluang, Yupaporn Sornnuwat, Ted A. Roland, Sr., and Violaine Weibel	48
Identifying termite resistant design works on Hong Kong buildings by investigating the ecology Of <i>Coptotermes formosanus</i> - Shing Kwong Cheng and Wai Tung Cheung	55
Efficacy And longevity of some soil termiticides under field trials in urban areas of Pakistan - Farkhanda Manzoor, Saadiya A. Malik and Ruhma Syed	60
Termite resistance of bamboo (intermediate results) - Gina Bachtiar	67
Resistance of Jabon timber modified with styrene and MMA against soil termites and dry wood termites - Lusita Wardani, Iwan Risnasari, Yasni, Yusuf Sudo Hadi	73
Physical barriers as prevention of subterranean termite attack on building - Indah Sulistyawati	79
Effect of sublethal exposure to fipronil in combination with the entomopathogenic fungus <i>Metarhizium anisopliae</i> on the subterranean termite <i>Coptotermes formosanus</i> Shiraki - Yuichi Yamamoto, Aya Yanagawa, Tsuyoshi Yoshimura and Kunio Tsunoda	85
The resistance of six Papua New Guinea woods to subterranean termite attack - YS Hadi, MY Massijaya, N Hadjib, M Niangu	91
Resistance of three small diameter logs to subterranean termite attack - N Hadjib, MY Massijaya, YS Hadi, D Hermawan	95

Application of liquid smoke to control termite infestation in oil palm plantation on peat land - Yuliati Indrayani	99
--	----

Session 2 - Physiology and Biochemistry

Isolation and identification cellulolytic bacteria from the termite <i>Coptotermes curvignathus</i> Holmgren and <i>Macrotermes gilvus</i> Hagen from secondary forest in West Kalimantan Indonesia - Farah Diba, Siti Khotimah and Utin Febriyana	105
Characterization of cellulase activities in wood-feeding termites of different evolutionary levels - Zhi-qiang Li, Wen-hui Zeng, Qiu-jian Li, Bing-rong Liu, Jun-hong Zhong	112
cDNA cloning and bioinformatics analysis of a novel endogenous cellulase from termite, <i>Reticulitermes flaviceps</i> (Isoptera; Rhinotermitidae) - Wenhui Zeng, Ruixian Liu, Zhiqiang Li, Bingrong Liu, Qiuqian Li and Junhong Zhong	118

Session 3 - Evaluation of bio-compounds and microbial agents

Preliminary research results on the ability of termite exocrine gland secretion resisting pathogenic microorganisms- Ta Kim Chinh	126
Termite feeding deterrents produced by a brown rot fungus <i>Fibroporia radiculosa</i> - Shota Nishizawa, Akiko Nakagawa-Izumi, Shuichi Doi, Tsuyoshi Yoshimura, Sakae Horisawa	130
Toxicity of volatile oil of <i>Corymbia citriodora</i> (Hook.) K.D. Hill & L.A.S Johnson against subterranean termites <i>Coptotermes vastator</i> Light and drywood termites <i>Cryptotermes dudleyi</i> Banks - Irma I. Palanginan and Carlos M. Garcia	135
Evaluation of the effects of <i>Metarhizium</i> (M1) for the control of subterranean termite <i>Coptotermes formosanus</i> - Nguyen Thi Phuong Thao, Nguyen Minh Duc, Vo Thu Hien and Dao Thi Lanh	141

Session 4 - Systematics and Biodiversity

Diversity and distribution of termite species on oil palm plantation at the PTP Nusantara VIII Bogor, West Java-Indonesia - Arinana, Noor Farikhah Haneda, Tinto Punto Kahar	148
Termite occurrence as pest of structures in Temenggong lake catchment area of northern Peninsular Malaysia - Aiman Hanis J. and Abu Hassan A.	156
Studies on termites (Isoptera) living in <i>Eucalyptus urophylla</i> S.T.Blade and <i>Acacia spp.</i> plantations in Vietnam - Nguyen Thi Bich Ngoc and Bui Thi Thuy	162
Biodiversity and distribution features of termites (Insecta: Isoptera) in Hanoi area, Vietnam - Nguyen Van Quang, Nguyen Hai Huyen, Ngo Kim Khue, Nguyen Thanh Huong, Trinh Van Hanh, Nguyen Thi My, Vo Thu Hien	169
The characteristic of the isomorphs (Sibling species) and the heteromorphy of isoptera. The discussion on the isopteran classification - Nguyen Duc Kham	178
Phylogeography of fungus-growing termite, <i>Macrotermes gilvus</i> (Blattodea: Termitidae) in South East Asia as inferred by mitochondrial DNA - G. Veera Singham, Ahmad Sofiman Othman and Chow-Yang Lee	181

Development and isolation of 17 microsatellite loci in *Coptotermes formosanus* Shiraki- 187
Bing-rong Liu, Jun-hong Zhong, Zhi-qiang Li, Qiu-jian Li and Wen-hui Zeng

Session 5 - Ecology and behavior

Evaluation of trail pheromone and attractants against drywood termite *Incisitermes minor* 192
(Hagen)(Blattodea: Kalotermitidae) - Emiria Chrysanti and Tsuyoshi Yoshimura

Parasitism by parasitoid *Misotermes mindeni* (Diptera: Phoridae) increases frequencies of 199
trophallaxis and allogrooming in *Macrotermes gilvus* (Blattodea: Termitidae) - Foong-Kuan
Foo, Ahmad Sofiman Othman and Chow Yang-Lee

Foraging behavior and territory of *Coptotermes formosanus* Shiraki (1909) in Hanoi city - 204
Trinh Van Hanh, Nguyen Quoc Huy, Nguyen Thi My, Nguyen Thuy Hien, Tran Thu
Huyen

Laboratory maintenance of a fungus-growing termite, *Macrotermes gilvus* (Blattodea: 210
Termitidae) - Ching-Chen Lee, Foong-Kuan Foo, and Chow-Yang Lee

The potential of subterranean termites *Macrotermes gilvus* Hagen (Blattodea: Termitidae) 216
in modifying soil in rehabilitation critical land - Niken Subekti

Do termites fear carcasses? Kok-Boon Neoh, Beng-Keok Yeap, Kunio Tsunoda, Tsuyoshi 220
Yoshimura, and Chow-Yang Lee

Plenary Session

Kunio Tsunoda Memorial Lectures

Innovations in termite control technology

Mark A. Coffelt

DuPont Professional Products

4417 Lancaster Pike, P. O. Box 80705, Chestnut Run Plaza, Laurel Run IN11 Wilmington, DE,
USA 19880-0705

Abstract

Termite control technologies have been utilized throughout the world for over a hundred years, with varying degrees of success. Early control efforts emphasized use of persistent, long residual compounds that were effective against termites but may have had mammalian toxicity concerns and negative effects on off target organisms. The liquid soil applied termiticides market was at one time dominated by the cyclodiene and organophosphate insecticides, and the market then shifted to pyrethroids. The nonrepellent termiticides started to secure market share in the middle to late 1990s with the launch of imidacloprid, and later with fipronil and chlorfenapyr. The newest entry into this nonrepellent market represents a new chemical class in the anthranilic diamides called chlorantraniliprole. At the same time as the nonrepellent termiticide entered the market, the first termite baits were being registered. These termite baits contain either insect growth regulators or slow acting toxicants, and many are available as below or above ground devices. The current termite control market is dominated by liquid applied soil termiticides and termite baits. This paper will provide an overview of these termite control technologies and discuss recent innovations and glimpses into the future.

Key words: termite control, liquid termiticides, termite baits, termite innovations

Introduction

Liquid soil applied termiticides consist today of six insecticide classes. These are the neonicotinoids, phenylpyrazoles, pyrethroids, pyrroles, anthranilic diamides and organophosphates. The repellent compounds are represented by the pyrethroids, while the nonrepellents contain the other classes. The toxicity of seven of the most recent nonrepellent termiticides was evaluated using both topical applications and substrate treatments (Mao et al. 2011). The organophosphate compound chlorpyrifos is considered nonrepellent, although various solvents in some of the formulations can make the product repellent when first applied. Liquid termiticides are commonly used throughout the world for both pre-construction and post-construction applications. Depending on the product and formulation, most liquid termiticides are considered economical in providing termite residual control. In the United States, liquid termiticides applied post-construction used to be applied primarily as a full, conventional application. This usually involved treating all termite entry areas, trenching and rodding around the structures, drilling into concrete to reach the soil, and interior applications (Potter, 2011). The volume required treating a structure with the new exterior perimeter and localized interior applications have reduced volumes around a structure by 30-50% (Potter, 2007). In addition, the launch of the first reduced risk termiticide with no signal word on the label with the active

ingredient chlorantraniliprole (Coffelt et al. 2011, Saran et al. 2011) has allowed a low hazard liquid termiticide to compete with the low environmental impact of termite baits. Chlorantraniliprole represents the first new insecticide class for termite control in nearly a decade with unique mode of action and an excellent environmental profile (Cordova et. al. 2006, Lahm et .al. 2005).

Termite bait technology consists of various active ingredients for termite control. Insect growth regulators include diflubenzuron, hexaflumuron, noviflumuron, bistrifluron and chlorfluazuron and slow acting inhibitors have included such active ingredients as sulfuramid and hydramethylnon. Key attributes required to make an effective termite bait include nonrepellancy, slow-acting toxicants, highly palatable bait matrix and ability for transmission of the compound to termite nest mates (Potter, 2011). Recent innovations in termite baits involve rigid, cellulose-based baits that are more durable and allow for longer inspection intervals and bait residual and palatability.

Materials and methods

Innovations in termite control technology were collected from many industry sources, including published scientific literature, popular articles, book reviews and termite industry consultants. Patents related to innovations were also reviewed. A summary of termite technology advancements were gathered and summarized for this article and presented.

Results and discussion

A historical perspective of termite control technology revealed that major innovations have occurred starting in the middle 1990s. These innovations involved development of nonrepellant active ingredients that allowed for transmission of the compound to termite nest mates and new applications methods that reduced termiticide volumes yet provided effective termite control. In addition, innovation around a new insecticide class, a new mode of action against termites, and improved environmental profiles compared to existing compounds were discovered. Innovations will continue to evolve in the liquid soil applied termiticide market. The termite bait market continues to show innovation around longer lasting termite bait matrices and devices to control termite populations.

Utilization of termite pheromones and other chemical cues are leading areas of research for many termite research organizations. In addition, genetic studies with termites may allow for new termite control technologies in the future. Future innovations are actively being researched throughout the world.

Conclusions

The cost of developing a new insecticide active ingredient and one that will be launched into the global termite market continues to rise. Many large agrochemical companies have been forced to reexamine the model for discovery and development of such expensive active ingredients. The latest estimates are that the discovery, development, registration, manufacturing and launch of such new active ingredients can easily exceed \$250 million USD. Therefore, the launch of a new insecticide active ingredient into the global termite market may be a rare event in the future.

Chlorantraniliprole represents such a new active ingredient and this novel chemistry and unique mode of action will allow termite control professionals to utilize innovative termite control technology. Additional innovations that may involve formulations, delivery devices, application methods, chemical cues and genetic attributes may provide a positive future for termite control technology.

References

Coffelt, M. A., C. Scherer and R. Saran. 2011 Chlorantraniliprole: A new insecticide for global termite control. Proc. 7th Intl. Conf. on Urban Pests. W.H. Robinson and A. Campos Eds., pp. 193-198.

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

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

Mao, L., G. Henderson and C. Scherer. 2011 Toxicity of seven termiticides on the Formosan and Eastern subterranean termites. *J. Econ. Entomol.* **104** (3), 1002-1008.

Potter, M. 2011 Termites. *In: Handbook of Pest Control*. Mallis Tenth Edition (S. Hedges and D. Moreland Eds.), pp. 292-441. The Mallis Handbook Company LLC.

Potter. M. 2007 The state of termite management. *Pest Control Technology* **35**(2), 39-56.

Saran. R., C. Scherer and M. Coffelt 2011. Chlorantraniliprole: A new termiticide. Proc. 7th Intl. Conf. On Urban Pests. W.H. Robinson and A. Campos Eds., pp. 179-182.

Cellulose digestion by termites and their symbionts

Gaku Tokuda

University of the Ryukyus, Nishihara, Okinawa 903-0213, Japan

Abstract

Most termites can survive on woody diet because of their efficient digestive system. The xylem of woody plants ingested by termites primarily consists of cellulose, hemicellulose, and lignin, which are the major constituents of the secondary cell wall of plants. An insoluble matrix formed by the complex structure of cellulose, hemicellulose, and lignin is termed lignocellulose. Termites digest lignocellulose with the aid of the intestinal microbial symbionts. However, termites also produce their own cellulolytic enzymes in the salivary glands and/or the midgut. It has been hypothesized that termites have a dual cellulolytic system (*i.e.* composed of endogenous and symbiotic digestive systems in the midgut and the hindgut) to accomplish the efficient hydrolysis of cellulose. In the present paper, the current knowledge on the detailed mechanisms by which termites digest cellulose is presented.

Key words: cellulase, endoglucanase, β -glucosidase, glycosyl hydrolase families,

Introduction

Cellulose is a linear polymer consisting of β -1,4-linked D-glucopyranosyl units. It is synthesized by all higher plants, which collectively fix $(6.0\text{--}6.3) \times 10^{16}$ g of carbon annually (Prentice et al. 2001).

Many microorganisms can survive on cellulosic materials. In contrast, it was believed that most animals could not utilize cellulose. However, recent and growing evidence indicates that cellulolytic enzymes are distributed in a wide range of invertebrate animals (Watanabe & Tokuda 2010). Some vertebrate animals (e.g., even-toed herbivores) utilize cellulosic biomass with the aid of intestinal symbionts. Xylophagous insects, especially termites, are well adapted to feeding on wood. Their body plans, including masticating organs, gut structures, digestive enzymes, and symbiotic systems, allow them to thrive on cellulosic substances. In this context, xylophagous insects possess efficient microscale bioconversion systems of cellulosic biomass in their bodies.

Under the current crisis of global warming due to the increasing use of fossil fuels, which results in significant carbon dioxide emissions, the cellulose-converting systems in these xylophagous termites may provide researchers with information about utilizing plant cellulosic biomass as a sustainable energy source. This paper summarizes the current status of studies on lignocellulose digestion in termites, the most efficient decomposers of wood on earth.

General machinery of cellulose hydrolysis

Cellulose is the most abundant compound in plant cell walls, contributing to about 20–40% of dry weight in the primary cell walls. It generally increases up to 50% in the secondary cell walls,

except for a few cases such as cotton seed hairs, which consist of 100% cellulose. Plant cell walls contain cellulose in the form of crystalline microfibrils. In native cellulose, crystalline and amorphous regions alternate in the cellulose microfibril (CMF). Such crystalline structures and the amount of cellulose vary among cell types and developmental stages. Other major components of the secondary cell walls are hemicellulose and lignin, which are colocalized mainly in the xylem of woody plants together with cellulose. The complex formed by cellulose, hemicellulose, and lignin is often called lignocellulose.

Cellulase is a general term for cellulolytic enzymes, of which three classes are recognized on the basis of the mode of enzymatic actions and the substrate specificities: endoglucanases (EGs) (Enzyme Commission [EC] 3.2.1.4), exoglucanases (EC 3.2.1.74 and 3.2.1.91), and β -glucosidases (EC 3.2.1.21) (Fig. 1). Endoglucanases (endo- β -1,4-glucanases or 1,4- β -D-glucan-4-glucanohydrolases) cleave amorphous sites of cellulose chains at random. Exoglucanases [1,4- β -D-glucan cellobiohydrolases (CBH) (EC 3.2.1.91) or 1,4- β -D-glucan glucohydrolases (EC 3.2.1.74)] act on the nonreducing or reducing termini of cellulose fibers to release either cellobiose (cellobiohydrolases) or glucose (glucohydrolases). β -Glucosidases (1,4- β -D-glucosidases or cellobiases) hydrolyze cellobiose or cello-oligomers to glucose from the nonreducing ends.

All enzymes are classified on the basis of their substrate specificities and mode of action (see <http://www.chem.qmul.ac.uk/iubmb/enzyme/>). Recently, cellulases and other carbohydrases have been grouped into a glycoside hydrolase (GH) family based on amino acid sequence similarities. Members in the same GH family are usually considered to share not only structural motifs and the catalytic machinery, but also an evolutionary origin (Henrissat & Davies 1997).

Endogenous cellulases from termites

RsEG (endo- β -1,4-glucanase of *Reticulitermes speratus*) was one of the first two animal cellulase genes to be cloned (Watanabe et al. 1998). Endogenous EG genes from plant parasitic nematodes were isolated at a similar time. A key question arising from the discovery of these EGs concerned their evolutionary origin. Unlike RsEG, the nematode EGs were from a completely different glycosyl hydrolase family (GHF5) (Watanabe & Tokuda 2001). This ruled out a common evolutionary origin for termite and nematode EGs. It is hypothesized that the nematode EGs have arisen from a horizontal gene transfer event from a bacterium (Mayer et al. 2011). Was horizontal gene transfer responsible for the presence of EGs in termites? Soon after the cloning of RsEG, the entire ~13 kb genomic sequence (including both flanking regions that encoded the typical eukaryotic promoter elements) of a GH9 EG from the higher termite *Nasutitermes takasagoensis* was determined, and found to contain 9 introns (Tokuda et al. 1999). The discovery of endogenous GHF9 genes from an abalone (*Haliotis discus*), and a sea-squirt (*Ciona intestinalis*) followed. A comparison of intron positions in these EG genes revealed that a number of them were identical among the three genes (Lo et al. 2003). This was highly suggestive that a gene with introns in the very same positions was present in the ancestor of all bilaterian animals. It is now clear that, contrary to the traditional belief that endogenous animal cellulases are non-existent or rare, GHF9 EGs are quite common animal enzymes, having

been inherited over hundreds of millions of years from a common ancestor (Davison & Blaxter 2005).

Endogenous β -glucosidase genes have been cloned from the lower termite *Neotermes koshunensis* (Tokuda et al. 2002), *Reticulitermes flavipes* (Scharf et al. 2010), *Coptotermes formosanus* (Zhang et al. 2012), and the higher termite, *N. takasagoensis* (Tokuda et al. 2009). These β -glucosidases are affiliated with GHF1, which also includes β -glycosidases reported from other insects. Although, to the best of my knowledge, no phylogenetic analysis of insect β -glycosidases has been available yet, it appears that these enzymes share a common ancestor.

Functional significance and expression sites of endogenous cellulases

The salivary glands were the only site of endogenous cellulase expression in lower termites (Table1) (Tokuda et al. 2004). The pattern is different in the phylogenetically apical Termitidae. Although EG expression occurs only in the salivary glands of the fungus grower *Odontotermes formosanus*, expression in the wood feeder *N. takasagoensis* and the soil feeder *Sinocapritermes mushae* has changed dramatically, now occurring only in the midgut (Table 1).

Table1. Distribution of EG activities and sites of expression in the gut of termites

Termite	Presence of protists	Distribution (%) of total EG activity in the gut				EG expression
		Salivary glands (SG)	Foregut (FG)	Midgut(MG)	Hindgut (HG)	
Macrotermitidae						
<i>Mastotermes darwiniensis</i>	+	49.7 ± 4.5	1.0 ± 0.1	17.1 ± 0.62	32.2 ± 2.6	GHF9/SG GHF5, 7,45/HG
Termopsidae						
<i>Hodotermopsis sjoestedti</i>	+	72.2 ± 1.7	0.2 ± 0.1	0.1 ± 0.1	27.5 ± 1.6	GHF9/SG GHF5, 7,45/HG
Kalotermitidae						
<i>Neotermes koshunensis</i>	+	85.7 ± 1.4	1.8 ± 0.3	0.1 ± 0.0	12.4 ± 1.1	GHF9/SG GHF5, 7HG
Rhinotermitidae						
<i>Reticulitermes speratus</i>	+	78.2 ± 1.5	0.3 ± 0.1	0.5 ± 0.1	21.0 ± 1.5	GHF9/SG GHF5, 7,45/HG
<i>Coptotermes formosanus</i>	+	44.6 ± 4.1	1.5 ± 0.3	15.9 ± 1.8	38.0 ± 3.4	GHF9/SG, MG? GHF5,7/HG
Termitidae						
Macrotermitinae						
<i>Odontotermes formosanus</i>	-	69.1 ± 1.0	0.3 ± 0.1	28.3 ± 1.1	2.3 ± 0.4	GHF9/SG
Nasutitermitinae						
<i>Nasutitermes takasagoensis</i>	-	0	0.4 ± 0.1	99.0 ± 0.2	0.1 ± 0.2	GHF9/MG GHF5,8,9,44,45,51/HG
Termitinae						
<i>Sinocapritermes mushae</i>	-	0	7.7 ± 0.3	88.5 ± 0.3	3.8 ± 0.0	GHF9/MG

Although endogenous termite cellulases are unable to exhaustively degrade cellulose, they do appear to play a significant role in providing energy for those termites that feed on wood. A recent study showed that inhibition of mRNA expression of an endogenous EG in *R. flavipes* by administration of dsRNA resulted in substantial mortality of the termites in a dose-dependent manner (Zhou et al. 2008), supporting an indispensable role of endogenous cellulases in termite physiology. In addition, notably, the amount of glucose produced by endogenous enzymes is likely to account for all, or most, of the CO₂ respired by each of these six wood-feeding termites (Tokuda et al. 2005). It is generally thought that EGs can only act in the amorphous regions of cellulose, but a recent finding suggests that the midgut of termites still hides an unknown mechanism that enhances cellulase activity against the crystalline regions of cellulose (Tokuda et al. 2012).

Cellulases from symbiotic microorganisms

Termites harbor numerous (prokaryotic and eukaryotic unicellular) microorganisms primarily in the hindgut and sometimes in the mixed segment. Although it has been almost a century since the presence of ecto-symbiotic cellulolytic agents in the guts of wood-feeders was first observed (Cleveland 1923), the symbiotic cellulase system was poorly understood until recent reports on cellulase genes of the symbiotic microbes.

Already, primarily three GH families 5, 7, and 45 have been reported only for the protistan cellulases of the termite hindgut (Table 1) (Todaka et al. 2010). The number of cellulase genes of symbionts identified continues to increase both in prokaryotes and eukaryotes. These cellulases probably do not freely exist in the lumen of the gut, since the symbiotic protists in the lower termites selectively take in wood particles by endocytosis and digest them in food vacuoles (Yamaoka & Nagatani 1977). Termites in the family Termitidae (higher termites) lack protistan fauna but have abundant prokaryote flora in the hindgut. The bacterial flora was thought to be noncellulolytic for a long time. Recently, the presence of cellulase in the bacterial insoluble fraction of the hindgut of the wood-feeding higher termite *Nasutitermes takasagoensis* and *N. walkeri* (Tokuda & Watanabe 2007) was demonstrated. Furthermore, genes belonging to 45 different GHFs (including putative GHF5, GHF8, GHF9, GHF44, GHF45, GHF51, and GHF74 cellulases as well as GHF1 and GHF3 β -glucosidases) were reported by pyrosequencing of the hindgut prokaryotic flora of a *Nasutitermes* termite (Warnecke et al. 2007). There is no indication that the microbial cellulase genes horizontally transferred to host termites, but it is likely that some bacterial cellulase genes horizontally transferred to the symbiotic protists in termites (Todaka et al. 2010).

Conclusions

Wood-feeding termites are efficient, microscale, biomass conversion systems that consist of mastication (comminution) and two independent sites of enzymatic degradation: endogenous and symbiotic. It is noteworthy that only a few enzymes constitute the endogenous cellulolytic system to obtain sufficient energy for insect survival without the need for thermomechanical and chemical pre-treatments. Thus, studies on the digestive systems of wood-feeding termites are expected to provide information after which an artificial bioreactor can be modeled.

Acknowledgement

This work was supported by the Program for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry (BRAIN) and by a grant-in-aid for Scientific Research Nos. 20380037 and 22658018 from the Japan Society for the Promotion of Science.

References

- Cleveland, L. R. 1923 Symbiosis between termites and their intestinal protozoa. *PNAS* **9**, 424-428.
- Davison, A. & Blaxter, M. 2005 Ancient origin of glycosyl hydrolase family 9 cellulase genes. *Mol. Biol. Evol.* **225**, 1273-1284.
- Henrissat, B. & Davies, G. J. 1997 Structural and sequence-based classification of glycoside hydrolases. *Curr. Opin. Struct. Biol.* **7**, 637-644.
- Lo, N., Watanabe, H. & Sugimura, M. 2003 Evidence for the presence of a cellulase gene in the last common ancestor of bilaterian animals. *Proc. R. Soc. Lond. B* **270 Suppl 1**, S69-72.
- Mayer, W. E., Schuster, L. N., Bartelmes, G., Dieterich, C. & Sommer, R. J. 2011 Horizontal gene transfer of microbial cellulases into nematode genomes is associated with functional assimilation and gene turnover. *BMC Evol. Biol.* **11**, 13.
- Prentice, I. C., Farquhar, G. D., Fasham, M. J. R. *et al.* 2001 The Carbon Cycle and Atmospheric Carbon Dioxide. In *Climate Change 2001: The Scientific Basis* (ed. J. T. Houghton, Y. Ding, D. J. Griggs, M. Noguer, P. J. van der Linden, X. Dai, K. Maskell & C. A. Johnson), pp. 193-237. Cambridge: Cambridge University Press.
- Scharf, M. E., Kovaleva, E. S., Jadhao, S., Campbell, J. H., Buchman, G. W. & Boucias, D. G. 2010 Functional and translational analyses of a beta-glucosidase gene (glycosyl hydrolase family 1) isolated from the gut of the lower termite *Reticulitermes flavipes*. *Insect Biochem. Mol. Biol.* **40**, 611-620.
- Todaka, N., Inoue, T., Saita, K., Ohkuma, M., Nalepa, C. A., Lenz, M., Kudo, T. & Moriya, S. 2010 Phylogenetic analysis of cellulolytic enzyme genes from representative lineages of termites and a related cockroach. *PLoS One* **5**, e8636.
- Tokuda, G., Lo, N. & Watanabe, H. 2005 Marked variations in patterns of cellulase activity against crystalline- vs. carboxymethyl-cellulose in the digestive systems of diverse, wood-feeding termites. *Physiol. Entomol.* **30**, 372-380.
- Tokuda, G., Lo, N., Watanabe, H., Arakawa, G., Matsumoto, T. & Noda, H. 2004 Major alteration of the expression site of endogenous cellulases in members of an apical termite lineage. *Mol. Ecol.* **13**, 3219-3228.

Tokuda, G., Lo, N., Watanabe, H., Slaytor, M., Matsumoto, T. & Noda, H. 1999 Metazoan cellulase genes from termites: intron/exon structures and sites of expression. *BBA* **1447**, 146-59.

Tokuda, G., Miyagi, M., Makiya, H., Watanabe, H. & Arakawa, G. 2009 Digestive β -glucosidases from the wood-feeding higher termite, *Nasutitermes takasagoensis*: intestinal distribution, molecular characterization, and alteration in sites of expression. *Insect Biochem. Mol. Biol.* **39**, 931-937.

Tokuda, G., Saito, H. & Watanabe, H. 2002 A digestive β -glucosidase from the salivary glands of the termite, *Neotermes koshunensis* (Shiraki): distribution, characterization and isolation of its precursor cDNA by 5'- and 3'-RACE amplifications with degenerate primers. *Insect Biochem. Mol. Biol.* **32**.

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

Tokuda, G., Watanabe, H., Hojo, M., Fujita, A., Makiya, H., Miyagi, M., Arakawa, G. & Arioka, M. 2012 Cellulolytic environment in the midgut of the wood-feeding higher termite *Nasutitermes takasagoensis*. *J. Insect Physiol.* **58**, 147-154.

Warnecke, F., Luginbuhl, P., Ivanova, N. *et al.* 2007 Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. *Nature* **450**, 560-5.

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

Watanabe, H. & Tokuda, G. 2001 Animal cellulases. *CMLS* **58**, 1167-1178.

Watanabe, H. & Tokuda, G. 2010 Cellulolytic systems in insects. *Ann. Rev. Entomol.* **55**, 609-632.

Yamaoka, I. & Nagatani, Y. 1977 Cellulose digestion system in the termite, *Reticulitermes speratus* (Kolbe). II. Ultra-structural changes related to the ingestion and digestion of cellulose by the flagellate, *Trichonympha agilis*. *Zool. Mag.* **86**, 34-42.

Zhang, D., Allen, A. B. & Lax, A. R. 2012 Functional analyses of the digestive β -glucosidase of Formosan subterranean termites (*Coptotermes formosanus*). *J. Insect Physiol.* **58**, 205-210.

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

Merging traditional taxonomy and molecular techniques to identify termite species - a blueprint for proper pest control in emerging Asian economies

Brian T. Forschler¹, Su Yee Lim¹, Beng Keok Yeap¹, Chow Yang Lee²

¹Department of Entomology, University of Georgia, Athens, GA 30602 USA

²Urban Entomology Laboratory, Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia.

Key Words: Systematics, morphology, standardized protocol, regional species repository

Abstract

The taxonomy of termites from Southeast Asia is confusing. This forum paper outlines the reasons for the difficulties surrounding consistent and proper identification of termites, provides examples from the authors experience with two genera, and presents a proposal for formation of a regional working group to develop a solution.

Introduction

Linnaeus and Leclerc laid the foundation for universal biological investigation over 250 years ago with their discussions on a systematic organization for naming animals that today is codified by the International Commission on Zoological Nomenclature (ICZN 2000). Correct identification of pestiferous species is the foundation of understanding their biology, and one of the fundamental principles of integrated pest management. The scientific community should be committed to providing timely and accurate information on pest identification and biology. The need to identify termite pest species is critical and nowhere more important than in tropical and subtropical Asia where there are over 350 species (Roonwall 1970, Tho 1992, Li 2000, Lee 2007). Urban development in the Pacific Rim has placed endemic and introduced termite species in increasing contact, and conflict, with human populations. This paper is intended to be a call to the research community represented by the participants here at the TRG to begin a formal dialog to develop a standardized processing protocol and repository for termite identification. This presentation is divided into three parts with the first involving definition of the issues surrounding termite identification, the second outlines lessons learned in recent investigations and lastly we issue a call for establishment of a consortium of researchers with the purpose of providing timely and accurate tools for termite identification.

The biology of termites presents difficulties in proper identification because the free-ranging adult stage is available for collection only at specific times of year after which they are difficult to locate and collect (Miller 1964, Weesner 1965, Nutting 1990, Pearce 1997). The majority of the life forms that can be collected year-round are immature stages that represent a wide range of phenotypic plasticity (Weesner 1965, Nutting 1990, Kirton 2005). The soldier caste is developmentally fixed but also present a range of phenotypes that complicates identification using objective measures (Banks and Snyder 1920, Weesner 1965, Nutting 1990). There are keys for identifying termites using morphological features of the various castes but these are

often regional and therefore not applicable to all parts of Southeast Asia. Molecular techniques have the advantage of being un-restricted by phenotype but are often not correlated with traditional taxonomic identification (Forschler and Jenkins 1999, Jenkins et al. 2000, Jenkins et al. 2001, Austin et al. 2005, Tripodi et al. 2006, Sillam-Dussès and Forschler 2010). The issues surrounding establishment of accurate species-level taxonomy have been outlined in numerous publications including; identification of the species concept used to name an organism, funding for and training of taxonomist, historic descriptions of regional focus without attention to type specimens, as well as, geographic and political constraints (Wheeler et al. 2004, Agnarsson and Kuntner 2007, de Carvalho et al. 2007, Wheeler 2008). The lessons learned by our experience with two genera of subterranean termites, one from the USA and the other from Southeast Asia highlight many of these constraints.

Lessons from recent research

The genus *Reticulitermes* Holmgren 1913 within the family Rhinotermitidae Froggatt, 1897, subfamily Heterotermitinae Froggatt, 1897 (Emerson 1971) is found in temperate regions in the northern hemisphere and represents the most economically important termite pests in North America (Holmgren 1913, Su and Scheffrahn 1990, Pearce 1997, Mallis 2011). There are seven valid species of *Reticulitermes* in North America: *R. flavipes* Kollar 1837, *R. virginicus* Banks 1907, *R. hageni* Banks 1920, *R. mallei* Clement 1986, *R. hesperus* Banks 1920, *R. tibialis* Banks 1920 and *R. nelsonae* Lim and Forschler 2011 (Kollar 1837, Banks 1907, Banks and Snyder 1920, Clément et al. 1986, Austin et al. 2007, Lim and Forschler 2011). There are 9 published keys for this genus in the USA, the most recent from 2009 yet none mention two species - *R. mallei* Clement 1986 and *R. nelsonae* Lim and Forschler 2011 (Banks 1946, Miller 1949, Snyder 1954, Weesner 1965, Nutting 1990, Scheffrahn and Su 1994, Austin et al. 2007, Wang et al. 2009, Mallis 2011). In addition, inter- and intraspecific phenotypic plasticity is observed for all morphological characters examined which are often confused because of poor descriptions of the methods used to obtain those measurements (Banks 1946, Miller 1949, Nutting 1990, Scheffrahn and Su 1994, Lim 2011).

Molecular data have been used to identify species and a recent review by Vargo and Hussenender (2009) listed a version the *Reticulitermes* species in the USA (Table 1). Their synopsis illustrates the potential danger of using genetic data, in isolation, to promote species designations.

Table 1: Species status of *Reticulitermes* in North America (reconstructed from Vargo & Hussenender 2009).

Species	Distribution	Status
<i>R. flavipes</i>	Throughout eastern and central United States	Valid
<i>R. arenincola</i>	Sandy soils near the Great Lakes	<i>Nomen dubium</i>
<i>R. virginicus</i>	Throughout eastern and central United States	Valid
<i>R. hageni</i>	Throughout eastern and central United States	Valid, but may be species complex
<i>R. mallei</i>	Eastern US	Valid

<i>R. tibialis</i>	Western and Midwestern United States	Valid, but may be species complex
<i>R. hesperus</i>	Western United States	Valid, but may be species complex
<i>R. okanaganensis</i>	Pacific Northwest	Valid

A review of the literature over the past 20 years provides three names frequently associated with *Reticulitermes* in North America; *R. santonensis*, *R. arenicola* and “*R. okanaganensis*” (Goellner 1931, Banks 1946, Jenkins et al. 2001, Ye et al. 2004, Austin et al. 2005, Szalanski et al. 2006). *R. santonensis* Feytaud 1924 was originally differentiated based on geographic separation and later by using soldier defense secretions (Bagnères et al. 1990) however, it has since been synonymized with *R. flavipes* and is now an accepted junior synonym supported by genetic data (Jenkins et al. 2000, Jenkins et al. 2001, Austin et al. 2005). Vargo and Hussender (2009) designated *R. arenicola* as *nomen dubium* because of genetic evidence (Austin et al. 2007), however, based on the International Code of Zoological Nomenclature *R. arenicola* should be termed a junior synonym of *R. flavipes* (ICZN 1999). The species designation “*R. okanaganensis*” (Szalanski et al. 2006), considered valid by Vargo and Hussender (2009), according to ICZN (2000) does not exist because it has never been formally described and published. Species identification is further hampered, in part because of the lack of updated taxonomic keys, by aberrations within the GenBank database for *Reticulitermes* that include sequences designated as *R. hageni* that actually reference the *R. nelsonae* species designation (Lim, 2011).

The genus *Coptotermes* is found in tropical and subtropical regions of the world and is the most economically important genus of urban pest insects in Asia and Australia (Su and Scheffrahn 2000, Lo et al. 2006, Takematsu et al. 2006, Lee 2007, Lee et al. 2007). Most of the morphological characters used for the identification of *Coptotermes* spp. provide values that have overlapping ranges and intraspecific variation influenced by, among other things, colony age and habitat (Light 1929, Tho 1992, Grace et al. 1995, Eggleton 1999, Kirton and Brown 2003, Kirton 2005, Scheffrahn et al. 2005, Hussender et al. 2008). The lack of standardized morphological characters and means of measuring them has made identification of *Coptotermes* across Asia difficult (Tho 1992, Eggleton 1999, Kirton 2005). Based on morphological characteristics, 24 species of *Coptotermes* have been described in China, despite overlap with previously described sympatric and allopatric species (Crosland 1995, Eggleton 1999). Kirton (2005) outlined species designations from what he termed the *C. gestroi* “complex” including: *C. javanicus*, *C. havilandi*, *C. vastator*, *C. heimi* and *C. ceylonicus*. Yeap et al. (2007) integrated molecular data with morphological characters and synonymized *Coptotermes vastator* Light with *Coptotermes gestroi* (Wasmann) yet more work needs to be conducted to verify or synonymize the remaining species designations. Yeap et al. (2009) also provided molecular data that supported designating *Coptotermes dimorphus* Xia & He, *Coptotermes cochlearus* Xia & He and *Coptotermes guangzhouensis* Ping as junior synonyms of *Coptotermes formosanus* Shiraki. Using a similar molecular approach, Yeap et al. (2010) also proposed *Coptotermes heimi* (Wasmann) as a junior synonym of *C. gestroi*. Geographic separation, political boundaries, and lack of standardized morphological measures continue to hinder the work needed to solidify those synonymies (Kirton 2005).

Proposed Pacific Rim termite identification advisory group

It should be recognized that an integrated approach using a thorough compilation of the pertinent literature, as well as, standardized quantitative morphology, standardized genetic and standardized behavioral data across the region is the only means of solving the Southeast Asian termite species dilemma. Morphological plasticity and the difficulty in obtaining adults are at the root of the convoluted taxonomic state for this group of insects. A lack of agreement on standardized morphological characters as well as few detailed methodologies on how to obtain objective quantitative characters further contribute to the confusion. Beyond that issue, which is the first step, it is imperative that genetic data include reference sequences in all phylogenetic analysis. Reference sequence for each species must be verified using described morphological and behavioral data from specimens - preferably collected from the same collection site - with the respective type specimens, type descriptions or validated keys (Lim 2011). Taxonomic keys need to be updated to reflect the most recent classification schemes and disseminated through the Internet using the new 'e-keys' (Walter and Winterton 2007, Pyle and Michel 2008, Wheeler 2008). The only way to accomplish such an endeavor is through a collaborative effort. We propose formation of a Pacific Rim Termite Identification Advisory Group to recognize a standard protocol for termite identification and establish a repository where specimens are archived by collection site/dates, measured using standardized methods and matched with appropriate sequence data to be deposited in GenBank. That body of knowledge would be used to generate e-keys designed for scientist, citizens and pest management professionals toward the goal of developing an understanding of the pest species complexes to affect emergent management tactics consistent with economically sustainable environmental stewardship.

References

- Agnarsson, I. and M. Kuntner 2007 Taxonomy in a Changing World: Seeking Solutions for a Science in Crisis. *Systematic Biology* **56**, 531-539.
- Austin, J. W., A. L. Szalanski, R. H. Scheffrahn, M. T. Messenger, S. Dronnet and A.-G. Bagnères 2005 Genetic Evidence for the Synonymy of Two *Reticulitermes* Species: *Reticulitermes flavipes* and *Reticulitermes santonensis*. *Annals of the Entomological Society of America* **98**, 395-401.
- Austin, J. W., A.-G. Bagnères, A. L. Szalanski, R. H. Scheffrahn, B. P. Heintschel, M. T. Messenger, J.-L. Clément, and R. E. Gold 2007 *Reticulitermes malletei* (Isoptera: Rhinotermitidae): a valid Nearctic subterranean termite from Eastern North America. *Zootaxa* **1554**, 1-26.
- Bagnères, A.-G., J.-L. Clément, M. S. Blum, R. F. Severson, C. Joulie, and C. Lange 1990 Cuticular Hydrocarbons and Defensive Compounds of *Reticulitermes flavipes* (Kollar) and *R. santonensis* (Feytaud): Polymorphism and Chemotaxonomy. *Journal of Chemical Ecology* **16**, 3213-3244.

- Banks, F. A. 1946 Species distinction in *Reticulitermes* (Isoptera: Rhinotermitidae). University of Chicago, Chicago.
- Banks, N. 1907 A new species of *Termes*. Entomological News and Proceedings of the Entomological Section Academy of Natural Sciences. *Philadelphia* **18**, 392-393.
- Banks, N., and T. E. Snyder 1920. A revision of the Nearctic termites, vol. **108**, 228 pp.
- Clément, J. L., R. Howard, M. Blum, and H. Lloyd 1986. Écologie Générale (Biogéographie). - L'isolement spécifique des termites du genre *Reticulitermes* (Isoptera) du sud-est des États-Unis. Mise en évidence grâce à la chimie et au portement d' une espèce jumelle de *R. virginicus* = *R. malleti* sp.nov. et d' une semi-species de *R. flavipes*. C. R. ACAD. SC. PARIS **302**, 67-70.
- Crosland, M 1995 Taxonomic splitters in China. *Isoptera Newsl.* **5**, 1.
- de Carvalho, M. R., F. A. Bockmann, D. S. Amorim, C. R. F. Brandão, M. de Vivo, J. L. de Figueiredo, H. A. Britski, M. C. C. de Pinna, N. A. Menezes, and F. P. L. Marques 2007 Taxonomic impediment or impediment to taxonomy? A commentary on systematics and the cybertaxonomic-automation paradigm. *Evolutionary Biology* **34**, 140-143.
- Eggleton, P. 1999 Termite species description rates and the state of termite taxonomy. *Insectes Soc.* **46**, 1-5.
- Emerson, A. E. 1971 Tertiary fossil species of the Rhinotermitidae (Isoptera), phylogeny of genera, and reciprocal phylogeny of associated Flagellata (Protozoa) and the Staphylinidae (Coleoptera). American Museum of Natural History.
- Feytaud, J. 1924 Le termite de Saintonge. C.R. Acad. Sc. Paris **178**, 241-244.
- Forschler, B. T. and T. M. Jenkins 1999 Evaluation of subterranean termite biology using genetic, chemotaxonomic, and morphometric markers and ecological data: a testimonial for multi-disciplinary efforts. *Trends in Entomology* **2**, 71-80.
- Goellner, E. J. 1931 A new species of termite, *Reticulitermes arenicola*, from the sand dunes of Indiana and Michigan, along the shores of Lake Michigan. *Proc. Ent. Soc. Wash.* **33**, 227-234.
- Grace, J. K., R. T. Yamamoto and M. Tamashiro 1995 Relationship of individual worker mass and population decline in a Formosan subterranean termite colony (Isoptera: Rhinotermitidae). *Environ. Entomol.* **24**, 1258-1262.
- Holmgren, N. 1913 Termitenstudien. 4, Versuch Einer Systematischen Monographie der Termiten der Orientalischen Region., vol. **50** (2). Almqvist & Wiksells Boktryckeri-A.-B., Uppsala & Stockholm.
- Husseneder, C., J. E. Powell, J. K. Grace, E. L. Vargo and K. Matsuura 2008 Worker size in the Formosan subterranean termite in relation to colony breeding structure as inferred from molecular

markers. *Environ. Entomol.* **37**, 400-408.

ICZN. 1999 Article 24. Precedence between simultaneously published names, spellings or acts. In W. D. L. Ride, H. G. Cogger, C. Dupuis, O. Kraus, A. Minelli, F. C. Thompson and P. K. Tubbs (eds.), International Code of Zoological Nomenclature, 4th ed. The International Trust for Zoological Nomenclature 1999, The Natural History Museum, London.

ICZN. 2000 International Code of Zoological Nomenclature, 4 ed, vol. International Commission on Zoological Nomenclature.

Jenkins, T. M., R. E. Dean, R. Verkerk, and B. T. Forschler 2001 Phylogenetic analyses of two mitochondrial genes and one nuclear intron region illuminate European subterranean termite (Isoptera : Rhinotermitidae) gene flow, taxonomy, and introduction dynamics. *Molecular Phylogenetics and Evolution* **20**, 286-293.

Jenkins, T. M., M. I. Haverty, C. J. Basten, L. J. Nelson, M. Page, and B. T. Forschler 2000 Correlation of mitochondrial haplotypes with cuticular hydrocarbon phenotypes of sympatric *Reticulitermes* species from the southeastern United States. *Journal of Chemical Ecology* **26**, 1525-1542.

Kirton, L. 2005 The importance of accurate termite taxonomy in the boarder perspective of termite management. Proceedings of the 5th international Conference on Urban Pests. C-Y Lee and W.H. Robinson (eds.). 1-7 pp.

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

Kollar, V. 1837 Naturgeschichte der Schadhlichen Insekten Verhandlungen Landwirthschaft Gesellschaft in Wien, **5**, 411

Lee, C.-Y. 2007 Perspective in urban insect pest management in Malaysia. Vector Control Research Unit, Universiti Sains, Malaysia.

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

Li, G. 2000 *Coptotermes*. pp. 299-341. In F. Huang, S. Zhu, Z. Ping, X. He, G. Li, and D. Gao [eds.], Fauna sinica, insecta vol. **17**: Isoptera. Science Press, Beijing, China.

Light, S. F. 1929 Notes on Philippine termites III. Philipp. *J. Sci* **40**, 421-452.

Lim, S. Y. 2011 Taxonomic review and biogeography of *Reticulitermes* (Rhinotermitidae) termites of Georgia. University of Georgia, Athens.

Lo, N., R. H. Eldridge and M. Lenz 2006 Phylogeny of Australian *Coptotermes* (Isoptera: Rhinotermitidae) species inferred from mitochondrial COII sequences. *Bull. Entomol. Res.* **96**, 433–437.

Mallis, A. 2011 Handbook of Pest Control, 10 ed, vol. The Mallis Handbook Company, Ohio.

Miller, E. M. 1949 A Handbook on Florida Termites, vol. University of Miami Press, Coral Gables, Florida.

Miller, E. M. 1964 Biology of Termites, vol. Coral Gables, Fla, University of Miami Press, Florida.

Nutting, W. L. 1990 Insecta: Isoptera, pp. 997-1032. In D. L. Dindal (ed.), Soil Biology Guide, vol. **33**. John Wiley & Sons, New York.

Pearce, M. J. 1997 Termites: Biology and Pest Management, vol. CAB International, Cambridge.

Pyle, R. L., and E. Michel 2008 ZooBank: Developing a nomenclatural tool for unifying 250 years of biological information. *Zootaxa* **39**, 2008.

Roonwal, M. L. 1970 Termites of the Oriental Region, pp. 315-391. In K. Krishna and F. M. Weesner [eds.], Biology of termites, vol. II. Academic, New York and London.

Scheffrahn, R. H. and N.-Y. Su 1994 Keys to soldier and winged adult termites (Isoptera) of Florida. *Florida Entomologist* **77**, 460-474.

Scheffrahn, R. H., J. Krecke, A. L. Szalanski and J. W. Austin 2005 Synonymy of Neotropical arboreal termites *Nasutitermes corniger* and *N. costalis* (Isoptera: Termitidae: Nasutitermitinae), with evidence from morphology, genetics, and biogeography. *Ann. Entomol. Soc. Am.* **98**, 273-281.

Sillam-Dussès, D., and B. T. Forschler 2010 A dominant and undescribed species of *Reticulitermes* in Sapelo Island (Georgia, USA). *Sociobiology* **46**, 137-147.

Snyder, T. E. 1954 Order Isoptera: The termites of the United States and Canada, vol. National Pest Control Association, New York.

Su, N.-Y., and R. H. Scheffrahn 1990 Economically important termites in the United States and their control. *Sociobiology* **17**, 77-94.

Su, N.-Y. and R. H. Scheffrahn 2000 Termites as pests of buildings, pp. 437-453. In T. Abe, D. E. Bignell, and M. Higashi [eds.], Termites: evolution, sociality, symbioses, ecology. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Szalanski, A. L., J. W. Austin, J. McKern and M. T. Messenger 2006 Genetic evidence for a new subterranean termite species (Isoptera: Rhinotermitidae) from Western United States and Canada. *Florida Entomologist* **89**, 299-304.

Takematsu, Y., T. Yoshimura, S. Yusuf, Y. Yanase, K. Kambara, A. Tashiro, S. Doi, M. Takahashi, P. Sukartana and T. Inoue 2006 Termite assemblages in urban areas of south east Asia-diversity and economic impacts, pp. 84–91. In Y. Imamura, T. Umezawa, and T. Hata [eds.], Sustainable development and utilization of tropical forest resources. RISH, Kyoto University, Kyoto, Japan.

Tho, Y. P. 1992 Termites of Peninsular Malaysia. *Malayan For. Rec.* **36**, 1–224.

Tripodi, A. D., J. W. Austin, A. L. Szalanski, J. McKern, M. K. Carroll, R. K. Saran and M. T. Messenger 2006 Phylogeography of *Reticulitermes* Termites (Isoptera: Rhinotermitidae) in California Inferred from Mitochondrial DNA Sequences. *Ann. Entomol. Soc. Am.* **99**, 697-706.

Vargo, E. L. and C. Husseneder 2009 Biology of Subterranean Termites: Insights from Molecular Studies of *Reticulitermes* and *Coptotermes*. *Annual Review of Entomology* **54**, 379-403.

Walter, D. E., and S. Winterton 2007 Keys and the Crisis in Taxonomy: Extinction or Reinvention? *Annual Review of Entomology* **52**, 193-208.

Wang, C., X. Zhou, S. Li, M. Schwinghammer, M. Scharf, G. Buczkowski and G. Bennett 2009 Survey and Identification of Termites (Isoptera: Rhinotermitidae) in Indiana. *Annals of the Entomological Society of America* **102**, 1029-1036.

Weesner, F. M. 1965 The Termites of the United States: A Handbook, vol. The Nat. Pest Control Association, Elizabeth, New Jersey.

Wheeler, Q. D. [ed.] 2008 The New Taxonomy. CRC Press, Ithaca, New York.

Wheeler, Q. D., P. H. Raven and E. O. Wilson 2004 Taxonomy: Impediment or Expedient? *Science* **303**, 285.

Ye, W., C.-Y. Lee, R. H. Scheffrahn, J. M. Aleong, N.-Y. Su, G. W. Bennett and M. E. Scharf 2004 Phylogenetic relationships of nearctic *Reticulitermes* species (Isoptera: Rhinotermitidae) with particular reference to *Reticulitermes arenicola* Goellner. *Molecular Phylogenetics and Evolution* **30**, 815-822.

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

Yeap, B.K., A.S. Othman and C.-Y. Lee 2009 Molecular systematics of *Coptotermes* (Isoptera: Rhinotermitidae) from East Asia and Australia. *Ann. Entomol. Soc. Am.* **102**, 1077–1090

Yeap, B.K., F.M. Dugal, A.S. Othman and C.-Y. Lee 2010 Genetic relationship between *Coptotermes heimi* and *Coptotermes gestroi* (Isoptera: Rhinotermitidae). *Sociobiology* **56**, 291 - 312

Entomopathogenic fungi as a termite control agent

Aya Yanagawa

Kyoto University, Uji 611-0011, Japan

Abstract

Disease-resistant behaviors of termites are known as commonly as other social insects, and often account for the difficulty in controlling termites by pathogens. On the other hand, several studies demonstrated that these social contacts induce the pathogen epizootics under certain conditions. Fungal agents have played significant roles in this area of research in termites. I would like to discuss here the past and the present studies about termite biological control and its future directions, together with our recent findings. More studies on termite ecology, physiology, genetics or behaviors are required to build an environment-friendly and sustainable control of termites.

Key words: termite, biological control, entomopathogenic fungi, social behavior

Introduction

Termites are classified officially in Blattodea though they are still described often as Isoptera. They play an important role in fields or forests as a decomposer. They are soft-bodied insects with cryptic habits, living in a social family unit called colony. In urban area, termites are serious problem since they attack houses or wood structures (Lax and Osbrink, 2003; Mulrooney et al., 2007). Biological control of termites using the pathogenic organisms, especially using fungal agents, has got attention to control termites in the 90s as an alternative of chemical control (Verma et al., 2009). However, researchers faced to the difficulties to provide the constant successful application because of environmental and conditional influences such as temperature, humidity, or termite colony size. Thus, the benefit of sociality against pathogen infection has started to be discussed in the late 90s. Now, termite hygiene-behavior occurred during the infection process was also count as important factor to overcome (Jackson et al., 2010). To my knowledge, there is still no report of colony dissolution by the infection of pathogenic microbe in the nature. It is important to understand the system, which termites use in the nature to protect them from the pathogenic infection.

Here I report the summary of previous studies on termite control using entomopathogens.

Biological control in termites

Various termiticidal chemicals have been used for the termite control, but the extensive use of them brought the concerns to cause significant environmental hazards (Zoberi, 1995; Faria and Wraight, 2007). Though the applications were not always successful, the laboratory experiments indicated the great potential in biological control of termites. After the several failures in field applications, Grace (2003) amended the possibility to the use of several pathogens or insecticide plus pathogen combination. Around the same time, the realization of the well-functioned pathogen resistant mechanism of termite discouraged the attempts and the biological control of

termite has been decreased gradually. However again, the recent studies bring new perspectives for biological control methods by natural products from pathogenenic organisms (Chouvenec and Su, 2010), molecular approaches (Bulmer et al., 2009) or termite chemical perception (Yanagawa et al., 2011b).

Entomopathogenic fungi as a termite control agents

Biological control of termites has been studied with nematode (Wilson-Rich et al., 2007), bacteria (Osbrink et al., 2001) and especially with entomopathogenic fungi (Culliney and Grace 2000; Rath, 2000; Sun et al., 2002; Verma et al., 2009). Termite lifestyle has some attributes that make fungal infection suitable. Firstly, termites occur in high densities that facilitate disease transmission. Second, most soil contains resident populations of entomopathogenic fungi (Yaginuma, 1990). Finally, the micro environment of the termite nest is characterized by the conditions of high humidity and moderate temperature, both of which are favorable for fungal infection (Vargo et al., 2003). However, as mentioned in previous paragraph, the attempts of fungal application have not always been successful. As pointed out by Roy et al. (2006), social function on eliminating microbes from a colony is not fully described yet. The benefit of social resistance has started to be realized (Traniello et al., 2002). It is important to clarify the interactions between termites and fungi in nature for the successful biological control of termites.

Social factor 1: Horizontal transmission

Fungal agents have been lightened as promising pathogen to control termite. According to Yoshimura and Takahashi (1998), *Beauveria brogniartii* -infected workers of *C. formosanus* could kill approximately equal numbers of untreated individuals by contagion. However, in nature, termite workers remove fungal conidia from the cuticle of their nestmates by grooming behavior, and fungal infection is prevented. The conflict between horizontal transmission and disease resistance by grooming behavior would be explained by the pathogen density, rates of

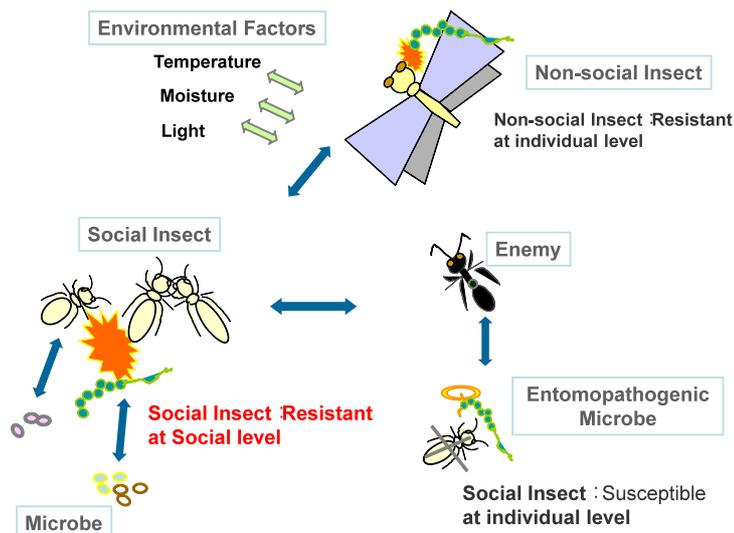


Fig. 1 Insect resistance in nature

Arrows indicate interactions among each factor.

infected population and the infection speed of pathogens. To overcome the resistance by hygiene behaviors and cause a fungal epizootic in fields, the fungi which possess the ability of conidia mass production, high attachment and quick infection plus strong tolerance to the environmental stresses should be discovered. The benefit of

grooming behavior to cause horizontal transmission is used mainly in the use of chemical insecticide (Kubota et al., 2008).

Social factor 2: Disease resistant behavior

Social behaviors against entomopathogenic fungi such as grooming behaviors are reported in several species including *Reticulitermes flavipes*, *Zootermopsis angusticollis*, *Coptotermes lacteus*, *R. speratus* and *C. formosanus* (Boucias et al., 1996; Rosengaus et al., 1998; Staples and Milner 2000; Rosengaus and Traniello, 2001; Shimizu and Yamaji, 2003, Yanagawa and Shimizu, 2007), but there is few comparative study on this defense mechanism among termites nor fungi. Worker termites remove fungal conidia from the nestmate cuticle with their glossae and excrete them after digestion. As a result, the termites are highly resistant to fungal infection. Myles (2002) reported that termite groomed a fungal-treated nestmate more. Some other studies also suggest that termites induce hygiene behaviors for the purpose of preventing disease epizootics in their colony (Rosengaus et al., 1999).

Olfactory perception of pathogen on termites

Since worker termites are blind, termites get the information of their living environments by auditory and chemically (Watson and Gay, 1991). To learn the mechanism of disease resistant behavior, it is essential to clarify the mechanism of pathogen perception in termites. Here, I would like to introduce the summary of our previous studies on olfactory perception to the odor of pathogenic fungi. When termites, *C. formosanus*, were treated with the odor solutions of entomopathogenic fungi (eg. *Metarhizium*, *Beauveria* and *Isaria*, Vega et al., 2009), the treatment induced significantly more grooming behavior in the population. Besides, termites cleaned more efficiently the conidia whose odor repelled them stronger (Yanagawa et al., 2011). Electrophysiological recordings on termite antennae suggested that termites can sense volatile substances emitted from fungal conidia (Yanagawa et al., 2010). All these results support that odor perception is involved somehow in this behavioral disease resistance in termites.

Conclusion

In conclusion, more studies are essential to overcome the difficulties or the effective operation of biological control. However it contains great possibility to provide environment-friendly and sustainable control methods targeted not only termites but also the other social insects.

References

- Boucias, D.G., C. Stokes, G. Storey and J.C. Pendland 1996 The effects of imidacloprid on the termites *Reticulitermes flavipes* and its interaction with the mycopathogen *Beauveria bassiana*. *Pflanzenschutz-Nachr. Bayer* **49**, 103–144.
- Blumer, M.S., I. Bachelet, R. Raman, R. Rosengaus and R. Sasisekharan 2009 Targeting an antimicrobial effector function in insect immunity as a pest control strategy. *PNAS* **106**(31), 12652-12657.

Chouvenc, T. and N-Y. Su 2010 Apparent synergy among defense mechanisms in subterranean termites (Rhinotermitidae) against epizootic events: limits and potential for biological control. *Journal of economic entomology* **103**(4), 1327-1337.

Culliney C.W. and J.K. Grace 2000 Prospects for the biological control of subterranean termites (Isoptera: Rhinotermitidae), with reference to *Coptotermes formosanus*. *Bull Entomol Res* **90**, 9–21.

Faria, M.R. de and S.P. Wraight 2007 Mycoinsecticides and Mycoacaricides: A comprehensive list with worldwide coverage and international classification of formulation types. *Biological Control* **43**, 237-256.

Grace, J.K. 2003 Approaches to biological control of termites. *Sociobiology* **41**(1), 115-121.

Jackson, M.A., C.A. Dunlop and A.T. Jaronski 2010 Ecological considerations in producing and formulating fungal entomopathogen for use in insect biocontrol. *BioControl* **55**, 129-145.

Kubota, S., Y. Shono, N. Mito and K. Tsunoda 2008 Lethal dose and horizontal transfer of bistrifluron, a benzoylphenylurea, in workers of the Formosan subterranean termite (Isoptera: Rhinotermitidae). *Journal of Pesticide Science* **33**(3), 243-248.

Lax A.R. and W.L.A. Osbrink 2003 United States Department of Agriculture - Agriculture Research Service research on targeted management of the Formosan subterranean termite *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). *Pest Management Science* **59**, 788-800.

Mulrooney, J.E., T.L. Wagner, T.G. Shelton, C.J. Peterson and P.D. Gerard 2007 Historical Review of Termite Activity at Forest Service Termiticide Test Sites from 1971 to 2004. *Journal of Economic Entomology* **100** (2), 488-494.

Myles, T.G. 2002 Alarm, Aggregation, and Defense by *Reticulitermes flavipes* in Response to a Naturally Occurring Isolate of *Metarhizium anisopliae*. *Sociobiology* **40** (2), 243-255.

Osbrink W.L.A., K.S. Williams, W.J. Connick Jr, M.S. Wright, A.R. Lax 2001 Virulence of bacteria associated with the Formosan subterranean termite (Isoptera: Rhinotermitidae) in New Orleans, LA. *Environmental Entomology* **30**(2), 443-448.

Rath, A. C. 2000 The Use of Entomopathogenic Fungi for Control of Termites. *Biocont. Sci. Tech.* **10**, 563-581.

Rosengaus R.B, A.B. Maxmen, L.E. Coates, J.F.A. Traniello 1998 Disease resistance: a benefit of sociality in the dampwood termite *Zootermopsis angusticollis* (Isoptera: Termopsidae). *Behav Ecol Sociobiol* **44**, 125-134.

Rosengaus, R.B., C. Jordan, M.L. Lefebvre. and J.F.A. Traniello 1999 Pathogen Alarm Behavior in Termite: A New Form of Communication in Social Insects. *Naturwissenschaften* **86**, 544-548.

Rosengaus, R.B. and J.F.A. Traniello 2001 Disease susceptibility and the adaptive nature of colony demography in the dampwood termite *Zootermopsis angusticollis*. *Behav. Ecol. Sociobiol.* **50**, 546-556.

Roy, H.E., D.C. Steinkraus, J. Eilenberg, A.E. Hajek, and J.K. Pell 2006 Bizarre interactions and endgames: Entomopathogenic Fungi and Their Arthropod Hosts. *Annu. Rev. Entomol.* **51**, 331-357.

Shimizu S and M. Yamaji 2003 Effect of density of the termite, *Reticulitermes speratus* Kolbe (Isoptera: Rhinotermitidae), on the susceptibilities to *Metarhizium anisopliae*. *Jpn J Appl Entomol Zool* **38**, 125-135.

Staples, J.A. and R.J. Milner 2000 A laboratory evaluation of the repellency of *Metarhizium anisopliae* conidia to *Coptotermes lacteus* (Isoptera: Rhinotermitidae). *Sociobiology* **36**(1), 133-148.

Sun, J., J.R. Fuxa and G. Henderson 2002 Sporulation of *Metarhizium anisopliae* and *Beauveria bassiana* on *Coptotermes formosanus* and in vitro. *Journal of Invertebrate Pathology* **81**, 78-85.

Traniello, J.F.A., R.B. Rosengaus and K. Savoie 2002 The development of immunity in a social insect: Evidence for the group facilitation of disease resistance. *PNAS* **99**(10), 6838-6842.

Vargo E.L., C. Husseneder and J.K. Grace 2003 Colony and population genetic structure of the Formosan subterranean termite, *Coptotermes formosanus*, in Japan. *Molecular Ecology* **12**(10), 2599-2608.

Verma, M., S. Sharma and R. Prasad 2009 Biological alternatives for termite control: A review. *International Biodeterioration & Biodegradation* **63**, 959-972.

Vega, F.E., M.S. Goettel, M. Blackwell, D. Chandler, M.A. Jackson, S. Keller, M. Koike, N.K.

Maniania, A. Monzon, B.H. Ownley, J.K. Pell, D.E.N. Rangel and H.E. Roy 2009 Fungal entomopathogens: new insights on their ecology. *Fungal Ecology* **2**, 149-149.

Watson, J.A.L and F.J. Gay 1991 Isoptera (*Termites*) In: The Insects of Australia 2nd ed., 20: 330-347, Melbourne University Press.

Wilson-Rich N., R.J. Stuart, R. Rosengaus 2007 Susceptibility and behavioral responses of the dampwood termite *Zootermopsis angusticollis* to the entomopathogenic nematode *Steinernema carpocapsae*. *Journal of Invertebrate pathology* **95**, 17-25.

Yaginuma, K. 1990. Detection of fungi to peach fruit moth, *Carposina niponensis* Walsingham from soil. *Bull. Fruit Tree Res. Stn.* **17**, 77–89 (in Japanese with English summary).

Yanagawa, A. and S. Shimizu 2007 Resistance of the termite, *Coptotermes formosanus* Shiraki to *Metarhizium anisopliae* due to grooming. *BioControl* **52** (1), 75-85.

Yanagawa, A., F. Yokohari and S. Shimizu 2010 Influence of fungal odor on grooming behavior of the termite, *Coptotermes formosanus* Shiraki. *J. Insect Sci.* **10**, article 4.

Yanagawa, A., N. Fujiwara-Tsujii, T. Akino, T. Yoshimura, T. Yanagawa and S. Shimizu 2011 Musty odor of entomopathogens enhances disease-prevention behaviors in the termite *Coptotermes formosanus*. *Journal of Invertebrate Pathology* **108**, 1-6.

Yoshimura, T. and M. Takahashi 1998 Termiticidal Performance of an Entomogenous Fungus, *Beauveria brongniartii* (SACCARDO) PETCH in Laboratory Tests. *Jpn. J. Environ. Entomol. Zool.* **9**(1), 16-22

Zoberi, M.H. 1995 *Metarhizium anisopliae* a fungal pathogen of *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). *Mycologia* **87**, 354–359.

Invasive termites

Theodore Evans

National University of Singapore, 117543, Singapore

Abstract

Invasive species are defined as ‘alien organisms that have established in a new area and are expanding their range’; currently there are 27 termite species that fulfil this definition. All 27 invasive species share three characteristics: wood for food, nesting in their wooden food, and a high capacity to generate secondary reproductives. These characteristics combine to increase probability of transportation of viable propagules; wood infested with reproductively capable transported by humans. These three characteristics are found most commonly in two families, the Kalotermitidae and Rhinotermitidae, which have 9 and 13 invasive species respectively. Three genera in these two families, *Cryptotermes*, *Heterotermes* and *Coptotermes*, comprise over half (58%) of all invasive species. The other families with invasive species are Mastotermitidae (1 species), Termopsidae (2 sp.) and Termitidae (2 spp.). The Termitidae is by far the largest termite family (ca. 1940 spp. whereas all other termite families combined have ca. 800), thus is very underrepresented, because few Termitidae species have all these three invasive characteristics. The biogeographic origins of the most invasive species is South and South East Asia (7 spp.), South America (6 spp.) and Australia (6 spp.). The most invaded biogeographic regions are the South Pacific islands (13 spp.) Caribbean islands (9 spp.) and North America (9 spp.), although the majority of species invading North America are found in south Florida, which can be considered more closely connected to the Caribbean in biogeographic terms. Islands have double the invasive species than continents, on average. Although most work on invasive species has been conducted in urban settings, some invasive species are found in natural habitats and have inland distributions. These wider distributions were discovered in part due to better identification methods. Only two invasive populations (*Coptotermes formosanus* in South Africa and *C. frenchi* in New Zealand) are likely to have been eliminated, due to a complete absence of new reports of these species post treatment.

Key words: *Coptotermes*, *Cryptotermes*, *Heterotermes*, invasion, , physical barrier

Introduction

Early work on invasive termites has been conducted primarily in urban areas, and therefore has focused on control in buildings rather than effects on native biodiversity, which is the purview of invasion biology (Elton 1958). Indeed the first review on invasive termites did not use the term ‘invasive’ but ‘introduced’, perhaps because these termites were believed to be found only in urban areas:

‘Introduced species frequently become of considerable economic importance, although they are rarely able to invade native habitats and are almost always restricted to the man-modified environments of houses, building, and cultivated crops’ (Gay 1969).

This distinction was used by Gay (1969) to distinguish native from introduced/invaded ranges, and has been used by others to help separate the endemic range from the invaded range for various termite species (e.g. Scheffrahn et al. 2009). However, this is of no consequence in invasion biology, according to the definitions of Falk-Petersen et al. (2006) (see Fig. 1):

- Native is a synonym of indigenous and of original, and means: ‘an organism occurring within its natural, past or present range and dispersal potential (organisms whose dispersal is independent of human intervention)’.
- Endemic means: ‘organism restricted to a specified region or locality’; i.e. native species with a restricted distribution.
- Introduction means ‘direct or indirect movement by human agency of an organism from its native, past or present range to a range outside its distribution potential’.
- Introduced is a synonym of alien, adventive, exotic, foreign, non-indigenous, non-native and novel, and means: ‘an organism occurring outside its natural, past or present range and dispersal potential including any parts of the organism that might survive and subsequently reproduce (organisms whose dispersal is caused by human action)’.
- Established means: ‘native or non-native organism that has obtained a self-sustaining population in an area where it previously did not occur’.
- Transfer is a synonym of translocation and transplantation and means ‘human mediated movement of an organism within its past or present range and dispersal potential’.
- Invasive means: ‘alien organisms that have established in a new area and are expanding their range’.
- Naturalized means: a non-native organism that has obtained a self-sustaining population.
- Pest means: ‘organisms [native, introduced or invasive] considered harmful to human activities’.

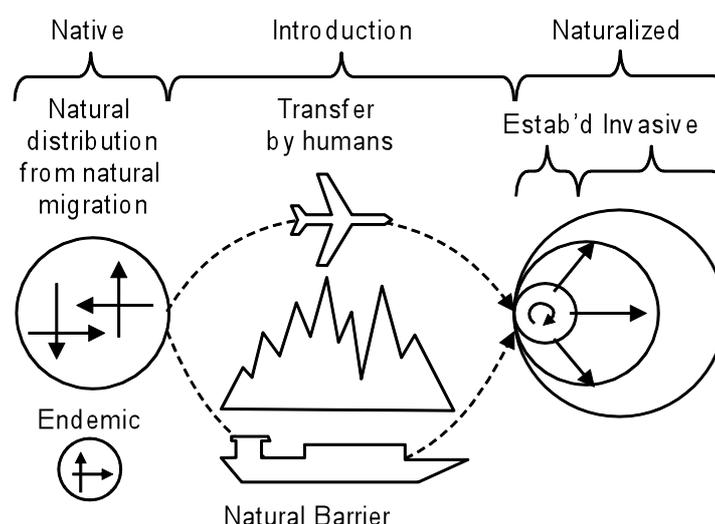


Figure 1. Schematic showing the definitions of invasion biology after Falk-Petersen et al. (2006). Not illustrated: pest, which means any organisms considered harmful to human activities.

Consequently most of the work on invasive populations has occurred without the theory houses (IBEC* 1998, 1999a, 1999b).

Materials and methods

Information on invasive termites was located through literature searches using the terms ‘dampwood, drywood, introduced, invasive, invasion, Isoptera, exotic, naturalized, pest, subterranean, termite, and white ant, along with all family names and generic names of species found by Gay (1969) (Web of Science, Google Scholar), searching government report databases, and discussions with academic, quarantine and museum professionals in multiple countries. Information was retained when the definition of Falk-Petersen et al. (2006) was met. Life history characteristics were gained from texts (Grassé 1982-1986; Krishna 1969-1970; Bignell et al. 2010) and scientific papers. Geographic regions (and localities, if present) were identified as the following: Caribbean (Greater Antilles, Lesser Antilles, North group, South group), North Atlantic (British Is., Islands off North Africa, Islands off West Africa), South Atlantic, North Pacific (North West Pacific, Micronesia, Hawaii), South Pacific (Melanesia, Polynesia, New Zealand, Galapagos), Indian Ocean (East islands, South West islands, Sri Lanka), North America (north temperate zone, south subtropical zone), South America (north tropical zone, south temperate zone), Europe, Africa (West Africa, East Africa, Southern Africa), Arabia, Asia (South & South East Asia (tropical), East Asia (temperate)), and Australia.

Results and discussion

Invasive species & distributions

Over 200 references were found (see Evans 2010 for full list); from these a list of 27 species were found that matched the criteria of Falk-Petersen et al. (2006), from five families. *Mastotermes darwiniensis*, the sole species in the Mastotermitidae from Australia has spread to nearby New Guinea. Two species of Termopsidae (the dampwood termites); have spread, one from Australia invading nearby islands and the other from the USA invading Japan (Table 1). The species appear to be spreading, sometimes despite extensive control methods, albeit slowly (Morimoto 2000; Thistleton et al. 2007; Phillip et al. 2008).

There were nine species in Kalotermitidae, the drywood termites: one *Kalotermes* species, one *Glyptotermes* species, two *Incisitermes* species, and five *Cryptotermes* species (Table 2). The *Kalotermes* and *Glyptotermes* species originate in Australia and have spread to nearby Pacific islands, and their spread in native forests in those islands is slow (Bain and Jenkin 1983). The *Incisitermes* species originate along the Pacific Ocean coasts of North and South America, and have been spread to Pacific Ocean islands as far as Japan, and one to the south eastern states of the USA (Indrayani 2005; Grace 2009).

Easily the most widespread kalotermitids are the five *Cryptotermes* species, especially *Cr. brevis*. From its endemic range along the Pacific Coast of Peru and Chili in South America, it has been spread to islands in all oceans except the Indian Ocean, across South America and much of North America, and with some locations in Africa, Europe and Australia. Asia is the only major region not invaded (Scheffrahn et al. 2009; Table 2). *Cryptotermes havilandi* from west Africa has spread to islands in the surrounding oceans, to the Caribbean, and to east Africa,

South America and South Asia (Scheffrahn and Krčec̃ek 1999; Schabel 2006). The three remaining species originate in South and Southeast Asia, and considered together, have been spread even more widely than *Cr. brevis* (more Pacific Islands, around Asia, but not Europe: Gray 1968; Williams 1976; Gay and Watson 1982; Bose 1984; Scheffrahn and Krčec̃ek 1999; Constantino 2002; Lal and Tuvou 2003; Schabel 2006; Grace 2009).

There were 13 species in Rhinotermitidae: two *Reticulitermes* species, five *Heterotermes* species, and six *Coptotermes* species (Table 3). One *Reticulitermes* species was moved from Europe to an island off the European coast (France to Britain; Jenkins et al. 2001), the other was moved further and more widely from the USA to Europe (France and Germany), South America (Chile and Uruguay) and one island in the Pacific (Galapagos) and another in the Caribbean (Grand Bahama) (Jenkins et al. 2001; Aber and Beltrami 2002; Austin et al. 2004, 2005). All but one species of *Heterotermes* follow the same pattern: originating on South America and spreading to nearby islands in the Caribbean, Galapagos and Florida (Araujo 1977; Szalanski et al. 2004). Two species have little information and likely fit this pattern as well: *H. perfidus* and *H. sp. n.* (see Table 3 and Evans 2010). Only *H. philippinensis* differs, as it originates from Southeast Asia (the Philippines) and was spread to Madagascar in the Indian Ocean (Cachan 1950).

The most widespread rhinotermitids are the six *Coptotermes* species. Four species have a limited spread to one or two invaded locations: *C. acinaciformis* and *C. frenchi* from Australia to New Zealand and/or Fiji in the Pacific Ocean (Bain and Jenkins 1982; Lal and Tuvou 2003); *C. curvignathus* from Southeast Asia to China (Xie et al. 2001); and *C. sjostedti* from west Africa to the Caribbean (Scheffrahn et al. 2004b). Two species have been spread far more widely. The east Asian (China and Taiwan) *C. formosanus* has spread in the North Pacific Ocean (Japan, Hawaii and nearby islands, Guam) and to the continental USA (nine states, mostly in the southeast) (Woodson et al. 2001; Austin et al. 2006). The Southeast Asia (Sundaland) *C. gestroi* has spread even further, to North and South Pacific Ocean islands (Taiwan, Polynesia (Hawaii, Midway, Marshalls, Marquesas), Micronesia (Yap, Guam)), the Caribbean (the Antilles), North America (Mexico and Florida) and South America, especially along the Atlantic cities of Sao Paulo and Rio de Janeiro (Scheffrahn et al. 1994; Woodrow et al. 2001; Constantino 2002; Ferraz and Montiel 2004; Scheffrahn and Su 2005; Jenkins et al. 2007; Li et al. 2009).

There were only two species of Termitidae found invasive; one from the subfamily Termitinae, ‘*Termes*’ clade and the other from the subfamily Nasutitermitinae. Both species originated from South America and the southern islands of the Caribbean, however they have different patterns of spread. *Termes hispaniole* has spread only nearby, to islands in the northern Caribbean (Scheffrahn unpub), whereas *Nasutitermes corniger* has spread to the north Caribbean islands and Florida, but to New Guinea in the South Pacific (Constantino 1998; Scheffrahn et al. 2005).

Characteristics of invasive species

The invasive species can be compared with all termites to discern common traits. Invasive species are rare, as there only 26 out of the ca. 2800 described termite species. It is clear that two families are over represented, Kalotermitidae and Rhinotermitidae in percentage of the clade

that is invasive, and in the percentage of invasive species (ignoring the very small families of Mastotermitidae and Termopsidae) (Table 5). All termites consume vegetation in some form, but the vegetation can be in a diverse array of forms. It is usually dead, can be sound and solid to rotted wood, leaves fresh, dry or mouldering, with varying states of decomposition, right to soluble organic matter mixed into mineral soil. Almost all of this diversity is found in the Termitidae; the lower termites almost always eat wood (the exception is the Hodotermitidae). All invasive species eat sound wood, including those in the Termitidae (Table 6).

The nesting locations are included in ‘lifeways’ types (Donovon et al. 2001; Eggleton and Tayasu 2001). Single-piece nesters feed on and nest in the same single piece of wood. Intermediate nesters are like single piece nesters, except they also forage away from their nest to find other pieces of wood; once the nest wood is exhausted, the colony relocates the nest to a new piece. Separate-piece nesters live a nest that is separate and distinct from their food; their forage away from their nest to eat wood, grass and litter. The location of the separate nest (i.e. hypogeal (below-ground), epigeal (aboveground), or arboreal) is unimportant. Donovan et al. (2001) defined four feeding groups based on gut morphology and food decomposition. Type I feeds on non-decomposed plant matter, easily recognised as wood, grass or litter, digested with the aid of gut flagellates (i.e. all families other than the Termitidae). Type II is the same as Type I, except they digest their food without the aid of gut flagellates (i.e. only Termitidae). Type III feeds on decomposed plant matter at the organic matter–soil interface, and Type IV feeds on highly decomposed plant matter (generally fine particles or soluble) in mineral soil (i.e. ‘true soil feeders’). Types III & IV (only higher termites) frequently have no nest as they live in the soil on which they feed and move through the soil, although many prominent epigeal mound-builders are also affiliated to these groups. It is clear from Table 6 that invasive species are either type I single piece nesters (termopsid and kalotermitid species: the ten species in *Porotermes*, *Cryptotermes*, *Incisitermes*, *Kalotermes* and *Glyptotermes*), type I intermediate piece nesters (*Mastotermes darwiniensis* and the twelve rhinotermitids species in *Reticulitermes*, *Heterotermes* and *Coptotermes*), or type II intermediate nester (*Termes hispaniolae*, *Nasutitermes corniger*). There are neither separate type nesters nor any type III or IV invasive termite species.

Primary reproductives in termites are defined as those imago (i.e. the adult winged form) individuals that fly from their natal nests to a new location, find a mate and then found a new nest and colony ‘independently’ (i.e. without assistance from helper castes). Secondary reproductives are those individuals that do not fly to start a nest independently, but retain assistance from their natal nest helper castes (Thorne 1996; Myles 1999; Roisin 2000). There are four fundamental secondary reproductive types: ergatoids, which develop from workers (i.e. excludes anything with wingbuds); nymphoids, which develop from nymphs (i.e. includes everything with wingbuds); adultoids, which develop from alates; and soldier-forms (only found in the termopsid, *Zootermopsis*).

Although knowledge of secondary reproductive is limited (ca. 200 species documented; Sieber 1985 ; Myles 1999), examples are known from all clades (except the Termitidae *Foraminitermes* group). Assuming the known species are representative, there are clear differences between

clade: the lower termites can produce ergatoids or nymphoids or both, whereas the higher termites mostly produce nymphoids or adultoids; three termitid clades produce only adultoids. All invasive species, including the two termitid species, are capable of forming ergatoid or nymphoid secondary reproductives (Table 7).

The crucial question for invasiveness is the opportunity of creating a viable propagule (sensu Mack et al. 2000; Lockwood et al. 2005) for transport to a new location. Species that eat wood have the best opportunity, as wood is traded by humans (dead and decomposing grass and soil much less so). Ergatoid reproductives have the highest potential to be transported foraging workers are available all year. Nymphoid reproductives have a medium to high potential to be transported, depending on how many months nymphs are present and whether they forage away from the nest. Adultoid reproductives have a low potential to be transported because alates are present for a short period, perhaps a month or two, and they are only found in the natal nest. Thus it is unsurprising that all invasive species are wood eating with ergatoid and/or nymphoid secondary reproductives.

Conclusions

Islands have been invaded more than continents. This is evident from Tables 1-4, which can be summarized as follows: the South Pacific Ocean is the most invaded region, with 13 species, followed by North America (9), the Caribbean Sea (8) and the North Pacific Ocean (8), the Indian Ocean (6), South America (5), Australia (4), East Asia (3) Africa (3), North Atlantic Ocean (3), South Atlantic Ocean (2), South and South East Asia (2) and Europe (1). North America has a high number of invasive species because of Florida; removing Florida (perhaps closer to the Caribbean than continental North America in biogeographical terms) reduces the North America total to three. The lower numbers in the Atlantic Ocean is partly due to the lower number of islands, especially in the south Atlantic, at least at latitudes suitable for termites.

Those termites that have invaded continents are moving steady from coastal fringes inland. *Coptotermes formosanus* has spread from the initial three coastal cities (Lake Charles LA, Charleston SC and Pasadena, TX) across ten states, with locations several hundred kilometres inland, mostly by human transport (Woodson et al. 2001; Jenkins et al 2002; Austin et al. 2006; Sun et al. 2007). Molecular identification tools has shown that *Reticulitermes flavipes* has spread deep into France, probably from alate flight as well as by human transport (Austin et al. 2005).

It is possible that continents are more invaded than these numbers suggest. Islands have lower diversity than continents thus a new (or indeed any) termite species may be more easily detected on an island than a continent. This appears to be true for (1) *C. formosanus* in the USA, which has relatively low termite diversity and no *Coptotermes* species, where identification can easily be made from the soldiers with their distinctive defensive secretion and (2) Uruguay and Chile, which also have low termite diversity and where invading *Reticulitermes* are readily detected. Species that are similar to native species are less easily undetected, for example France has native *Reticulitermes* species and New Guinean has native *Nasutitermes* species, thus the invasive species in these countries were not detected until recently using molecular tools.

Perceived patterns may be affected by the availability of taxonomic expertise. Perhaps it is not mere coincidence that the most invaded state in the USA also has the greatest concentration of termite specialists. If so, then invasions in much of Africa, Asia and South America will be more difficult to detect. Similarly, invasions in urban areas with immediate damage to houses may be detected more readily than invasions of natural or semi-natural habitats.

Any novel invasive species will likely resemble the existing 27 invasive species: wood-eating, type I single piece nester or type I or II intermediate nester, with ergatoid or nymphoid or both secondary reproductives, and probably from an area with a high volume of trade. If so, then more kalotermitids (especially *Cryptotermes* species), rhinotermitids (especially *Reticulitermes*, *Heterotermes* and *Coptotermes* species), and termitids (*Nasutitermes*, but perhaps also type II intermediate nester species from *Microcerotermes* and *Amitermes*) may join the list. Future new invasive termite species may be native pests that live in urban habitats. Such termites are pre-adapted to living with humans. For example, 85% of house infestations in urban areas of peninsula Malaysia are from *Coptotermes gestroi*, which becomes less common in suburban and semi rural areas, and is rare in natural forests (Kirton and Azmi 2005). Similar patterns are known for *C. formosanus* in China and Taiwan (e.g. collecting sites of Li et al. 2009). Perhaps novel invasive species are appearing in new urban areas around the world, awaiting trade to move them to new locations. A focus on trade routes and better identification will help to find invasive termites before they establish yet further territory.

References

- Aber, A. and Beltrami, M. 2002 *Reticulitermes lucifugus* in urban households in Uruguay (Isoptera: Rhinotermitidae). *Sociobiology* **40**,153-162
- Araujo, R.L. 1977 *Catálogo dos Isoptera do Novo Mundo*. Academia Brasileira de Ciências, Rio de Janeiro (in Portuguese).
- Austin, J.W., Szalanski, A.L. and Cabrera, B.J. 2004 A phylogenetic analysis of the subterranean termite family Rhinotermitidae (Isoptera) using the mitochondrial cytochrome oxidase (COII) gene. *Annals of the Entomological Society of America* **97**,548-555.
- Austin, J.W., Szalanski, A.L., Scheffrahn, R.H., et al. 2005 Genetic evidence for the synonymy of two *Reticulitermes* species: *Reticulitermes flavipes* and *Reticulitermes santonensis*. *Annals of the Entomological Society of America* **98**,395-401.
- Austin, J.W., Szalanski, A.L., Scheffrahn, R.H., et al. 2006 Genetic evidence for two introductions of the Formosan subterranean termite, *Coptotermes formosanus* (Isoptera: Rhinotermitidae), to the United States. *Florida Entomologist* **89**, 183-193.
- Bain, J. and Jenkin, M.J. 1983 *Kalotermites banksiae*, *Glybltotermes brevicornis*, and other termites (Isoptera) in New Zealand. *New Zealand Entomologist* **7**, 365-371

Bose G (1984) Termite fauna of southern India. *Records of the Zoological Survey of India* **49**. Zoological Survey of India, Delhi.

Bignell, D.E. Roisin, Y. and Lo, N 2010. *Biology of Termites: A Modern Synthesis*. Springer, Berlin.

Cachan, P. 1950 *Les termites des Madagascar et leur dégats*. L'Institut de Recherche Scientifique, Tananarive-Tsimbazaza (in French).

Constantino, R. 1998 Catalog of the living termites of the new world (Insecta: Isoptera). *Arquivos de Zoologia de Sao Paulo* **325**, 135-230.

Constantino, R. 2002 The pest termites of South America: taxonomy, distribution and status. *Journal of Applied Entomology* **126**, 355-365.

Donovan, S.E., Eggleton, P. and Bignell, D.E. 2001 Gut content analysis and a new feeding group classification of termites (Isoptera). *Ecological Entomology* **26**,356-366.

Eggleton, P. and Tayasu, I. 2001 Feeding groups, lifetypes and the global ecology of termites. *Ecological Research* **16**, 941–960.

Evans, T.A. 2010 Invasive termites. In: Bignell, D.E., Roisin, Y. & Lo, N. (eds) *Biology of termites, a modern synthesis*, pp 519-562. Springer, Berlin.

Ferraz, M.V. and Montiel, J.T.M. 2004 First record of a subterranean termite, *Coptotermes havilandi* Holgrem (Isoptera, Rhinotermitidae), on the west coast of North America (Mexico). *Acta Zoológica Mexicana* **20**, 39-43.

Gay, F.J. 1969 Species introduced by man. In: Krishna, K. and Weesner, F.M. (eds) *Biology of termites, vol 1*, pp 459-494. Academic Press, N.Y.

Gay, F. and Watson, J.A.L. 1982 The genus *Cryptotermes* in Australia (Isoptera : Kalotermitidae). *Australia Journal of Zoology (Suppl)* **30(88)**, 1-64.

Grace, J.K. 2009. What can fecal pellets tell us about cryptic drywood termites (Isoptera: Kalotermitidae)? The International Research Group on Wood Protection, IRG-WP doc 09-20407.

Grassé, P.P. 1982-1986 *Termitologia, Tomes 1 -3*. Masson, Paris.

Gray, B. 1968 Forest tree and timber insect pests in the territory of Papua and New Guinea. *Pacific Insects* **10**, 301-323.

Indrayani, Y., Yoshimura, T., Fujii, T. et al. 2005. A case study of *Incisitermes minor* (Isoptera: Kalotermitidae) infestation in Wakayama Prefecture, Japan. *Sociobiology* **46**, 45-63.

Inward, D.J.G., Vogler, A.P. and Eggleton, P. 2007 A comprehensive phylogenetic analysis of termites (Isoptera) illuminates key aspects of their evolutionary biology. *Molecular Phylogenetics and Evolution* **44**, 953-967.

Jenkins, T.M., Dean, R.E., Verkerk, R. and Forschler, B.T. 2001 Phylogenetic analyses of two mitochondrial genes and one nuclear intron region illuminate European subterranean termite (Isoptera: Rhinotermitidae) gene flow, taxonomy, and introduction dynamics. *Molecular Phylogenetics and Evolution* **20**, 286-293.

Jenkins, T.M., Dean, R.E. and Forschler, B.T. 2002 DNA technology, interstate commerce, and the likely origin of Formosan subterranean termite (Isoptera: Rhinotermitidae) infestation in Atlanta, Georgia. *Journal of Economic Entomology* **95**, 381-389.

Jenkins, T.M., Jones, S.C., Lee, C.-Y. et al. 2007 Phylogeography illuminates maternal origins of exotic *Coptotermes gestroi* (Isoptera: Rhinotermitidae). *Molecular Phylogenetics and Evolution* **42**, 612–21.

Kirton, L.G. and Azmi, M. 2005 Patterns in the relative incidence of subterranean termite species infesting buildings in peninsular Malaysia. *Sociobiology* **46**, 1-15.

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

Krishna, K. and Weesner, F.M. (eds.) 1969-1970 *Biology of Termites, vol 1 -2*. Academic Press, N.Y.

Lal, S and Tuvou, L. 2003 Development of Forest Health Surveillance Systems for South Pacific Countries and Australia; country report – Fiji. Australian Government, ACIAR, Canberra.

Li, H.F., Ye, W., Su, N.-Y. and Kanzaki, N. 2009 Phylogeography of *Coptotermes gestroi* and *Coptotermes formosanus* (Isoptera: Rhinotermitidae) in Taiwan. *Annals of the Entomological Society of America* **102**, 684-693.

Lockwood, J.L., Cassey, P. and Blackburn, T. 2005 The role of propagule pressure in explaining species invasions. *Trends in Ecology and Evolution* **20**, 223–228.

Mack, R.N., Simberloff, D. and Lonsdale, W.M. et al. 2000 Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications* **10**, 689-710.

Morimoto, K., 2000 On the damp-wood termite genus *Zootermopsis* introduced to Japan. *Shiroari* **122**, 3-8 (in Japanese).

Myles, T.G. 1999 Review of secondary reproduction in termites (Insecta: Isoptera) with comments on its role in termite ecology and social evolution. *Sociobiology* **33**, 1-91.

Phillip, B., Pearson, H. and Bennett, S. 2008 Australian termites in New Zealand. *Biosecurity Magazine* **82**, 12-13.

Roisin, Y. 2000 Diversity and evolution of caste patterns. In: Abe, T., Bignell, D.E. and Higashi, M. (eds) *Termites: evolution, sociality, symbioses, ecology*. Kluwer Academic Publishers, Dordrecht, pp 95-119.

Schabel, H.G. 2006 *Forest entomology in East Africa: forest insects of Tanzania*. Springer, Dordrecht.

Scheffrahn, R.H. and Krčec̃ek, J. 1999. Termites of the Genus *Cryptotermes* Banks (Isoptera: Kalotermitidae) from the West Indies. *Insecta Mundi* **13**, 111-171

Scheffrahn, R.H. and Su, N.-Y. 2005 Distribution of the termite genus *Coptotermes* (Isoptera: Rhinotermitidae) in Florida. *Florida Entomologist* **88**, 201-203.

Scheffrahn, R.H., Darlington, J.P.E.C., Collins, M.S. et al. 1994 Termites (Isoptera: Kalotermitidae, Rhinotermitidae, Termitidae) of the West Indies. *Sociobiology* **24**, 213-238.

Scheffrahn, R.H., Krčec̃ek, J., Maharajh, B. et al. 2004 Establishment of the African termite, *Coptotermes sjostedti* (Isoptera: Rhinotermitidae), on the Island of Guadeloupe, French West Indies. *Annals of the Entomological Society of America* **97**, 872-876.

Scheffrahn, R.H., Krčec̃ek, J., Szalanski, A.L. et al. 2005 Synonymy of two arboreal termites (Isoptera: Termitidae: Nasutitermitinae): *Nasutitermes corniger* from the neotropics and *N. polygynus* from New Guinea. *Florida Entomologist* **88**, 28-33.

Scheffrahn, R.H., Krčec̃ek, J., Ripa, R. and Luppichini, P. 2009 Endemic origin and vast anthropogenic dispersal of the West Indian drywood termite. *Biological Invasions* **11**, 787–799.

Sieber, R. 1985 Replacement reproductives in Macrotermitinae (Isoptera: Termitidae). In: Watson, J.A.L. and Okot-Kotber, B.M. (eds) *Caste differentiation in social insects*. Pergamon Press, Oxford, pp 201-208.

Sun, J.-Z., Lockwood, M.E., Etheridge, J.L., et al. 2007 Distribution of Formosan subterranean termite (Isoptera: Rhinotermitidae) in Mississippi. *Journal of Economic Entomology* **100**, 1400-1408.

Szalanski, A.L., Scheffrahn, R.H., Austin, J.W., Krěček, J., Su, N.-Y. 2004 Molecular phylogeny and biogeography of *Heterotermes* (Isoptera: Rhinotermitidae) in the West Indies. *Annals of the Entomological Society of America* **97**,574-585.

Thistleton, B.M., Neal, M., Peki, M. and Dobunaba, J. 2007 *Mastotermes darwiniensis* in the Lae area of PNG: an assessment of current and potential status of the incursion, and options for management, final report. Australian Government, Australian Centre for International Agricultural Research, Canberra

Thorne, B.L. 1996. Termite terminology. *Sociobiology* **28**, 253-61.

Woodson, W.D., Wiltz, B.A. and Lax, A.R. 2001 Current distribution of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in the United States. *Sociobiology* **37**, 661-671.

Woodrow, R.J., Grace, J.K. and Higa, S.Y. 2001 Occurrence of *Coptotermes vastator* (Isoptera: Rhinotermitidae) on the Island of Oahu, Hawaii. *Sociobiology* **38**, 667-673.

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

Table 1. Invasive species in Mastotermitidae, Termopsidae and Kalotermitidae, their natural and invaded ranges. A ‘?’ indicates uncertainty.

Species	Invaded Regions	Invaded localities
<i>Mastotermes darwiniensis</i> Australia	S Pacific O	New Guinea (Lae)
<i>Porotermes adamsoni</i> Australia	S Pacific O	New Zealand
<i>Zootermopsis nevadensis</i> W N America	N Pacific O	Japan

Table 2. Invasive species in Kalotermitidae, their natural and invaded ranges. N = north, S = south, E = east, W = west, O = ocean, Is = island or isle (incl. plurals), Gtr = Greater, Lsr = Lesser, ? = unknown, uncertain or disputed. States in the USA & Australia indicated with standard two letter abbreviations.

Species	Invaded Regions	Invaded localities
<i>Incisitermes immigrans</i> S America ¹	N Pacific O	Galapagos, Hawaii, Polynesia (Marquesas, Kiribati: Fanning & Jarvis Is)
<i>Incisitermes minor</i> N America (Mexico, USA-CA)	N Pacific O	Japan, Ryukyu Is, Hawaii
	E Asia	China, Zhejiang
	N America	USA (TX, AK, LA, OK, SC)
<i>Cryptotermes brevis</i>	Caribbean	North Is, Gtr Antilles, Lsr Antilles, S Is

S America (Peru, Chile)	N Atlantic O	Azores, Canary Islands
	S Atlantic O	Saint Helena Island
	N Pacific O	Hawaii, Midway
	S Pacific O	Melanesia (Fiji, New Caledonia, Tonga); Polynesia (Marquesas, Easter Is); New Zealand
	N America	USA (NY-FL, WI-TN, CA), Panama to Mexico, El Salvador
	S America	Venezuela, Guiana, Brazil, Peru, Ecuador, Uruguay
	Europe	Portugal
	Africa	Congo, Sierra Leone, S Africa
<i>Cryptotermes cynocephalus</i> SE Asia (Philippines)	Australia	Qld
	N Pacific O	Hawaii
	S Pacific O	PNG, New Britain
	Indian O	Sri Lanka
<i>Cryptotermes domesticus</i> SE Asia?	Australia	Qld, Torres Strait Islands
	N Pacific O	Guam
	S Pacific O	Melanesia (Fiji, New Britain, New Guinea, Samoa, Solomon Is); Polynesia (Samoa, Marquesas, Pitcairn, Tahiti)
	N Pacific	Micronesia (Kiribati-Fanning & Flint Is)
	N America	Panama
	E Asia	China (Hainan)
<i>Cryptotermes dudleyi</i> S & SE Asia?	Australia	N Coast of Australia, Qld, Torres Strait Is
	Caribbean	Gtr Antilles (Jamaica); S Is (Trinidad)
	N Pacific O	Guam, Marshall Islands
	Indian O	Cocos / Keeling Islands; Madagascar, Mauritius
	N America	Costa Rica, Nicaragua, Panama
	S America	Brazil, Colombia
	S Asia	India; Bangladesh
	Africa	Kenya, Tanzania, Somalia, Uganda
<i>Cryptotermes havilandi</i> Africa	Australia	NT (Darwin), Torres Strait Islands
	Caribbean	Lsr Antilles
	S Atlantic O	African Is (Fernando Póo Is)
	Indian O	Comoro, Europa Is, Madagascar
	S America	Brazil, Guiana, Surinam
	S Asia	Bangaldesh, India
<i>Kalotermes banksiae</i> Australia	Africa	Tanzania
	S Pacific	New Zealand
<i>Glyptotermes brevicornis</i> Australia	S Pacific	Fiji, New Zealand

1 Unknown, probably Central & South America (Constantino 1998).

Table 3. Invasive species in Rhinotermitidae, their natural and invaded ranges. N = north, S = south, E = east, W = west, O = ocean, Is = island or isle (incl. plurals), Gtr = Greater, Lsr = Lesser, ? = unknown, uncertain or disputed. States in the USA & Australia indicated with standard two letter abbreviations.

Species	Invaded Regions	Invaded localities
<i>Heterotermes convexinotatus</i>	Caribbean	Gtr Antilles, Lsr Antilles
	S Pacific O	Galapagos?

S America	N America?	USA (FL)?
<i>Heterotermes perfidus</i> ¹ S America?	S Atlantic O	Saint Helena Island
<i>Heterotermes philippinensis</i> Philippines	Indian O	Madagascar, Mauritius
<i>Heterotermes tenuis</i> S America	Caribbean	Lsr Antilles
<i>Heterotermes</i> n. sp. Caribbean	N America	USA-FL
<i>Reticulitermes flavipes</i> N America	Caribbean	N Is
	S Pacific O	Galapagos
	Europe	France, Germany
	N America	Canada
<i>Reticulitermes grassei</i> Europe (France)	S America	Uruguay, Chile
<i>Reticulitermes grassei</i> Europe (France)	N Atlantic O	England, Azores
<i>Coptotermes acinaciformis</i> Australia	S Pacific O	Fiji, New Zealand
<i>Coptotermes curvignathus</i> SE Asia	East Asia	China
<i>Coptotermes formosanus</i> ² E Asia (China, Taiwan)	N Pacific O	Japan, Polynesia (Hawaii, Marshall Is, Midway), Micronesia (Guam)
	Indian O	Sri Lanka
	N America O	USA (AB, CA, FL, GA, LA, MS, NC, SC, TN, TX)
	(Africa?)	(S Africa?)
<i>Coptotermes frenchi</i> ³ Australia	(S Pacific?)	(New Zealand?)
<i>Coptotermes gestroi</i> ⁴ SE Asia	Caribbean	N Is, Gtr Antilles, Lsr Antilles
	N Pacific O	Polynesia (Hawaii, Midway, Marshalls), Micronesia (Yap, Guam), Taiwan
	S Pacific O	Polynesia (Marquesas)
	Indian O	Mauritius, Reunion
	N America	Mexico, USA (FL)
	S America	Brazil
<i>Coptotermes sjostedti</i> Africa	Caribbean	Lsr Antilles

1 Gay (1969) believed this to be a valid species, but more likely either *H. convexinotatus* or *H. tenuis*.

2 *Co. formosanus* probably eradicated from South Africa.

3 *Co. frenchi* probably eradicated from New Zealand.

4 Includes *Co. havilandi* and *C. vastator*, as these names have been synonymised under *Co. gestroi* (Kirton & Brown 2003; Yeap et al. 2007)

Table 4. Invasive species in Termitidae, their natural and invaded ranges. N = north, S = south, E = east, W = west, O = ocean, Is = island or isle (incl. plurals), Gtr = Greater, Lsr = Lesser, ? = unknown, uncertain or disputed. States in the indicated with standard two letter abbreviations.

Species	Invaded Regions	Invaded localities
<i>Termes hispaniolae</i> S America & Antilles	Caribbean	N Is, Lsr Antilles
<i>Nasutitermes corniger</i> S America & Antilles	Caribbean	Lsr Antilles
	S Pacific O	New Guinea
	N America	USA (FL)

Table 5. Main termite evolutionary clades showing number and percentage of invasive species. ‘Total #’ = in the clade, ‘# Invasive’ = number of invasive species in clade, ‘% Clade invasive’ = percentage of clade that is invasive, ‘% of invasive spp’ = percentage of invasive species in clade.

Clade (Family / subfamily / group) ¹	Species			
	Total #	# Invasive	% Clade invasive	% of invasive spp
Mastotermitidae	1	1	100	3.8
Hodotermitidae	20			
Termopsidae	20	1	5	3.8
Kalotermitidae	430	9	2.1	34.6
Serritermitidae	2			
Rhinotermitidae	330	13	3.9	50
Termitidae				
Macrotermitinae	330			
<i>Foraminitermes</i> group	10			
Apicotermitinae, <i>Anoplotermes</i> group	160			
Apicotermitinae, <i>Apicotermes</i> group	35			
Termitinae, <i>Amitermes</i> group	305			
Termitinae, <i>Termes</i> group	285	1	0.4	3.8
<i>Cubitermes</i> group	165			
Nasutitermitinae	650	1	0.2	3.8

¹ after Donovan et al (2001); Eggleton and Tayasu (2001); Inward et al (2007).

Table 6. Main termite evolutionary clades showing food and life way types, which include nesting behaviour. Food: w = wood (dw = dry, sound wood, ww = wet, rotted wood, vw = wood in various states), g = grass, l = litter, f() = fungus culturing, o = (degraded) organic material–soil interface, s = mineral soil. Life way types: Sin = single piece nester, Int = intermediate nesters, Sep = separate piece nester, I = non-humified plant food (w g l), with gut flagellates; II = non-humified plant food (w g l) without gut flagellates; III = degraded organic material–soil interface; IV = mineral soil; n.b. III & IV have no nest as they live in soil.

Clade (Family / subfamily / group)	Food		Life way types	
	Total	Invasive	Total	Invasive
Mastotermitidae	dw	dw	Int I	Int I
Hodotermitidae	g		Sep I	
Termopsidae	ww	dw	Sin I	Sin I
Kalotermitidae	dw	dw	Sin I	Sin I
Serritermitidae	w		Sep I	
Rhinotermitidae	vw	dw	Sin I, Int I, Sep I	Sin I, Int I
Termitidae				
Macrotermitinae	f (w g l)		Sep II	
<i>Foraminitermes</i> group	o		III	
Apicotermitinae, <i>Anoplotermes</i> group	o s		III, IV	
Apicotermitinae, <i>Apicotermes</i> group	s		IV	
Termitinae, <i>Amitermes</i> group ³	vw g l o		Int II, Sep II, III	
Termitinae, <i>Termes</i> group ^{3,4}	vw o s	dw	Int II, Sep II, III, IV	Int II
<i>Cubitermes</i> group	s		IV	
Nasutitermitinae ⁵	vw g l o s	dw	Int II, Sep II, III, IV	Int II

Table 7. Main termite evolutionary clades showing secondary reproductive types. Secondary reproductive types: e = ergatoid (excludes any form with wingbuds), n = nymphoid (includes any form with wingbuds), a = adultoid (all forms, includes retained alates), s = soldier, ? = uncertain. #Species (en) = number of species with ergatoids or nymphoids; %Clade (en) = percentage of species in clade with ergatoids or nymphoids, %Invasive (en) = percentage of invasive species in clade with ergatoids or nymphoids.

Clade (Family / subfamily / group)	Secondary reproductives			
	Type	#Spp (e n)	%Clade (e n)	%Invasive (e n)
Mastotermitidae	e	1	100	100
Hodotermitidae	n	4	21	
Termopsidae	n s	14	70	100
Kalotermitidae	n a	51	12	100
Serritermitidae	e n			
Rhinotermitidae	e n	38	11	100
Termitidae				
Macrotermitinae	a			
<i>Foraminitermes</i> group	?			
Apicotermitinae, <i>Anoplotermes</i> group	a			
Apicotermitinae, <i>Apicotermes</i> group	a			

Termitinae, <i>Amitermes</i> group	e n a	34	11	
Termitinae, <i>Termes</i> group ¹	n a	10	4	?
<i>Cubitermes</i> group	n a	1	<1	
Nasutitermitinae ²	e n a	24	4	100

1 ergatoid neotenic reported for four species only: *Amitermes hastatus*, *Microcerotermes biroi*, *M. papuanus* & *M. strunkii*.

2 ergatoid neotenic reported for thirteen species: *Lacessitermes batavus*, *Leptomyxotermes doriae* and 13 *Nasutitermes* spp. including *corniger*.

Session 1

Evaluation of termite management products, compounds and resistant materials

Are results on the termite resistance of plastic materials comparable between countries? - Summary report of a field study with *Coptotermes*, *Mastotermes* and *Reticulitermes* (Isoptera)

Michael Lenz¹⁾, Brad Kard²⁾, James W. Creffield³⁾, Theodore A. Evans⁴⁾, Kenneth S. Brown⁵⁾, Edward D. Freytag⁶⁾, Jun-Hong Zhong⁷⁾, Chow-Yang Lee⁸⁾, Boon-Hoi Yeoh⁹⁾, Tsuyoshi Yoshimura¹⁰⁾, the late Kunio Tsunoda¹⁰⁾, Charunee Vongkaluang¹¹⁾, Yupaporn Sornnuwat¹¹⁾, Ted A. Roland, Sr.¹²⁾, and Violaine Weibel¹³⁾

¹⁾CSIRO Ecosystem Sciences, GPO Box 1700, Canberra, ACT 2601, Australia

Email: Michael.Lenz@csiro.au

²⁾Department of Entomology and Plant Pathology, 127 Noble Research Center, Oklahoma State University, Stillwater, OK 74078-3033, USA

³⁾Onwood Entomology Pty Ltd., 22 Davis Drive, Mt. Eliza, VIC 3930, Australia

⁴⁾Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543

⁵⁾BASF Corporation, 26 Davis Drive, Research Triangle Park, North Carolina 27312, USA.

⁶⁾City of New Orleans Mosquito, Termite, and Rodent Control Board, 6601 Stars and Stripes Blvd., New Orleans, LA 70126, USA

⁷⁾Guangdong Entomological Institute, 510260, Guangzhou, China

⁸⁾Urban Entomology Laboratory, Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

⁹⁾Bentz Jaz Singapore Pte Ltd., 48 Toh Guan Rd. East, #06-139 Enterprise Hub, Singapore 608586

¹⁰⁾Research Institute for Sustainable Humanosphere, Kyoto University, Uji, Kyoto 611-0011, Japan

¹¹⁾Royal Forest Department, Bangkok 10900, Thailand

¹²⁾United States Department of Agriculture (Retired), Picayune, Mississippi, USA

¹³⁾Arkema Japan., Kyoto Technical Center, SCB# 3, Kyoto Research Park, 93 Chudoji Awatacho, Shimogyo-ku, Kyoto 600-8815, Japan

Abstract

Cable sheathings of Low- and Medium-density polyethylene, a Development product on polyamide-basis and Polyamide 12 (Nylon) were exposed to termites from six countries (five species of *Coptotermes*, *Mastotermes darwiniensis* and *Reticulitermes flavipes*). Low-density polyethylene was the most susceptible, Polyamide 12 the most resistant material. The Australian species *C. acinaciformis* and *M. darwiniensis* were the only ones causing a rating of “destroyed”. The economically most important global pest species *C. formosanus* (USA, Japan, China) and *C. gestroi* (Thailand, Malaysia) caused only minor damage (“nibbles”). *R. flavipes* (USA) failed to damage the cables. In at least some countries, e.g. Australia, plastic materials declared resistant even when based on trials with *C. formosanus* and *C. gestroi*, will have to be re-assessed against the local termite fauna.

Key words: *Coptotermes formosanus*, *Coptotermes gestroi*, *Coptotermes acinaciformis*, polyethylene, polyamide, plastic materials, termite resistance, field test

Introduction

Termites have a cellulose-based diet, but they are also able to damage many materials that lack nutritional value, including several plastic materials. The susceptibility of plastic materials to termite damage varies with their chemical structure, surface finish and hardness. Results may also differ between species of termite (Beal and Bultman 1978; Watson et al. 1984).

Differences between termite species regarding the susceptibility of a given material clearly have significant practical and economic implications. A material may be assessed in one country as termite resistant. However, when exposed to a different termite fauna in another country, the same material may prove susceptible.

We provide a brief summary of the key results from a field study evaluating the response of several major termite pest species from six countries to four plastic materials in the form of cable sheathings using below-ground (Lenz et al. 2012) and above-ground (Creffield et al. 2012, in prep.) exposure methods. A formal manuscript detailing complete study findings is being prepared for publication.

Materials and methods

The following polymers with different termite resistance rankings based on earlier field and laboratory studies in Australia (M. Lenz unpubl.) were assessed: Low-density polyethylene (LDPE), highly susceptible; Medium-density polyethylene (MDPE), susceptible to resistant; Development Product on Polyamide-basis (DPPA), limited susceptibility to resistant and Polyamide 12 (PA 12), resistant.

Coptotermes formosanus in China, Japan and the USA, four other species of *Coptotermes* in Thailand, Malaysia and Australia, *Reticulitermes flavipes* in the USA, and *Mastotermes darwiniensis* in Australia, were included in this study (Table 1).

Plastic sheathings in the form of tubes (i.e. without the cable wire inside), 20 cm long with an outside diameter of 0.9 cm and a 0.1 cm thick wall, were capped at each end with a metal dome nut. Thus, only the ability of termites to attack the smooth sheathing surface was evaluated. To simulate possible damage to the surface of a cable during installation in service, samples were given a shallow 10 cm long scratch to their surfaces.

Samples were exposed to termites below-ground, either within trenches or in buried containers (Table 1). In the above-ground trial against *C. formosanus* in the USA, samples were placed within stainless steel containers at termite aggregation sites (Creffield et al. 2012, in prep.).

Plastic samples have no inherent food value for termites. Therefore, the samples were installed side-by-side in direct contact with highly palatable wood (bait wood) in order to attract and sustain termite activity in the trenches and containers (for details see Lenz et al. 2012; Creffield et al. 2012, in prep.). The below-ground trials lasted one year. In an attempt to increase termite pressure against the experimental materials, the below-ground samples were removed, cleaned and re-installed in contact with new bait wood every three to six months for the duration of one year (see Table 1). Samples were recovered at the end of the trial, then visually inspected and

rated for damage (see Table 2). The above-ground trial was terminated when termites had consumed all the bait wood.

Table 1. Location of field sites, target species of termite, below-ground exposure method, and intervals between replenishment with new bait wood within a 12 month period.

Location	Coordinates	Climate	Target species of termite	Method of exposure	Bait wood replacement
Stennis Space Ctr., McNeill, MS, USA	30°21'N, 89°37'W	Subtropical	<i>Coptotermes formosanus</i>	Trench/ Container	4 months
New Orleans, LA, USA	30°03'N, 90°05'W	Subtropical	<i>C. formosanus</i>	Trench	4 months
Guangzhou, China	23°10'N, 113°23'E	Subtropical	<i>C. formosanus</i>	Trench	3 months
Kagoshima Pref., Japan	31°00'N, 130°23'E	Warm-temperate	<i>C. formosanus</i>	Trench	6 months
Phuket, Thailand	8°00'N, 98°22'E	Tropic	<i>C. gestroi</i>	Trench	4 months
Penang, Malaysia	5°21'N, 100°18'E	Tropical	<i>C. gestroi</i>	Container	3 months
Penang, Malaysia	5°21'N, 100°18'E	Tropical	<i>C. curvignathus</i>	Container	3 months
Penang, Malaysia	5°21'N, 100°18'E	Tropical	<i>C. kalshoveni</i>	Container	3 months
Griffith, NSW, SE Australia	32°54'S, 146°14'E	Semi-arid	<i>C. acinaciformis</i> , tree-nesting	Trench/ Container	3 months
Darwin, N.T., N. Australia	12°36'S, 131° 16'E	Tropical	<i>C. acinaciformis</i> , mound-building	Trench	3 months
Darwin, N.T., N. Australia	12°36'S, 131° 16'E	Tropical	<i>Mastotermes darwiniensis</i>	Trench	3 months
Gulfport, MS, USA	30°37'N, 89°08'W	Subtropical	<i>Reticulitermes flavipes</i>	Trench	4 months

Table 2. Visual rating system for damage to cable sheathings.

Damage rating	Abbreviation	Definition
Not damaged	OK	No damage
Nibbled	N	Surface roughened or pitted very shallowly (<0.3 mm), and only in a few, restricted regions ≤100mm ²
	SN	As for N, but only along the scratch line
Attacked	A	Surface shallowly or deeply pitted, over extensive areas (>100 mm ²), but material not penetrated.
	SA	As for A, but only along the scratch line
Destroyed	D	Material penetrated so that the metal core is exposed, Providing the potential for system failure

Results

The proportions of samples rated in each damage category for each plastic for all species of termite (averaged across sites for each species) are shown in Fig 1.

LDPE sustained the highest amounts of damage and PA12 sustained the least. LDPE and MDPE had ‘D’ and ‘SD’ damage, whereas DPPA and PA12 did not. DPPA experienced a small number (3 of 285) of A and SA damage, whereas PA12 sustained few (15 of 285) ‘N’ and ‘SN’ – the lowest damage ratings.

Termite species could be separated into four tiers capable of causing decreasing amounts of damage to the plastic samples. The first tier of the most damaging species included *C. acinaciformis* (mound-building form) and *M. darwiniensis*, both from tropical Australia; these were the only species that caused ‘D’ or ‘SD’ ratings. The second tier comprised *C. acinaciformis* (tree-nesting form) from temperate Australia and *C. kalshoveni* from SE Asia, which both caused some ‘A’ and ‘SA’ damage ratings. The third tier included *C. curvignathus* and *C. gestroi* from Southeast Asia, plus *C. formosanus* from China, Japan and the USA, which caused some ‘N’ and ‘SN’ only. The fourth tier was only *R. flavipes* from the USA, which caused no damage.

The effect of introducing an initial scratch on the surface of the materials was important for the resulting damage by some species. For *C. acinaciformis* (60 sets), ‘N’ did not differ significantly from ‘SN’, neither did ‘A’ from ‘SA’. However, ‘D’ was higher than ‘SD’, showing that this species did not require a scratch to initiate damage to the surfaces of the plastic samples.

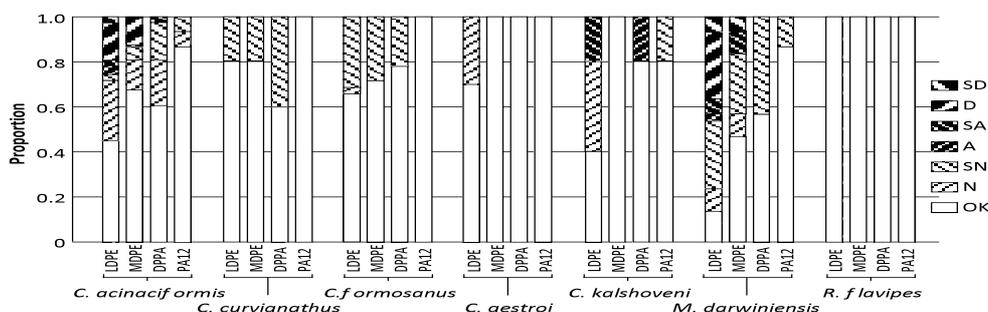


Figure 1. Damage to each plastic type by termite species (data averaged across sites for each species). See Table 2 for details of damage ratings and text in Materials and methods for plastic types).

For *C. formosanus* (97 sets), the opposite was true: ‘N’ was significantly lower than ‘SN’, showing that this species did need a scratch to gain purchase on the surfaces of the plastic samples. The other three *Coptotermes* species showed no differences.

Neither the method of placement nor the wood replacement frequency was found to have any effect (*C. acinaciformis* – temperate tree-nesting form and *C. formosanus*).

Discussion

There are two clear patterns in the data: plastic type, and termite species.

Among the plastics, low density polyethylene clearly had the least resistance to termite attack, with medium density and development product polyamide with an intermediate level of resistance, and polyamide 12 (nylon) showing the greatest resistance.

C. acinaciformis (tropical mound-building form) and *M. darwiniensis* caused the most damage. *C. acinaciformis* (temperate tree-nesting form) and *C. kalshoveni* caused less damage, but more so than *C. curvignathus*, *C. formosanus* and *C. gestroi* whereas *R. flavipes* was not able to damage any samples.

This study has illustrated major differences in the ability of various termite species to attack the tested types of plastic cable sheathings using a number of different assessment protocols. Interestingly, the two species considered globally to be the most damaging to wooden structures - *C. formosanus* and *C. gestroi* - had little impact on the plastic materials despite making extensive contact with the samples as well as destroying all the adjacent bait wood. However, it would be inaccurate to assume that these species are incapable of causing damage to such plastics. Once their workers have a point of vantage for their mandibles on imperfections in the cable surface, such as scratches or folds (e.g. as a result of buckling), damage may be possible as indicated in this study and also in laboratory trials (Watson et al. 1984; Tsunoda et al. 2010) although such damage caused by *C. formosanus* and *C. gestroi* has never reached the extent as reported for Australian *C. acinaciformis* and *M. darwiniensis* in this study and elsewhere (Gay and Wetherly 1962, 1969; Watson et al. 1984; Lenz et al. 2012).

It would seem imperative that plastic materials exposed to termites in their end-use and deemed “termite resistant” based on assessment with non-Australian species of termite, need to be re-evaluated before they can be used in Australia.

The current lack of detailed knowledge on the capacity of many species of termite to damage plastic materials is especially pronounced for *C. formosanus* and *C. gestroi*. In order to obtain a better idea of the economic impact a given species may have it would be prudent as a first step to collect all documented or anecdotal accounts of termite damage to plastics and the types of susceptible plastics.

Acknowledgement

We thank Arkema Japan (Kyoto) for providing the samples, technical advice and for some financial support. Messrs. R. Eldridge (Sydney) and B.C. Peters (Brisbane) gave valuable comments on an earlier draft.

References

- Beal, R.H. and J.D. Bultman 1978 Resistance of polymeric cable coverings to subterranean termite attack after eight years of field testing in the tropics. *International Biodeterioration Bulletin* **14**, 123-127.
- Creffield, J.W., M. Lenz, D.K. Scown, T.A. Evans, J.-H. Zhong, B. M. Kard, J.R.B. Hague, K.S. Brown, E. D. Freytag, J.P. Curole, W.R. Smith and T.F. Shupe (in prep. for *Journal of Economic Entomology*) International field trials of pyrethroid-treated timber exposed to *Coptotermes acinaciformis* in Australia and *C. formosanus* (Isoptera: Rhinotermitidae) in China and the USA.
- Gay, F.J. and A.H. Wetherly 1962 Laboratory studies of termite resistance. IV. The termite resistance of plastics. Division of Entomology (CSIRO Melbourne, Australia) Technical Paper No. 5, 31 pp.

Gay, F.J. and A.H. Wetherly 1969 Laboratory studies of termite resistance. V. The termite resistance of plastics. Division of Entomology (CSIRO Melbourne, Australia) Technical Paper No. 10, 49pp.

Lenz, M., J.W. Creffield, T.A. Evans, B. Kard, C. Vongkaluang, Y. Sornnuwat, C.-Y. Lee, T. Yoshimura and K. Tsunoda 2012 Resistance of polyamide and polyethylene cable sheathings to termites in Australia, Thailand, USA, Malaysia and Japan: A comparison of four field assessment methods. *International Biodeterioration & Biodegradation* **66**, 53-62.

Tsunoda, K., G. Rosenblat and K. Dohi 2010 Laboratory evaluation of the resistance of plastics to the subterranean termite *Coptotermes formosanus* (Blattodea: Rhinotermitidae). *International Biodeterioration & Biodegradation* **64**, 232-237.

Watson, J.A.L., Z-R. Dai and R.A. Barrett 1984 Comparative studies on the resistance of materials to species of *Coptotermes* (Isoptera: Rhinotermitidae). I. The susceptibilities of various plastics to *C. acinaciformis* (Froggatt) from Australia and *C. formosanus* Shiraki from China. *Bulletin of Entomological Research* **74**, 495-503.

Identifying termite resistant design works on Hong Kong buildings by investigating the ecology Of *Coptotermes formosanus*

Shing Kwong Cheng and Wai Tung Cheung

BST, City University of Hong Kong, 83, Tat Chee Avenue, Kowloon Tong, Hong Kong SAR, China

Abstract

Termite control is still a big problem around the world. In the metropolitan Hong Kong, although buildings are built of concrete, termites can enter houses and destroy timber contents causing severe economic damage. Consequently, the typical termite can still threaten the structure of buildings if inappropriate design work is applied.

Although there are many methods to treat the termites, some methods are not effective enough to eliminate the colony. Therefore, it is essential to find out some of the effective ways to treat them. In this research, it will mainly focus on the design works of treating the termites. We use the structured interview and case study as the support evidence of our theory. We have interviewed four experts and conducted one case and found those methods by following on the ecology of termites. These methods became effective design works for treating them.

Key words: *Coptotermes formosanus*, Design works, Hong Kong buildings, ecology, foraging, moulting

Introduction

Termite is classed as Insecta and ordered by Isoptera under the kingdom of Animalia. There are seven families of termites, they are Mastotermitidae, Kalotermitidae, Termopsidae, Hodotermitidae, Rhinotermitidae, Serritermitidae and Termitidae. In the metropolitan part of Hong Kong, termites can still affect the building structure and content significantly, it is necessary to identify some design works so as to stop the termite attack.

The aim of this research is to find out the effective termite resistant design works so that the other researchers can improve their works on treating *Coptotermes formosanus*.

The objective of this study is shown below:

1. Find out the life pattern and style of termite, i.e. *Coptotermes formosanus* in Hong Kong;
2. Investigate different treatments of reducing the effect of the termite on buildings;
3. Compare their advantages and disadvantages as seen from the interviewees; and
4. Finalize the result and assess the effective termite resistant design works.

It is expected that some of the effective design works can emanate from this research.

Material and methods

In this research, I have conducted four face-to-face interviews and one fieldwork observation. The interviews concerned mainly the effectiveness of the design work on treating the termites.

The effectiveness factors include the required time to eliminate termite, cost, environmental friendliness; feasibility and possibility to apply to the Hong Kong situation. We have found six methods through this research, they are: soil chemical barrier, in-structure chemical barrier, physical barrier, baiting system, choice of material and ventilation and moisture-proofing method.

The interviewees included the termites' company personnel and the staff of an academic institution in Hong Kong who have had the experience of treating the termites.

Field observation

The purpose of the field observation is to find the problems and solutions for treating termites. We have visited the Student Residence of City University of Hong Kong which was recorded to have a serious termite past problem.

Results and discussion

After collecting the comments and literature review about the design work against the termites, there are six types of design works that are possible to use to prevent and eliminate the termites in Hong Kong buildings. The following is the methods of the design work and the related feedback.

For the soil chemical barrier, according to the comments from the interviewees, it is cheap yet the chemical has low potency today that is of around three years' durability. It cannot cover the underground pipe work properly. Also, the infiltration is blocked by rock and there is a possibility of it washing away by rain.

For the in-structure chemical barrier, similar to the previous one, it is cheap. Nevertheless, it will be washed away easily and it is smelly.

For the physical barrier, it is environmental friendly yet termites can go inside the crack of barrier easily and the cost of the comprehensive installation is high.

For the baiting system, it is easy and flexible to install. It can attract termites easily and the maintenance cost of the building cost can be lowered gradually. However, it needs a regular maintenance which can be time consuming and therefore costly.

For the choice of material, there are two types to prevent termites attack. They are the non-cellulose material and treated wood. For the non-cellulose material, like stone or metal, it can stop termites destroying the building but these materials are usually high in the renovation cost. For treated wood, it can stop the fungi decay yet it cannot stop the termites entering the structure to find other untreated cellulose.

For the method of ventilating and removing moisture, it is effective to remove moisture substantially but some areas cannot be blown by wind and the moisture will remain in that area, like the shoe horn shape of a ship cabin.

The followings are the comments in detail:

Soil chemical barrier

For this type of design work, it is true that the drawback is greater than the benefit. This analysis can be supported by the study of Rudolf (1998). He indicated that soil chemical barrier is not effective enough when there is a large amount of termites. Moreover, although the cost of the soil chemical barrier is cheap, according to Rudolf's study (1998), the termiticide is more expensive than those in the past and is less persistent than Cyclodienes.

Although soil chemical barrier has an immediate effect of killing termites, it cannot eliminate the whole colony and the cost is not as low as in the past. In addition, the longevity is not as durable as the other design works. Therefore, it is not effective enough for treating the problem of termites in Hong Kong.

In-structure chemical barrier

Many interviewees said that the chemical for the in-structure chemical barrier is smelly and it dissolves in and is brought away by water easily. Nevertheless, according to the study of Lloyd (2011), he said that one particular chemical has provided a better infiltration and is less smelly. He provided the recorded test result from the Louisiana State University and Mississippi State University, and concluded that the performance meets the requirement. It seems that the disadvantage commented by the interviewees are not applicable to this type of chemical design work.

In addition to the lower cost, there are more advantages on this type of design work. Lloyd (2011) has indicated that apart from the barrier, it has the performance of baiting and preservative. It is important since the baiting performance can help to eliminate the colony through their foraging behavior of the termites. It can increase the effectiveness of this design work. With reference to the interviewees and literature review the in-structure chemical barrier can meet the factors of time, cost, amount of killing, longevity and even the foraging behavior. Therefore, it is an effective method to treat termites.

Physical barrier

Many interviewees said that physical barrier could result in cracks easily. Moreover, many researchers also mentioned this kind of disadvantage in their research. For example, Rudolf (1998) and Tai (2004) said that crack will create due to careless installation and termites can go into the building through these cracks. Since the physical barriers are constructed under the ground slab, it is difficult to find and to repair them. Furthermore, the barrier is expensive. Although it is environmental friendly because of its non-chemical nature and the longevity duration than other methods, it cannot meet the requirements of cost, elimination and time as suggested by Lloyd (2011). Consequently, it is not an effective way to treat termites.

Baiting system

This design work uses the *Coptotermes formosanus* ecology in the foraging and moulting behavior. This was supported by the statements of Tai (2004), Tso (2011), Yuen (2011) and Tong (2011). They indicated that effectiveness of the system is after following the *Coptotermes*

formosanus behavior, it can finally eliminate the whole colony. Also, according to a case study of the campus building, after using the baiting system, the problem of termites on City University of Hong Kong has been resolved. Therefore, it proves that it is the effective method to eliminate them.

Nevertheless, time and cost maybe a problem when using this type of design work. According to the comments of Tso (2011) and Kong (2011) the effectiveness will show only after three to four months. Furthermore, it has to maintain the system regularly so as to retain the function of the system. Therefore, other design works may need to be combined so as to eliminate the drawback of the baiting system.

Choice of material and removing moisture

Both types of design work possess the advantages and disadvantages. It is suggested that both types are used as the secondary control method. The reason is that it may not be effective enough to eliminate *Coptotermes formosanus*. Lloyd (2011) mentioned that '*An in-structure barrier, soil barrier or other system should be used apart from the treated wood.*'

Moreover, many interviewees said that ventilation is not effective enough since it cannot remove moisture at places where it is far away from the window. Consequently, removing moisture can only be used as a supplementary measure where other method such as the in-structure chemical barrier is the primary one for termite control.

Conclusion

It is suggested that in-structure chemical barrier, baiting system are the most effective termite resistant design works on treating the termites' problems for the Hong Kong buildings. Meanwhile, the choice of material and removing moisture are used as the secondary defence line.

It is no doubt that the effective methods are premised on the ecology of the termite. For instance, the baiting system is using *Coptotermes formosanus* foraging behavior to attract them to go into the station. The chemical and wood inside the station can cause the foraging termites fail to moult and then starve to death. Finally, the colony can be eliminated because of the shortage of food.

Although the design works are classified as effective or not effective enough, none of the methods should be used independently. It is because all the design works have their own drawbacks. If multiple design works are used, it can provide a comprehensive control on termites. The comment on whether the design work is effective is only for reference.

Moreover, because of the time limitation, this research is conducted between January to April, it is not the breeding period of *Coptotermes formosanus* in Hong Kong. It is difficult to conduct the experiment on them and observe their behavior on different types of design work. More data could have been obtained through the experiment and observational activities.

References

- Alan R.L. and L.O. Weste 2003 United States Department of Agriculture—Agriculture Research Service research on targeted management of the Formosan subterranean termite *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). *Pest Management Science* **59**, 788-800.
- Ashok R., I.P. Yong, G. Dale 2008 Moulting in workers of the Formosan subterranean termite *Coptotermes formosanus*. *Journal of Insect Physiology* **54**, 155 – 161.
- Campus Development and Facilities Office 2011 <http://www6.cityu.edu.hk/cdfo/?lang=Eng&pageID=obrr> (accessed on 1/3/2011)
- Cheng S. K. E., Cho K.C. 2010 *The Preservation of Heritage Buildings through the Weakening of Termite Attack* Ph.D. City University of Hong Kong, conference paper.
- 刘源智,江涌,苏祥云,彭心赋,魏翰均,史文鹏,唐国清,1998 *中国白蚁生物学及防治*. 成都: 成都科技大学出版社, (E-book).
- Grace J.K., Yates J.R., 1999 Termite resistant construction and building materials, USA, Honolulu. University of Hawaii, conference paper.
- 百仕佳虫害防治有限公司 2011 <http://www.pestguy.com.hk/ctc.asp> (accessed on 16/4/2011)
- Hill D.S., H. Phyllis and I.W.B. Thornton 1982 *Insects of Hong Kong*. Hong Kong: Hong Kong University Press.
- Kubal M.T. 2008 *Construction Waterproofing handbook 2nd ed.* New York, USA: The McGraw-Hill Companies
- 李雄生, 李永忠 2000 白蟻種間及種內群體的相容性初步研究. 杭州市: 中國白蟻防治研究會 **17**(4), 1-5
- 李桂祥,戴自榮,李棟,1989 *中國白蟻與防治方法*. 北京:科學出版社, (E-book).
- 黃亮文 1994. 家白蟻初建群體的生態學及生物學特性的研究. 杭州市: 中國白蟻防治研究會 **11**(3), 1-8
- 黃復生,李桂祥,朱世模, 1989 *中國白蟻分類及生物學 (等翅目)*. 西安: 天則出版社
- 廣州市昆蟲研究所, <http://www.gdei.gd.cn/> (accessed on 10/2/2011)
- 載自榮,陳振耀, 2004 *白蟻防治教程(第二版)*. 廣州: 中山大學出版社
- SentriCon 2011 <http://www.sentricon.com/us/mission/> (accessed on 1/3/2011)
- Wikipedia 2011 <http://en.wikipedia.org/wiki/Termite> (accessed on 14/4/2011)

Efficacy and longevity of some soil termiticides under field trials in urban areas of Pakistan

Farkhanda Manzoor, Saadiya A. Malik and Ruhma Syed

Department of Zoology, Lahore College for Women University, Lahore

Abstract

Efficacy and longevity of commercially available soil termiticides (Fipronil, Imidacloprid, Pirate, Chlorfenapyr, Cadusafos and Biflex (Bifenthrin)) were studied under two field experiments, i.e., Ground Stake Test (GST) and Modified Ground Board Test (MGBT). Five different infested sites were selected for studying underground activity of subterranean termites. *Populus deltoides* stakes were used for studying termite infestation. After two years period, *Populus deltoides* stakes treated with Biflex (Bifenthrin) showed no signs of infestation by termites and proved to be most effective in its efficacy. Soil samples collected from test sites were bioassayed both for mortality and for tunneling activity of *Heterotermes indicola* (Wasmann) as the most economically important species in Pakistan. No termite mortality and tunneling were observed for Biflex (Bifenthrin) at all sites and at all concentrations.

Key words: Termiticides, subterranean termites, *Populus deltoides*, efficacy.

Introduction

Termites are considered to be the most important and economically hidden wood damaging pests of the world that can cause havoc without any alarm. It is estimated that out of the damages caused by pests, 70% are of termites and it attacks usually wood, fallen logs, paper or any cellulose containing material (Eggleton, 2000). Soil termiticide treatments have been used since 1900s and are generally inexpensive and easy to use (Richman *et al.*, 2006). Currently available soil termiticide treatments degrade and may require re-application after five or more years to maintain long-term protection of structures (Su *et al.*, 1999). Organophosphates and synthetic pyrethroid insecticides have been the promising alternative compounds as termite-control agents, because most of them have exceptionally high insecticidal activity, low mammalian toxicity and low environmental pollution (Inoue *et al.*, 2000). Evaluation of their termiticidal efficacy have been carried out in laboratory and long-term field test in the United States, Europe, Australia and Japan against the destructive subterranean termites, *Coptotermes* and *Reticulitermes* spp. either in wood treatment or in soil treatment (Thorne and Lenz, 2001). However, data available cannot be applied to Pakistan due to difference in soil texture, pH, microbial activity and weathering conditions. The present study was carried out to evaluate the efficacy and longevity of termiticides available in the market

Materials and methods

Test termiticides: Five commercial soil termiticides belonging to different chemical groups: Synthetic pyrethroid Bifenthrin (Biflex), 2.5% EC, Fipronil (Agenda) 2.5% EC, Imidacloprid 20% SC, Chlorfenapyr (Pirate) 36%SC and Cadusafos 88% Technical. Water was used as

solvent for three insecticides Fipronil, Chlorfenapyr and imidacloprid. Acetone was used for Cadusafos and Biflex (Bifenthrin). Different test concentrations of each test termiticide were prepared in their relevant solvent.

Wooden blocks and stakes: Wooden blocks and stakes of *Populus deltoides* were used as feeder stakes that were in contact with termiticide-treated soil.

Field test sites: Five different areas (infested with termites) were selected as field test sites in Lahore. Location, climatic conditions, soil types and termite species were also observed.

Field test methods: Two test methods were used to evaluate the persistence of soil termiticides in field as by reported by Sornnuwat *et al* (1996) with little modifications.

1. Ground Stake Test (GST): In the selected area of study, five trenches each measuring (100cm x 100cm x 50cm deep) were prepared by digging the soil from it. Soil was brought to the laboratory and after cleaning, crushing and oven drying; it was tested for soil type, pH and moisture content. After that soil was treated (sprayed) with an aqueous solution of formulated termiticides by spraying at 5 l/m². After this treatment, the treated soil was brought back into the trench. A wood stake of *Populus deltoids* measuring 5cm x 3cm x 30cm length was placed into the center of the treated area such that the upper 10cm of the stake was left unburied, as shown in figure 1 (a and b). Three replicates were prepared for each treatment unit.

2. Modified Ground Board Test (MGBT): In this method, treatment of soil was same as that of GST method. A Polyvinyl chloride (PVC) pipe (10cm diameter by 15cm long) was placed upright in the center of treated area. A concrete slab of 100cm x 100cm with 10cm thickness was prepared by pouring the concrete over the treated area. A wooden block of poplar (5cm x 5cm by 3cm thick) was then inserted within the pipe by keeping it intact with the treated soil as shown in figure 1(c & d).

Monitoring of termiticidal performance: After the establishment of field experiments, wooden blocks and stakes were visually monitored to check the presence of termites whether they penetrate into the soil or not. Each unit of GST and MGBT was inspected after every 3 months to estimate the efficiency of termiticides for two year in all. In both test methods, each treatment was replicated three times and was arranged in complete block design.

Results and Discussion

The soil type was loamy with pH 8 to 8.4 and organic matter was 0.5 to 0.8 %. During the visual inspection of test sites, it was noted that degree of termite activity was higher in control plots and almost 85-90% of the untreated control plots were severely infested by subterranean termites. Observed termite species were *Odontotermes obesus*, *Odontotermes guptai*, *Heterotermes indicola*, *Microtermes obesi*, *Microtermes mycophagus*, *Coptotermes heimi*, *Heterotermes indicola*, *Microtermes obesi* and *Microtermes mycophagus*.

Severity of the termite activity was also observed high during the months of high humidity at the test and control sites. The experiment was initiated in September 2009, a considerable infestation

were observed at the second month after installation of control stakes, and all popular stakes were damaged after six months of installation in control plots. Although both experimental plots were covered by fast growing vegetation at both test sites i.e., Ground Stake Test and concrete slab at Modified Ground Board Test, yet most of the activity and severity of termite attack was observed in Ground Stake Test plots as compared to the termite activity observed in wooden blocks of Modified Ground Board Test. The reason for higher termite activity at Ground Stake Test and least termite activity at Modified Ground Board Test sites might be related to the foraging preference of the termites at open places than the covered one. Plots experiencing Ground Stake Test were having naturally more severe conditions because the treated soil was subjected to sunlight and rain that ultimately resulted in gradual degradation and running off termiticides.

From table 1, it was estimated that Cadusafos 88%, a representative of the organophosphate class proved to be effective for shorter span of time and maximum protection lasted for only 12 months in GST and 18 months in MGBT as compared to other chemicals. It was also observed that among all the tested termiticides Cadusafos protection was the first to be breached by termites. Apart from Cadusafos, the remaining test chemicals were sufficiently effective to guard for at least one year even in Ground Stake Test. The second last chemical in performance was the Imidacloprid shielded the stakes for maximum 18 months with its highest concentration applied in MGBT and minimum for 12 months in GST. Here our results confirm the assessments of Kard *et al.* (1989), Kard (1991), Tamashiro *et al* (1990), Sornnuwat (1996) and Vongkaluang *et al* (2005) that endurance of chlorinated pesticides surpasses the organophosphates. Among the other tested termiticides, 0.1% concentration of Biflex (bifenthrin) and fipronil 2.5 EC were ranked high at all sites in both test GST and MGBT while 0.5% concentration of both these chemicals in MGBT had showed 100% defense against termites and no invasion was observed even after two years. These results are also in conformity with (Sornnuwat (1996) and Vongkaluang *et al* (2005). Regarding efficacy from the data shown in table 1 the termiticides can be ranked as Biflex > fipronil > chlorphenapyr > imidacloprid > Cadusafos.

The results of present study indicate that termiticides degrade more rapidly when exposed to open field conditions in Ground Stake Test (GST) rather than modified Ground Board Test (MGBT), as shown in figure 2.1. Similar results were obtained by Sornnuwat (1996), Vongkaluang *set al.* (2005) and Horwood (2007), who reported that rapid degradation of termiticides occur in soil exposed to.

Ground Stake Test (GST) and the termiticide treated soil of Modified Ground Board Test (MGBT) provided long term prevention against termite infestation in wooden blocks. This might be due to the reason that conditions of Ground Stake Test (GST) were naturally more severe than that of Modified Ground Board Test (MGBT) because the treated soil was directly subjected to sunlight and rain.

In the durability of these termiticides physical, chemical and biological factors definitely participate. As the GST sites are open and exposed, hence are subject to direct sunlight, rainfall,

microbial activity, weathering and many other environmental factors contribute collectively to degrade or leach down the termiticides applied to the soil. These factors limit the prolonged efficacy of our test termiticides in the open field environment. However the assumption of the Vongkaluang *et al* (2005) that the termiticides formulation and the applied concentrations may also play role in termiticides degradation in the soil and their resultant performance, cannot be neglected.

The chemically treated soil in MGBT test was not openly exposed to dry weather conditions and remained unaffected from climatic variations throughout the experimental period. Rapid degradation of termiticides did not occur in MGBT test sites because of the concrete slab covering over the treated plots, making it a closed space and controlling several other limiting factors. So longevity of the applied termiticides efficacy is favoured and make the MGBT a success. As a result, all the termiticides remained effective more than 12 months, and proved to be a strong protective barrier against termite activity in the field conditions. However, it can be inferred that formulation of the solution played an important role in its performance while preventing termite penetration.

The study is still in progress and information obtained from this study would help in termite control practices in urban areas of Pakistan.

References

- Eggleton, P. 2000 Global patterns of termite diversity. In: *Termites: Evolution, Sociality, Symbioses, and Ecology* (ed. by Abe, T., Bignell, D. E. and Higashi, M.) Kluwer Academic Publishing, Dordrecht. 25-51 pp.
- Inoue, T. K., Yoshimura, T. and I. Yamaoka. 2000 Symbiotic associations with protists. In: *Termites: Evolution, Sociality, Symbioses, and Ecology*. (ed. by Abe, T., Bignell, D. E. and Higashi, M.) Kluwer Academic Publishing, Dordrecht, The Netherlands. 275-288 pp.
- Kard, B., M. J.K., Mauldin, and S.C Jones. 1989. *Pest Control*. **57**(10) 58, 60-88.
- Kard, B.M. 1991 *Pest Management* **8**(5), 20-21.
- Richman, D.L., C.L. Tucker and P. G. Koehler 2006 Influence of port land cement amendment on soil pH and residual soil termiticide performance. *Pest Management Science* **62**, 1216-1223.
- Sornnuwat, Y. 1996 Studies on Damage of Constructions Caused by Subterranean Termites and Its Control in Thailand. *Wood Research* **83**, 59-139.
- Su, N. Y., P.M. Ban and R.H. Scheffrahn 1999 Longevity and efficacy of pyrethroid and organophosphate termiticides in field degradation. *Japanese Journal of Entomology*, **1**(1), 20.
- Tamashiro, M., J.R. Yates, R.H. Ebesu and R.T. Yamamoto 1990 Research Extension Series II9, University of Hawaii, Honolulu.

Thorne, B. L. and M. Lenz 2001 Population and colony structure of *Stolotermes inopinus* and *S. ruficeps* (Isoptera; Stolotermitinae) in New Zealand. *The New Zealand Entomologist* **24**, 63-70.

Vongkaluang, C., K. Charoenkrung and Y. Sornnuwat 2005 Field trials in Thailand on the efficacy of some soil termiticides to prevent subterranean termites. Proceedings of the Fifth International Conference on Urban Pests, Malaysia. 417-429 pp.

Table 1: Number of months that termiticides have been effective against subterranean termites in Modified Ground Board Test (MGBT) and Ground Stake Test (GST) in field (during 2011).

No.	Termiticides	Conc (%AI)	Longevity of termiticides (months)									
			Ground Stake Test (GST)					Modified Ground Board Test* (MGBT)				
			Site 1	Site 2	Site 3	Site 4	Site 5	Site 1	Site 2	Site 3	Site 4	Site 5
1	Chlorfenapyr (Pirate) 36% SC	0.5	18m *	18m *	21m *	18m *	18m *	24m *	21m *	24m **	24m **	24m **
		1.0	21m *	18m *	21m *	18m *	21m *	24m **	24m *	24m **	24m *	24m **
		2.0	24m *	24m *	24m *	24m *	24m *	24m *	24m *	24m *	24m **	24m **
2	Fipronil (Agenda) 2.5% EC	0.025	18m *	18m *	18m *	24m **	18m *	18m *	24m **	21m *	21m *	24m **
		0.05	21m *	24m *	21m *	24m *	24m *	21m *	24m **	21m *	24m **	24m **
		0.1	21m *	21m *	24m **	24m **	24m **	24m **	24m *	24m **	24m *	24m **
3	Bifenthrin (Biflex) 2.5% EC	0.025	24m *	18m *	24m **	21m *	24m **	24m **	24m **	24m **	24m **	24m **
		0.05	24m *	21m *	24m **	24m *	24m **	24m **	24m **	24m **	24m **	24m **
		0.1	24m **	24m *	24m **	24m *	24m **	24m **	24m **	24m **	24m **	24m **
4	Imidacloprid 20% SC	0.125	12m *	12m *	15m *	12m *	12m *	15m *	18m *	18m *	21m *	18m *
		0.25	12m *	15m *	15m *	18m *	15m *	18m *	15m *	21m *	21m *	18m *
		0.5s	15m *	18m *	18m *	18m *	18m *	21m *	21m *	21m *	21m *	18m *
5	Cadusafos 88% (w/w)	0.5	6m* *	12m *	9m* *	6m* *	9m* *	12m *	12m *	15m *	12m *	12m *
		1.0	6m* *	9m* *	12m *	9m* *	12m *	12m *	15m *	15m *	18m *	15m *
		2.0	12m *	12m *	9m* *	12m *	15m *	15m *	18m *	18m *	18m *	18m *

*denotes the end of the experiment due to the complete consumption of the stake by termites.

** denotes the continuation of the experiment due to the incomplete/non consumption of the stake by termites.

AI active in gradient concentration in the test formulation.



Figure 1 a: Burial of wooden stake in Ground Stake Test (GST) in field.

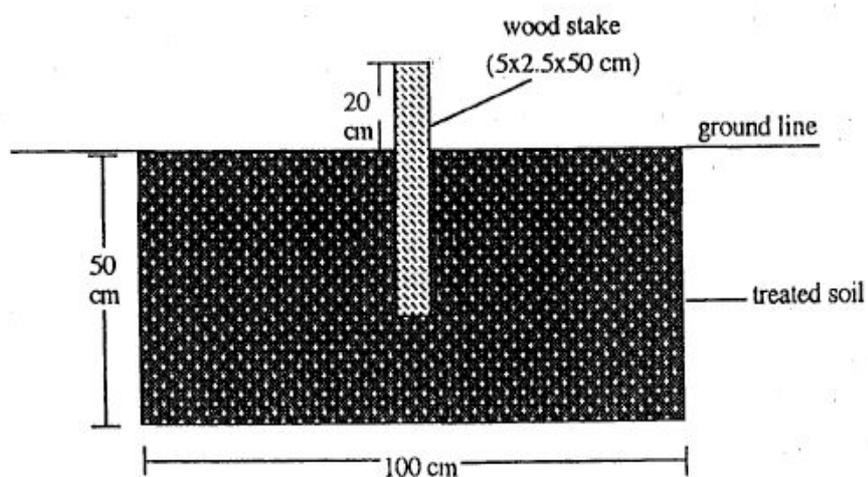


Figure 1 b: Standard Ground Stake Test (GST).



Figure 1 c: Establishment of Modified Ground Board Test (MGBT) in field.

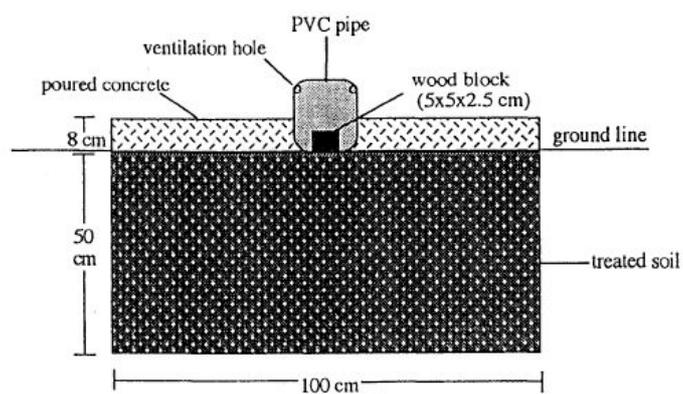


Figure 1 d: Standard Modified Ground Board Test (MGBT) or Concrete Slab Test

Termite resistance of bamboo (intermediate results)

Gina Bachtiar

Civil Engineering Department, State University of Jakarta, Rawamangun, Jakarta 13220,
Indonesia

Abstract

One of several bamboo species used as building materials is tali bamboo (*Gigantochloa apus* Kurz) that often used as components of the roof structure, columns and wall. Bamboo as a building material should have a long service life. One disadvantage in using bamboo as a building material is a relatively short service life due to bamboo liable to termites, beetles and fungi. Among the several types of termites often attacked the building, subterranean termites is the most species of termites found in Jakarta. The results of treatment in conjunction with *Gigantochloa apus* on an area that attacked by termites for one month showed that so long the sample has jet not been attacked.

Key words: tali bamboo, roof truss, building materials, service life

Introduction

Bamboo is a plant of the grass family (Graminae) which are often found in people's lives, including in Indonesia. Traditionally bamboo used for various purposes such as building materials, household tools and crafts, while the young bamboo (bamboo shoots) used as food ingredients.

Bamboo as building material, widely used in rural areas. Its use such as columns, roof structure, beams and rafters, as well as the framework of the bridge. The technology used is hereditary experience. They learn these technologies by seeing, observing existing technologies then applied the technology in buildings they made. Because bamboo is generally used for simple house in rural area as shown in Figure 1, when the public opinion refers bamboo to poverty. In India, bamboo is referred to as 'wood for the poor' (Frick, 2004). Therefore, people are reluctant to live in bamboo houses.



Figure 1. Bamboo house in rural area

The development of the population related in an increased requirement for housing, which also means increased the need for building materials. One of building material is wood that is deemed that the wood in the form of plywood is also used as a source of the state income. Demand for wood will lead to excessive logging of forests in large quantities and endanger the sustainability of forests. For the preservation of forests, it is necessary to look for other building materials as a substitute for timber. To overcome these problems, we have to do some research on alternative building materials that can be used. One of them is the bamboo that has been widely used for buildings, especially in rural areas. The choice of bamboo as an alternative material for construction of buildings, because bamboo has several advantages, namely fast-growing, easily available, cheap, long culm and relatively easy to be processed. The bamboo mechanical properties in a direction parallel to fibers have mechanical properties better than wood. Bamboo as a building material is very potential, because the bamboo for construction needs could be harvested after the age of 3 ~ 5 years. This means faster than even the fast-growing wood. Wood as building material harvested after about 30 years old. In general, after the timber-producing trees are cut, then it needs to be planted again in order to produce more wood in the future.

While the bamboo plant, after a single cut the clump can be harvested repeatedly. Bamboo as a building material disadvantage is due to near-spherical shape with various diameters and wall thickness made it difficult making the connection. Also as a result of similar material of wood, bamboo contains starch that makes it preferred by termites, beetles, and fungi. The use of bamboo as a building material is very lack of support for applied scientific research. While the current timber supply crisis, bamboo is expected to enter the market materials to replace wood as building material alternatively. Bamboo and bamboo-based products should be improved so that bamboo will equivalent to other building materials. For that, the necessary research and development in order to be optimally must be performed.

According to Widjaja (2001), bamboo is a plant belonging to the family *Bambusoideae*, one member of the sub family of grasses (*Gramineae*) that grows in tropical and sub tropical regions ranging from the valley to the hills. Bamboo is easy to distinguish from other plants, because the bamboo culm-shaped cylindrical tube with a diameter up to 30 cm and can reach 35 meters in length. Bamboo culm are generally hollow and is divided into sections (internode) is limited by the node. Unique ramifications, because every node branched. Bamboo grow rapidly. In its infancy, some bamboo can grow vertically 5 cm / hour, or 120 cm / day. Utilization of bamboo need to consider age, because the older the age of bamboo, the greater the weight of its kind. The greater the weight of its kind, the greater the mechanical strength. According to Sattar et al. (1991), the maximum achievable density bamboo at age 3 years, after which its density does not increase anymore. Therefore, for the use of construction is generally used bamboo aged 3 to 6 years which cut immediately after the growth of new shoots. As grasses family, harvesting bamboo clump will not turn off the clumps. Clumps can continue to produce new seeds.

Of the approximately 143 species of bamboo that grows in Indonesia is known about 60 species of which grow on the island of Java. The number of bamboo on the island of Java, making use of bamboo is very diverse. Bamboo utilized from the roots to the leaves. The roots of bamboo is generally used for carving, while their culms use for building materials, agricultural implements,

handicrafts, and musical instruments and the leaves are used for food packaging. *Gigantochloa apus* Kurz bamboo grows in tight clump. Their culms can reach 22 meters while on the base until the middle of the culm is straight and a curved on the top end. Branches starting at 1.5 m from ground level, consisting of 5-11 branches, one branch of the lateral larger than the others. The young culms are hairy brown reeds, but the whole when it is older the hair drop and the reeds become green. Internode length 20-60 cm, diameter about 4 cm~15 cm and wall thickness up to 15 mm. Sized leaves are 13-49 cm x 2 -9 cm (Widjaja, 2001).

There are several types of bamboo used as bulding materials such as: tali bamboo (*Gigantochloa apus Kurz*), petung bamboo (*Dendrocalamus asper*), black bamboo (*Gigantochloa atroviolacea Widjaya*), Gombong bamboo (*Gigantochloa pseudoarundinacea Widjaya*), Ampel bamboo (*Bambusa vulgaris Schrad*) and thorn bamboo (*Bambusa blumeana Schultes*). Among these species, tali bamboo is an easily obtainable bamboo. In addition to the island of Java, tali bamboo also found in South Sumatra, Central Sulawesi and Central Kalimantan. Bamboo straps are widely used for building materials, such as for walls (woven), floor, ceiling and roof frame (Figure 2). According to Sulthoni research (Sulthoni, 1988), bamboo powder straps are not vulnerable even if not preserved, because it does not contain much starch.

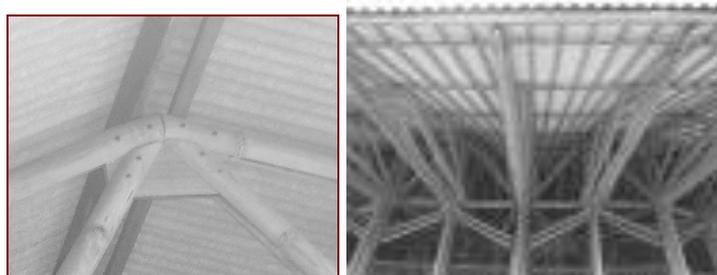


Figure 2. Bamboo as building materials (roof truss)

The bamboo culm consist of internodes and nodes. In the internodes, cell are axially oriented, where as nodes provide the transversal interconnections. The gross anatomical structure of transverse section of any internodes is determined by vascular bundles. At the pheripheral zone of the culm, the vascular bundles are small and numerous, but at the inner part larger and fewer (Figure 3). Within the the culm, the total numer of vascular bundles decrease from the bottom to the top.

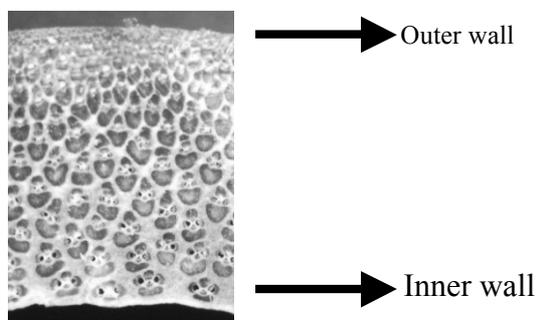


Figure 3. Anatomy of bamboo

As building materials, factors that greatly influence the bamboo is the physical properties of bamboo (round) is quite difficult in the process, because its dimensional variations and long

segments are not uniform and easily attacked by destructive organisms such as powder, termites and fungi (Purwito, 2008)

The disadvantage of bamboo as building materials was its durability, since bamboo is liable attack by fungi, and insect such as termite and beetle (Liese, 1980). The durability of bamboo depends on climatic conditions and the environments. As building materials durability is one thing that must be known or it could only be used as a temporary building material like people opinion nowadays.

Building damaging pest termites are generally classified as two categories, those are subterranean Termites and drywood Termites. In the Jakarta area, according to data there are about 15 species of termites as building pest, of which 13 species are subterranean termite (Nandika, 1999). This paper told about an intermediate study about durability of tali bamboo, especially natural bamboo durability as well as treated bamboo, as a part of the research on the utilization of *Gigantochloa apus* Kurz as the space truss elements (Bachtiar, 2008).

Materials and methods

Sample of this study is *Gigantochloa apus* bamboo grows in West Java, Indonesia and the age of 3 years with a diameter of about 6 cm. Samples prepared by cutting the bamboo clumps in a length equal to the diameter as sample for compression test match to ISO 22157-2004 (Figure 4). This study will be examined weight loss which occurs after 1 month, 2 months and 3 months as well as decrease in compression strength.



Figure 4. sample

Since subterranean termites were the most common termites in the area of Jakarta, the termites used in this study is *Coptotermes*. Treatment of samples is done by placing the sample on the area being attacked by termites in building. It is given that the use of bamboo as a roof truss is not exposed to rain fall and sunlight nor connected to the soil.

Results and discussion

The sample apply to tali bamboo with such properties as 0.71 gr/cm^3 density and 12.7 MPa compressive strength. After the bamboo samples were treated for 1 month, the results obtained that the termites live in the bamboo. In addition to visible presence of termites in the outside and inside walls the sample (Figure 5a), there is also the termite stains both on the inside walls, or on the outer wall of bamboo (Fig. 5b), but there aren't any holes which indicates that the sample has been attacked by termites. This means up to one month after treatment has not happened to lose weight. Therefore, it is assumed that the reduction in compressive strength has not happened. From the results of research shows that termites prefer other food ingredient instead of bamboo. In this study they prefer to eat paper as paper content high cellulose and even not hard as bamboo.



Figure 5. Sample after one month. (a) termite activities on bamboo. (b) spot of dirt on the inner and outer wall

In addition, based on research conducted Sulthoni (1983) *Gigantochloa apus* is the lowest starch content of most small when compared with petung bamboo (*Dendrocalamus asper*), black bamboo (*Gigantochloa atroviolacea* Widjaya) and Ampel bamboo (*Bambusa vulgaris* Schrad). Even the power as the main predator don't even like tali bamboo, because tali bamboo harder other bamboo used as building material.

Conclusions

Based on the intermediate result of the study for a month can be concluded that if there are other food choices, termites the soil will choose another food to be eaten. To find out more about how *Gigantochloa* attacked by termites, further research needs to be done with much longer timescales reply. In addition to placing the sample in conditions as close as usage, research by using insulation and Graveyard test will be performed. Similarly to cryptothermes, even if the spread in the study area (Jakarta) is not so much. It is given that cryptothermes is also one destroyer bamboo.

References

- Bachtiar, G. 2008 Utilization of Bamboo as Space Truss Elements. Thesis. Bogor Agricultural University, Bogor Indonesia (in Indonesian Language).
- Frick, H. 2004 Bamboo structural construction, Arsitectural structure series 7. Kanisius, Publisher, Yogyakarta. (in Indonesian Language).
- Liese W. 1980 Preservation of Bamboo. Bamboo Research in Asia, Proceedings of a Workshop. Singapore: 28-30 May 1980. Singapore : International Development Research Center and the International Union of Forestry Research Organizations, 165 – 172 pp.
- Morisco 2006 Bamboo Technology, Post Graduate Program On Building Technolgy, Gajah Mada University, Yogyakarta(in Indonesian Language).
- Nandika, D., Surjokusumo and S dan Rismayadi 1999 Status Of Termite Attack Dangerous On Building Structure In Indonesian. Porciding Of Nasional Symposium On Termite Attack In Indonesia. Jakarta, Agustus, 1999(in Indonesian Language).
- Purwito 2008 Standardization of Bamboo as building materials instead of wood. Prociding of PPI Standardization 2008, Jakarta, November 25, 2008 (in Indonesian Language).

Sattar M.A., M.F. Kabir and D.K. Bhattacharjee 1991 Bamboo on Their Physical and Mechanical Properties. Proceedings 4th International Bamboo Workshop on Bamboo in Asia and The Pacific. Chiangmai : 27-30 November 1991. Thailand : Forsa Publicaton, hlm 271 -275.

Sulthoni 1983 Final Report of research on Bamboo Handling in the rural areas of Yogyakarta (Indonesia) with special reference to its traditional preservation. Yogayakarta (Indonesia: Gajah Mada University).

Widjaya, E.A. 2001 Identification of bamboo species in Java. Biology Unit Indonesian Institute of science, Bogor. (in Indonesian Language).

Resistance of Jabon timber modified with styrene and MMA against soil termites and dry wood termites

Lusita Wardani¹⁾, Iwan Risnasari¹⁾, Yasni³⁾ Yusuf Sudo Hadi³⁾

¹⁾²⁾ Postgraduate Student of Dept. of Forest Products, IPB Bogor

²⁾ Research Institution of Forest Product, Bogor

³⁾ Dept. of Forest Products, Faculty of Forestry, IPB Bogor

Abstract

Modification of wood with polymeric materials is an effort to increase the resistance of wood against biological destruction. Red Jabon (*Anthocephalus macrophyllus*) and White Jabon (*Anthocephalus cadamba*) are the types of fast growing timbers with a very low resistance to attacking organisms (Class IV-V). This study modified the timber with a polymer solution of styrene and MMA added with the Mepoxe initiator with the compositions of 60/38, 70/28, 80/18 (v / v) and the Mepoxe solution of 2%, using the impregnation method for 45 minutes at the pressure of 600 mmHg. The modified wood was then fed on soil termites (*Coptotermes curvignathus* Holmgren), dry wood termites (*Cryptotermes cynocephalus* Light). The design was based on the SNI. 01.7207-2006. The study results showed an increased resistance of the modified wood against various termite attacks, that is, the increase in polymer loading could raise resistance to the termite attack in the laboratory tests. The weight loss due to the soil termite attack was 3.10 - 7.26%, and due to the dry wood termites 0.12 to 0.48%. The degree of soil termite attack was 0.56 to 8.58%, and 0-20% of dry wood termites. The mortality of 100% was in the soil termites, and 80-96% in the dry wood termites. The scores of modified wood for the resistance against the attack were I-II for soil termites, and A – B for dry wood termites.

Keywords: Modified Jabon, Durable Class

Introduction

The superiority of wood as an organic material would be more complete if the wood we use has a resistance to damage by either biological factors or others. Because of these biological attacks, not only the usage life of wood but also its sale value will decrease and currently the timber industry has been processing timber products from industrial forest of plants that are relatively young with very low resistance to biological attacks (durable class IV- V).

The technological development to extend the usage life of wood has been very fast. Initially, we only used wood directly, and now the wood processing technology has contributed quite a lot to improving the usage life and sale value of processed wood products so that they are more efficient and effective. Wood is modified or processed with an acetylation-based technology to improve the quality of wood products. Yet, this requires further development so that we really get better products of modified timber and especially also of environmentally friendly products.

Hadi.YS., *et al* (2005) states the death rate of dry wood termites *Cryptotermes cynocephalus* treated with Furfuryl alcohol on pine wood, agathis and sengon reached 62.8-100% compared to

only 26.8% in the control without the modification of additional material. The weight loss of wood is 4.2-0% wood and that of the control 10%. Further, the same treatment of 3 months, 6 months and one year showed that the weight loss due to soil termite attack (*Coptotermes curvignathus* Holmgren) were 7-2%, whereas on the control wood it reached 100%.

The trial use of DMDHEU (dymethyloldihydroxyl-ethyleneurea) in the modification of beech and pine woods fed on soil termites found lower results than in the field. The modified pine wood was better than beech (H. Militz S. Schaffert *et al*, 2010).

Kartal.SN, *et al* (2003), tested the use of MMA and DOT with AGE on sugi wood against fungal and soil termite attack and the result was that in addition to improving dimensional stability of wood, the resistance of sugi wood against fungal and termite attack was increased. Yildis (2005) stated that the WPC with a mixture of styrene and MMA (65% : 28%) (V/V%) gave the best results in physical and mechanical properties compared to the WPC with styrene and/or MMA alone.

The solutions of styrene and MMA with the impregnation of 3 compositions to obtain the ideal composition refer to the trial by Yildis, 2005. The different species of jabon timber was to respond to the challenge that the red jabon has better properties than white jabon because of its higher gravity, Can polymerization by an impregnation method improve the quality of the wood type and more greatly improve the white jabon?

Research Objectives:

The research was to modify red jabon timber (*Anthocephalus macrophylla*) and white jabon (*Anthocephalus cadamba*) with impregnation of three mixed compositions of styrene and MMA solutions with the initiator of mepoxe against the attack of soil termites and dry wood termites in a laboratory test and on the field. The resistance properties were tested and compared against the attack of termites *Coptotermes curvignathus* Holmgren and *Cryptotermes cynocephalus* Light.

Materials and Methods

Materials

Red Jabon (A1) and white Jabon (A2) with the dimensions of 20 x 20 x 10 mm for soil termites (*Coptotermes curvignathus* Holmgren) of 200 in number per test sample and the dry wood termites (*Cryptotermes cynocephalus* Light) in 50 in number for each with the sample dimensions of 20 mm x 20 mm x 50 mm. Solutions used were Styrene, Methyl Metha Acrylyte (C₅H₈O₂), and mepoxe catalyst with the compositions: B1 (60 :38 :2) ; B2(70 :28:2) ; (80 :18 :2) (v/v%). This was done in the laboratory of Forest Product, IPB Darmaga, Bogor.

Polymerization

Samples of red Jabon and white Jabon before impregnation and soaking were first dried at 70⁰ C for 24 hours or until kiln dried, and then the dimensions and weight were measured. Next, the test samples were put into the impregnator containing a mixed solution of styrene and MMA with the catalyst mepoxi and then vacuumed for 30 minutes at a pressure of 600 mm Hg. Then,

the samples were wrapped in aluminum foils and dried at a temperature of 60° C for 24 hours for polymerization and conditioning at the room temperature for 2 weeks or until the typical polymer odor disappeared.

Results and Discussions

Polymer loading

The modification of Jabon wood with a polymer makes it have an extremely stinging smell because of the emitted polymer. The number of monomers that could fill the cavities in the wood cell wall is shown in the value of the polymer loading (Figure 1). Polymer loading of white wood jabon was higher than that of the red jabon. This is because white jabon has a lower density (0.22 to 0.34) than the red jabon (0.45 to 0.47). According to Yildiz *et al.* (2005), wood with lower density has a large number of cell cavities (thinner cell wall), which causes the wood to be more porous than the wood of higher density.

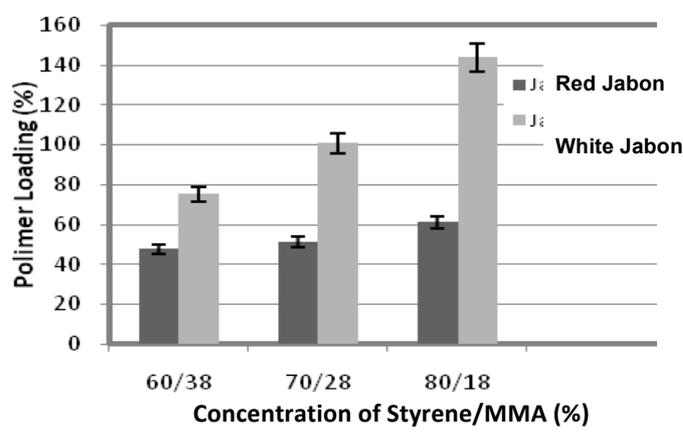


Figure1. Polymer Loading in Modified Red Jabon and White Jabon

On the white jabon, the polymer loading was 75% for A1B1 treatment, 100.89% for A2B2 and 143.66% for A3B3; 47.41% for A2B1, 51.28% for A2B2 and 60.88% for A3B3. The amount of polymer loading on the white jabon would later greatly affect the durability of this modified wood. Wood density also increased between 36.2 to 80.6% with the reduced weight percentage (3.28 to 7.26%) for white jabon and 3.1 to 6.84% for red jabon, and this can be said that white jabon showed a better improved quality of durability. This is because the modified wood, in addition to being extremely dense, have become more stable.

MMA composition also plays a role in increasing the polymer loading – the higher the concentration of MMA in the mixed solution, the higher the polymer loading on each of the test samples.

Resistance Properties of Modified Red Jabon and White Jabon

The durability or resistance class of the wood against the termite attack is determined by weight loss after the wood is fed on termites for 3 months. The test results in the tabulation show that although the mortality of termites on red jabon was equal to that of termites on the white jabon, the attack intensity was higher on the white jabon. This could mean that with or without modification, the red jabon remains harder than the white one, thus it is difficult for termites to attack.

Table 1. Weight Loss (%) Modified Red Jabon and White Jabon by Termite Attack

Testing	Control	Styrene 60/38	Styrene 70/28	Styrene 80/18
Soil termites				
Rubber	39.89			
Red Jabon/A1	16.61	3.10	6.84	5.82
White Jabon/A2	32.40	7.26	5.82	3.28
Dry Wood Termites				
Rubber	1.06			
Red Jabon/A1	0.40	0.48	0.21	0.19
White Jabon/A2	0.70	0.45	0.46	0.12

This test also proved that with a chemical modification, wood of low durability can be improved, especially the properties of its resistance to termite attack. (Hadi *et al*, 2005) this resistance to termite attack does not mean that the modified wood has become non-degradable material, but only the usage life of the wood becomes longer or more durable and this reduces the maintenance costs during use.

Table 2: Percentage of Termite Mortality (%) on Modified Red Jabon and White Jabon

Testing	control	Styrene 60/38	Styrene 70/28	Styrene 8/18
Soil Termites				
Rubber	3.6	-	-	-
Red Jabon/A1	19.8	100	100	100
White Jabon/A2	32.4	100	100	100
Dry Wood Termites				
Rubber	39	-	-	-
Red Jabon/A1	60	80	94	92
White Jabon/A2	40	90	90	96

The total mortality of soil termite on the modified wood was 100%; even the termites were dead on the observations after 1(one) month. In the unmodified timber, the mortality was 3.6% for the tested rubber wood, 19.8% for red jabon and 32.4% for white jabon. The mortality of dry wood termites also increased, but not as drastically as that of the soil termites. (Table 2)

The modified wood at the beginning of the treatment produced a strong odor due to the emission of chemicals, which was then overcome by conditioning the sample before it was fed on the

termites, but this is still very likely to produce undesirable smell for the termites so that the termites would die because of no material / substrates for them to eat.

Table 3: Percentage of Termite Attack Intensity (%) against of Modified Red Jabon and White jabon

Testing	Control	Styrene 60/38	Styrene 70/28	Styrene 8/18
Soil Termites				
Rubber	100	-	-	-
Red Jabon/A1	47	0.66	3.6	8.58
White Jabon/A2	84	7.5	6.1	0.56
Dry Wood Termites				
Rubber	70	-	-	-
Red Jabon/A1	40	20	20	0
White Jabon/A2	40	20	20	0

Table 4: Resistance of Modified Red Jabon and White Jabon against Termite Attack

Testing	Control	Styrene 60/38	Styrene 70/28	Styrene 8/18
Soil Termites				
Rubber	100 (V)	-	-	-
Red Jabon/A1	70 (III)	0(I)	40 (II)	40 (II)
White Jabon/A2	100(V)	40 (II)	40 (II)	0 (I)
Dry Wood Termites				
Rubber	70©	-	-	-
Red Jabon/A1	40 (B)	40(B)	0(A)	0(A)
White Jabon/A2	70©	40(B)	0(A)	40(B)

The results of compiled weight loose and attack intensity determine the score for the basis of classifying the wood durability or resistance to termite attack. The total values obtained by classify the modified red jabon and white jabon into Class I-II, that is, from resistant wood to very resistant wood to the attack of *C. curvignathus* Holmgre) because the values (percentage) of weight loss ranged between 0.66 – 7.26%. A significant increase occurred in the white jabon from the durable Class V to Class I-II, also in the red jabon from Class IV to I-II. The resistance class against the attack of *C. cynocephalus* Light also improved from Class CB to AB.

Conclusions and Suggestions

The results of this study take the conclusion that:

- Polymerization of Jabon timber with the solutions of Styrene, MMA and Mepoxy can improve the resistance properties of Red Jabon and White Jabon timber.
- MMA composition also plays a role in increasing the polymer loading.
- The increased dosage of MMA has no effect on the percentage in the reduction of attack intensity and weight.

- d. The durability value of Jabon increases more significantly than its natural properties compared to the red Jabon, i.e. the durable Class IV-V to Class I-II against the attacks of *C. curvignathus* Holmgren and A-B against the attack of *C. cynocephalus* Light

References

Aikfei, A., Z. Ashaari, E.S. Bakar and M.H. Sahri 2009 Enhancing the Properties of Mahang (*Macaranga* spp.) Wood through Acrylic Treatment in Combination with Crosslinker. Faculty of Forestry, Universiti Putra Malaysia 43400, Serdang, Selangor, Malaysia.

Baki, H., O. Yalcin, A. Hakki 1993 Improvement of wood properties by impregnation with macromonomeric initiators. *J. Appl. Polym. Sci.* **47**, 1097.

Yildiz, U.C., S. Yildiz and E.D. Gezer 2005 Mechanical properties and decay resistance of wood-polymer composites prepared from fast growing species in Turkey. *Bioresource Technology* **96**, 1003–1011.

Hill, C.A.S. 2006 Wood Modification: Chemical, Thermal and Other Processes. John Wiley & Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex PO19 8SQ, England.

Hadi, Y.S., M. Westin, and E. Rasyid 2005 Resistance of furfurylated wood to termite attack. Forest Product, http://goliath.ecnext.com/coms2/gi_0199-5013183/Resistance-of-furfurylated-wood-to.html.

Hadi, Y.S., D.S.Nawawi, E.N. Herliyana and M. Lawniczak 1998 Polystyrene poland woods resistance to biodegradation. *Forest Product Journal* **48**(9), 60-62.

Risnasari, I., L. Wardani, F. Febrianto and Y.S. Hadi 2011 Physical and mechanical properties of Modified Jabon Wood through Impregnation with a solution of Styrene and Methyl Methacrylate. MAPEKI Seminar 2011, Faculty of Forestry, University of Gajah Mada Yogyakarta.

Kartal, S.N., T. Yoshimura and Y.Imamura 2004 Decay and Termite resistance of boron-treated and chemically modified wood by in situ co-polymerization of allyl glycidyl ether (AGE) with Methyl methacrylate (MMA). *International Biodeterioration & Biodegradation* **53**, 111-117.

Physical barriers as prevention of subterranean termite attack on building

Indah Sulistyawati

Faculty of Civil Engineering and Planning, Trisakti University, Indonesia
email: indahusakti@yahoo.com

Abstract

The problems of building damage due to termite attacks never ending. As an observation destroys here is subterranean termites. They are an important group of urban insects pests in tropical countries. Physical barrier is used to prevent termite attack. The type of physical barrier are graded stones, stainless steel mesh, and steel strip shielding. This study was conducted with the aim to know the appropriate and reason application of three kind physical barrier as preventing termite attack. The result showed that the physical barriers as graded stone, stainless steel mesh, steel strip shielding are a good choice to prevent subterranean termite attack if applied appropriately; the action as a barrier to encourage the termites out into the open where they can be noticed when and appropriate action taken to destroy the nest.

Keywords: physical barriers, steel mesh, subterranean

Introduction

Buildings are required to provide security, safety, protection and comfort for occupants and possessions. During their life, buildings, building materials and building contents may be subjected to one of a hazards, it is termite attack. In areas where a termite hazard exists, its can be threatened to the structure safety. The protection against termite infestation should be concerned.

Subterranean termites are an important group of urban insects pests in tropical countries (Lee 2002, Lee *et al.* 2007). This colony is located in the ground. Subterranean termites build characteristic mud tubes for movement between nests.

The appearance of these tubes are often the first sign of infestation. Its biology, aggressiveness, and hidden, unpredictable invasiveness make this insect difficult to be detected and controlled. Detection can become difficult if such tubes are hidden inside walls, or termites are entering in cracks occurring in concrete slabs or foundations.

In certain circumstances, the people do not use pesticides around the home and prefer non-chemical control methods. The other alternative techniques of termite attack used is physical barriers. Physical barriers are gaining popularity world-wide as methods of preventing attacks on structures by subterranean termites (Yulian *et al.*, 1999).

The intended function of physical barrier systems is as an aid to the detection of termites attack. The physical barriers need to be done to give an indication for the home owner or supervisor of the termites movement that termite infestation will be done and resulting building damages.

Construction methods utilising “slab on ground” or subfloor masonry walls and or piers, leads to a different level of risk of termite entry into the building. These construction methods can use the treatment barriers as special construction techniques utilising physical barriers in those areas where termites are a threat to buildings.

The application each type of physical barrier should be done as effectively and efficiently as possible. The wrong treatment means waste of energy and money, so that the purpose of preventing building from termite attack is not reached. It should be done based on structural engineering of thinking that are prevented from termite attack.

Materials and methods

This study was conducted using the data compilation. The preventing data of termite attack that result of the researchers in journals, blogs, and comments was collected from the internet network accessed. Most data was the results of its review the preventing of subterranean termite attack knowledge. Based on the data and knowledge has been obtained, it was made a discussion of the placement and a reason used each type of physical barrier as preventing termite attack.

Results and discussion

Subterranean termites generally nest underground in the soil, build earthen mounds, or nest in the root crowns or trunks of living and dead trees. Having to maintain contact with soil or a reliable water source to obtain sufficient moisture to survive, subterranean termites construct protective mud shelter tubes from their nests to their food source - living trees, seasoned timber, books, furniture etc.

Eliminating sources of chronic moisture in the home is one of the most important factors in managing subterranean termites. Moist soil is necessary for termites to survive. Termites travel back between soil and food sources because they must obtain moisture from the soil.

Attacks on buildings are usually initiated from the nest below the ground from which the termites build galleries over piers or walls to attack wood and wood products in buildings. Usually, the nest is outside the building perimeter but occasionally a nest may be buried in the soil beneath the building.

One of the most common ways subterranean termites get in the home is through wood-to-ground contact, including door frames, deck posts, and porch steps or supports. The termites also enter homes through cracks in the foundation and cracks in brick mortar. Sometimes, they even use the holes in concrete blocks to travel through foundation walls.

Areas subject to moisture build-up, such as bathrooms, should be given special attention since they are likely to be attacked areas. Areas under tubs and drains leading to the exterior such as air conditioner drains should be considered.

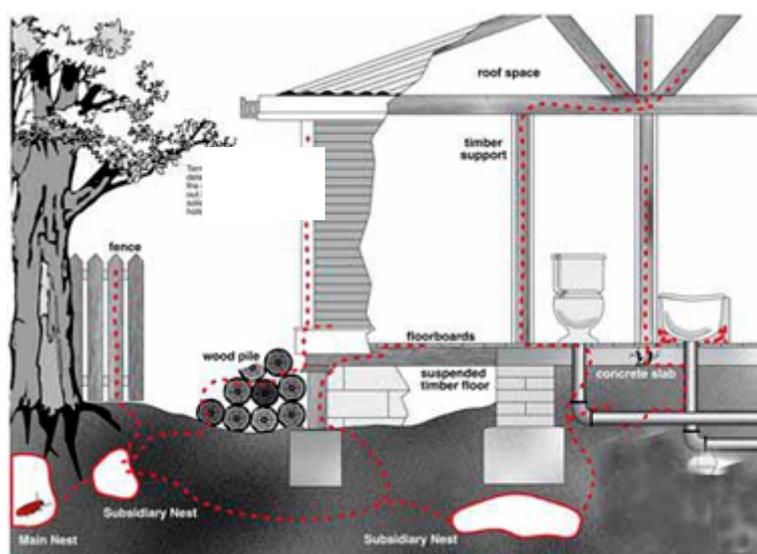


Figure 1. Route of Subterranean termite attack to the building

The main types of physical barrier systems are:

1. Graded stone
2. Stainless steel mesh.
3. Steel strip shielding.

Each type of physical barrier can discussed as follows:

1. Graded Stone

Subterranean termites have difficulty penetrating layers of certain granular materials, depending on the material's physical size, smoothness, shape, weight, and hardness. Studies in Hawaii and elsewhere have found that for various termite species, suitable materials include crushed basalt, granite, or quartz, silica sand, and even glass shards.

Extensive laboratory and field tests found that crushed, screened basaltic gravel is suitable as a barrier to the Formosan subterranean termite, it has done by research at College of Tropical Agriculture & Human Resources (CTAHR) University of Hawaii at Manoa. The Hawaii studies used irregularly shaped particles in sizes ranging from less than 1/10 inch to about 1/5 inch (0.2–4.8 mm). The laboratory technique used a glass tube with about 1 1/2 inch of the test material sandwiched between layers of agar. Termites were put in one end of the tube and allowed to bore through the agar to the material. It was found that the termites were unable to penetrate basaltic particles sized from 7/100 to 9/100 inch (1.7–2.4 mm), even when they had access to the material for as much as five years (Julian *et al.*, 1999).

Slab-on-ground construction requires more permanent protection methods due to inaccessible areas such as under the slab. The barrier can be used as a fill before pouring a concrete slab foundation, be placed around the perimeter of an existing concrete slab, or can be placed beneath and around foundation retaining walls during new construction (Figure 2).

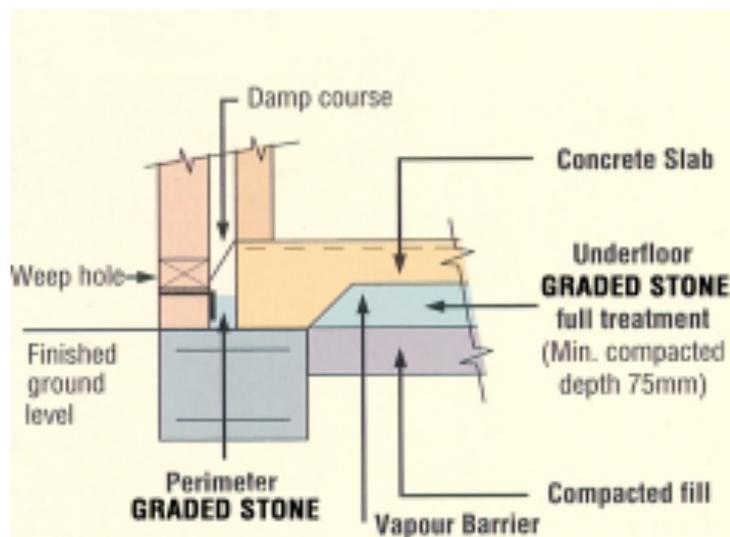


Figure 2: Graded stone under concrete slab

The system works on the principle that the particles, when placed and compacted, will not allow the termites to find a path through them. The particles are too hard to eat and are too small to allow the movement of termites.

2. Stainless steel mesh

Stainless steel mesh barriers incorporate finely woven, marine grade stainless steel mesh, placed as a partial barrier around the perimeter of the building. It can be installed beneath concrete slabs, around plumbing and electrical conduits, to seal concrete cracks and cold joints, to prevent penetration through hollow-tile retaining walls, and as a “sock” or “boot” to protect wooden fence posts and utility poles.

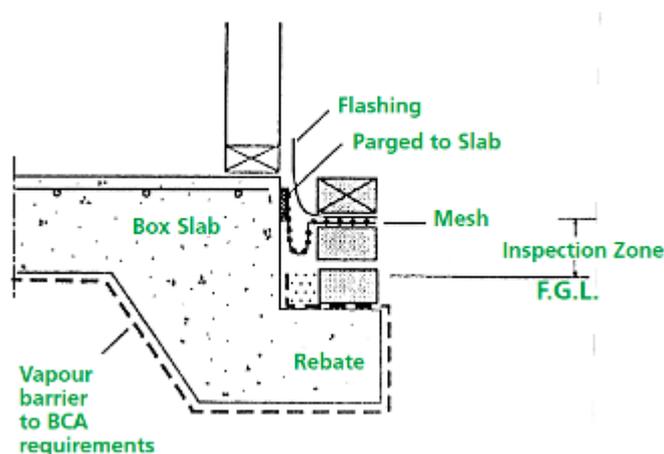


Figure 3 The placement of stainless steel mesh

Stainless steel mesh to be effective as a barrier against the subterranean termite, provided that the mesh was properly installed and seamlessly bonded to other elements of the construction, such as concrete blocks, so that termites could not circumvent the barrier.

The grid pattern of the mesh is fine enough to not allow the termites through and is strong enough to prevent them from chewing through it. Being stainless steel, it is unlikely to be affected by the corrosive nature of some concrete and ground conditions.

3. Steel strip shielding

Steel termite shields are used primarily on elevated houses that has concrete foundation or masonry piers as supporting structural building. Soil should always be from 12 to 18 inches below any wood member, the greater the distance, the better.

It prevent termites from entering through the interior cracks of masonry walls or foundation blocks, Figure 4. A good metal shield placed on body of foundation and piers may prevent mud tubes from reaching the wood above them, but will more likely cause termites to build around the shield, making their mud tubes easily detected and destroyed (Crouse B, 2002).

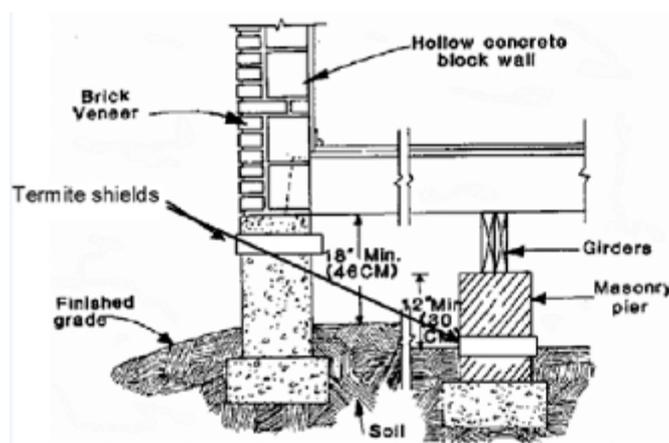


Figure 4 Steel strip shielding on the masonry pier and concrete foundation

Steel strip shielding will not prevent termites from getting into the building. They will act as a barrier to encourage the termites into the open where they can be noticed when inspections are carried out.

Conclusions

From discussion above, it could be concluded that the physical barriers as graded stone, stainless steel mesh, steel strip shielding are a good choice to prevent subterranean termite attack if applied appropriately; the action as a barrier to encourage the termites out into the open where they can be noticed when and appropriate action taken to destroy the nest.

References

Anonim. Termite Management System.

http://www.qbpr.com.au/pdf/Termite_Management_System. Pdf. Download: December 20, 2011.

Crouse, B. 2002 Taking the Terror Out of Termites. *A Guide to Home Termite Management* **22**, 20-23.

Yates, J.R III, J. K. Grace and M. Tamashiro 1999 New Technology for Managing the Formosan Subterranean Termite. Household and Structural Pests. HSP-3.

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

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

Effect of sublethal exposure to fipronil in combination with the entomopathogenic fungus *Metarhizium anisopliae* on the subterranean termite *Coptotermes formosanus* Shiraki

Yuichi Yamamoto, Aya Yanagawa, Tsuyoshi Yoshimura and Kunio Tsunoda*

Kyoto University Research Institute for sustainable Humansphere, Gokasho, Uji, Kyoto, 611-0011, Japan

*deceased

Abstract

The effect of sublethal dose of fipronil on grooming activity of the termite *Coptotermes formosanus* Shiraki, and its impacts on mortality with entomopathogenic fungus *Metarhizium anisopliae* were evaluated. Termites exposed to fipronil have significantly inactivated their grooming at 1 week after treatment except the 0.1 ppm×1 hour (fipronil concentration × exposure time) combination. Synergistic effects on the mortality were observed in the combinations of 0.1 ppm×24 hour or 1 ppm×1 hour exposure treated with 1.01×10^6 /ml fungal conidia, and 5 ppm×1 hour exposure treated with 1.01×10^4 or 1.01×10^6 /ml fungal conidia.

Key word: soil treatment, fipronil, *Coptotermes formosanus*, *Metarhizium anisopliae*, combination treatment

Introduction

Recently, non-repellent insecticides such as fipronil and imidacloprid have been used in the soil treatment for subterranean termite control. They are non-repellent and relatively slow acting (Gahlhoff and Koehler 2002). The mechanism of these insecticides in the soil treatment is transferring the insecticide from the termites, which contact to the treated soil to their nestmates through the social behavior and contact, resulting in colony collapse.

The termites taken a high dose of the insecticide die of the insecticide. However, it is not known how the termites taken a non-lethal dose of the insecticide change the behavior, and its impacts on mortality in the soil treatment are not clear.

In the laboratory experiment, the horizontal transfer of lethal dose of fipronil was reported to be limited as ≤ 5 m (Su 2005). In the field experiment, it is reported that the monitoring points, where were 0.3 to 4.0 m apart from approximately 10 ppm fipronil treated area were inactive (Potter and Hillery 2001). On the other hand, the longest linear distance of foraging was reported as 10 m in *Reticulitermes speratus*, which is an economically important termite in Japan (Tsunoda et al. 1999). We should reconsider the soil treatment from more effective point of view for subterranean termite control with the insecticides, which target a large area.

Subterranean termites live in the soil. In general there are many microorganisms including entomopathogens that attack the termites. The termites maintain their colony stability from entomopathogens like fungi through the social behaviors such as allogrooming. One of the

functions of allogrooming in termites is to clean the attached entomopathogens from the surface of their nestmates by mouth (Yanagawa and Shimizu 2008). As a result, the density of pathogens in the colony will decrease, and the colony is protected from an epizootic by pathogens. Considering these activities, we examined the hypothesis that colony collapse will occur by entomopathogens if the grooming activity is restrained or lost in the colony.

Chouvenc and Su (2010) suggested that seven factors including avoidance, grooming activity, antifungal gut activity prevent an epizootic by the entomopathogenic fungus *M. anisopliae* in termite colony. In those seven factors, the effects of grooming activity can be handled by a sublethal dose of insecticide. In their study, they estimated the effect of three factors on termite survivorships, and indicated that the absence of grooming in defense mechanism caused a problem in termite immune system, and in consequence, the termite survivorship became low. It means that the insecticide did not only kill the termites but also helped the colony immune system to be ruined. The synergistic effects with insecticide and entomopathogens could be another important factor to drive a colony into collapse.

The objectives of this study are to evaluate the grooming activity of the subterranean termite *Coptotermes formosanus* Shiraki, which are exposed to the soil treated with several concentrations of fipronil and the synergistic effects between the insecticide and the entomopathogenic fungus *M. anisopliae* on the termite mortality. We examined the synergistic effects on termite mortality with fipronil and *M. anisopliae* by the grooming frequencies at different concentrations of insecticides in the soil treatment.

Materials and methods

Termites

Matured workers from one laboratory colony of *Coptotermes formosanus* in the Deterioration Organisms Laboratory (DOL) of the R. I. S. H., Kyoto University, Kyoto, Japan were used in this study. The colony is maintained at 28 ± 2 °C and $\geq 85\%$ R.H.

Insecticide

A 1 % stock solution of fipronil was prepared by adjusting Agenda SC (9.1% fipronil; Bayer Crop Science) with distilled water. This stock solution was used for making a desired concentration of fipronil solution.

Soil Treatments

Air-dried sandy loam passed 20 meshes was used. To prepare 5, 1, 0.1, 0 ppm (w/w) fipronil treated soil, 10 g of desired percentages of fipronil aqueous solution was mixed with 50 g of sandy loam in a glass petri dish, and the treated soil was dried for 1 week at the room temperature.

Entomopathogenic fungus

An isolate of entomopathogenic fungus *Metarhizium anisopliae* KM25 was used in this study. Its virulence to the termite *C. formosanus* was reported as follows; the mortality of 5 termites in a petri dish at 1 day, 2 day, 3 day, and 4 day after application was 0, 20, 50, and 60 %, respectively

(Yanagawa and Shimizu 2005). The fungus has been maintained on potato dextrose agar (Potato extract, 0.4 %; Glucose, 2.0%; Ager, 1.5%) in a petri dish at 25°C.

Preparation of conidial suspension

The conidia of *M. anisopliae* KM25 were harvested from 14-days old cultures. The conidial suspensions of 0, 1.01×10^4 , and 1.01×10^6 conidia/ml of the fungus were prepared in a 0.025% aqueous solution of Tween 20 by counting with a Thoma hemocytometer.

Termite grooming frequencies

5.0, 1.0, 0.1, 0 (control) ppm of fipronil solutions were applied in this experiment. The termite exposure time to the treated soil was set as 5.0 ppm for 1 h, 1.0 ppm for 1 h, 0.1 ppm for 1 h and 24 h, and 0 ppm for 1 h and 24 h as controls. Twenty workers were exposed to 5 g treated soil with fipronil of each concentration in a petri dish (I.D. 6 cm). Water content was adjusted as 20% (wt: wt soil). Then, exposed termites were transferred into another petri dish (I.D. 9 cm), which contained a wet filter paper disc and maintained for four weeks in a dark chamber at 28°C and 85 % R.H. The grooming activity of the termites was recorded by a video camera for one hour at one day, one week, and two weeks after the exposure. Five replicates were prepared for each exposure condition. Dead termites were removed from a petri dish every day.

The grooming activity was counted at every minute for an hour, summed up and divided by the number of living termites in the petri dish in order to obtain the frequency of the grooming activity of one termite (times / a termite / hour). We counted “one grooming” when one termite groomed another termite at observed points.

The mean frequencies of termite grooming for five replicates of each exposure condition were analyzed with Turkey’s method by the parameters; time and fipronil concentrations.

Interaction between insecticide and fungus on the termite mortality

Twenty termites treated by the six exposure conditions were prepared as described above. About 5 h after exposure, the twenty termites were replaced from a petri dish to a microcentrifuge tube (1.5 ml) containing 1 ml of the conidial suspensions of each concentration (0, 1.01×10^4 and 1.01×10^6 conidia/ml). The termites were submerged in a suspension and gently stirred for five seconds. Then they were taken out and allowed to dry on a paper towel. The termites were put back into the former petri dish (I.D. 9 cm) and maintained in a dark chamber at 25°C for 2 weeks. The mortality was recorded and dead termites were removed every day. Five replicates were conducted for each combination of the exposure conditions of fipronil and the conidial concentrations of *M. anisopliae*.

Data from the five replicates were pooled before analysis. The mortality of each exposure condition in each conidial concentration was calculated. Four series of fipronil treatments (5ppm for 1h exposure, 1 ppm for 1 h, 0.1 ppm for 1 h and 0.1 ppm for 24 h) were analyzed by two-way ANOVA on the termite mortality by day. 2×3 factorial design with the fipronil exposure condition (0 ppm for x hour and y ppm for x hour) and the conidial concentrations (0, 1.01×10^4 and 1.01×10^6 /ml, 1ml) as main parameters were used. After each two-way ANOVA, the percentage of variation of each parameter (fipronil, *M. anisopliae*, interaction, error) by day was calculated by dividing the sum of squares for each parameter by the total sum of squares from ANOVA (Fig 1).

- I Fipronil (0 ppm) effected by Meta
- II Fipronil (x ppm) effected by Meta
- III Meta (0 /ml) effected by fipronil
- IV Meta (1.0li 10⁴ /ml) effected by fipronil
- V Meta (1.0li 10⁶ /ml) effected by fipronil

		B (fipronil)	
		b1(0)	b2(x)
A (Meta)	a1(0)	III	I
	a2(10 ⁴)	IV	II
	a3(10 ⁶)	V	

Fig 1 5 patterns of interaction

Results and discussion

Termite grooming frequencies

The grooming frequencies under the different concentrations of fipronil for 1 h exposure and the different exposure time to 1ppm fipronil are shown in Table 1 and Table 2. Termites exposed to the higher concentrations (5.0, 1.0 ppm for 1 h) and for long periods (24 h in 0.1 ppm) showed the lower grooming frequencies on each day than those of control and the 0.1 ppm fipronil with 1 h exposure. It is suggested that the quantity of the insecticide that termites ingested from the treated soil affected the grooming activity in a population. On each exposure condition except the control and the 0.1 ppm for 1h condition, the grooming frequencies had declined after 1 week. Termites treated with insecticides generally show the decline of the capacity of grooming (Neves and Alves. 2000). The results of this experiment showed the significant inactivation of termite grooming behavior with the passage of time when termites took more insecticides.

Table 1 The number of grooming frequency at 1 hour exposure to fipronil treated soil per termite per hour

	control	0.1 ppm	1.0 ppm	5.0 ppm
1 day	2.2±0.5 a AB	2.7±0.5 a A	1.8±0.2 ab A	0.4±0.1 b A
1 week	3.2±0.5 a AB	1.5±0.6 ab A	0.3±0.1 b B	0.0±0.0 b B
2 weeks	3.4±1.0 a B	1.5±0.5 ab A	0.1±0.1 b B	0.0±0.0 b B

Table 2 The number of grooming frequency at 0.1 ppm exposure to fipronil treated soil per termite per hour

	1h	24h
1 day	2.7±0.5 a A	1.5±0.4 a A
1 week	1.5±0.6 a A	0.4±0.1 a B
2 weeks	1.5±0.5 a A	0.2±0.0 b B

Comparisons among concentrations are labeled with letters and times with capitals.

Concentraions and times with different alphabets is significantly different, $P=0.05$ Tuckey's way

Interaction between insecticide and fungus on the termite mortality

The termite mortality (%) with four series of fipronil exposure conditions analyzed with 2×3 factorial design is shown in Fig 2. The combination treatments of fipronil and *M. anisopliae* had enhanced the termite mortality in 3 exposure conditions (5.0 ppm for 1 h, 1 ppm for 1h, 0.1 ppm for 1 h). It is said that grooming activity plays an important role to protect nestmates from entomopathogenic fungi (Yanagawa et al. 2008). Our results clearly demonstrated that the fipronil applications enhanced the termite susceptibility to the pathogen by restraining the allogrooming activity. In the exposure condition of 5 ppm \times 1 h, the parameter of fipronil was appeared as the main factor in the variability of mortality. On the other hand, in the case of 0.1 ppm \times 1 h, the error parameter was appeared as the main factor. In the exposure conditions of 0.1 ppm \times 24 h and 1 ppm \times 1 h, the similar results was obtained in variation (%), and both parameters, *M. anisopliae* and interaction, had a peak on 5 to 7 days after treatment. Then, the variation (%) of both parameters decreased, and that of fipronil increased and took over. Ramakrishnan *et al.* (1999) stated that the variation (%) of the interaction parameter on imidacloprid and *M. anisopliae* increased at 10 days after treatment in the sterile soil treatment

with fungus. We considered that the variation (%) of interactions in our experiments decreased after 7 days depending on the experimental condition; termites were exposed to *M. anisopliae* only once.

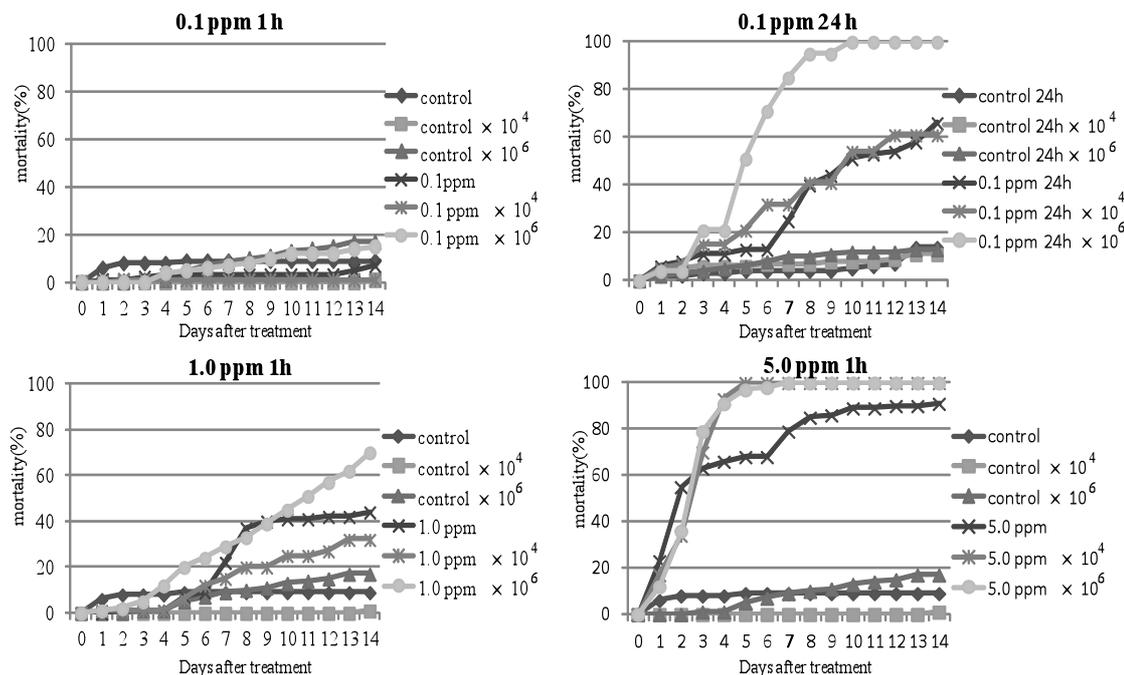


Fig 2 The termite mortality (%) in four series of fipronil exposure conditions

The termite mortality by the fipronil treated soil was significantly increased only with the 1.01×10^6 /ml conidial concentration of *M. anisopliae* from 5 days after treatment in the exposure conditions of 0.1 ppm × 24 h and 1 ppm × 1 h. Whereas, in the condition of 5 ppm fipronil for 1 h exposure, it was influenced by both 1.01×10^4 and 1.01×10^6 /ml of *M. anisopliae* from 4 days after the treatment (data not shown). These results indicated that termites exposed to the higher dose of insecticides were strongly affected on their grooming activity during an early time after the treatment, and died even by the lower dose of the fungal conidia. Therefore, we consider that termite mortality in the soil treatment could be improved by the proper combination of a quantity of insecticides termites ingested from, conidial concentrations in the soil and its virulence.

Conclusion

Termites exposed to the fipronil treated soil decreased their grooming ability. As a result, the exposed termites were more susceptible to the virulent fungus along with the degree of the lack of grooming.

References

Chouvenc, T. and N-Y. Su 2010 Apparent synergy among defense mechanisms in Subterranean termites (Rhinotermitidae) against epizootic events: limits and potential for biological control. *J. Econ. Entomol.* **103**(4), 1327-1337.

Gahlhoff, J. E. and P.G. Koehler 2001 Penetration of the Eastern Subterranean Termite into Soil Treated at Various Thicknesses and Concentrations of Dursban TC and Premise 75, Household and Structural Insects, *J. Econ. Entomol.* **94**(2), 486-491.

Nevas, P. M. O. J. and S.B. Alves 2000 Grooming capacity inhibition in *Cornitermes cumulans* (Kollar) (Isoptera:Termitidae) inoculated with entomopathogenic fungi and treated with imidacloprid. *An. Soc. Entomol. Bras.* **29**(3), <http://dx.doi.org/10.1590/S0301-80592000000300016>.

Potter, M. and A.E. Hillery 2001 Exterior-targeted liquid termiticides: an alternative approach to managing subterranean termites (Isoptera: Rhinotermitidae) in buildings. *Sociobiology* **39**(2), 373-405.

Ramakrishnan, R., D.R. Suiter, C.H. Nakatsu, R.A. Humber and G. W. Bennett 1999 Imidacloprido-enhanced *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) susceptibility to the entomopathogen *Metarhizium anisopliae* *J. Econ. Entomol.* **92**(5), 1125-1132.

Su, N-Y 2005 Responce of Formosan Subterranean Termites (Isoptera: Rhinotermitidae) to Baits or Nonrepellent termiticides in Extends Foraging Areas, Household and Structural Insects. *J. Econ. Entomol.* **98**(6), 2143-2152.

Tsunoda, K., H. Matsuoka, T. Yoshimura,, M. Tokoro 1999. Foraging populations and territories of *Reticulitermes speratus* (Isoptera: Rhinotermitidae). *J. Econ. Entomol.* **22**(5), 1113-1117.

Yanagawa, A. and S. Shimizu 2005 Defence strategy of the termite, *Coptotermes Formosanus* Shiraki to the Entomopathogenic Fungi, *J. Environ. Entomol. Zool.* **16**(1), 17-22.

Yanagawa, A., K. Iiyama, C. Aoki and S. Shimizu 2008 Innate humoral immunity of the termite, *Coptotermes formosanus* Shiraki to *Metarhizium anisopliae*. *Entomotech.* **32**, 51-54, (in Japanese).

The resistance of six Papua New Guinea woods to subterranean termite attack

YS Hadi¹⁾, MY Massijaya¹⁾, N Hadjib²⁾, M Niangu³⁾

¹⁾Bogor Agricultural University, Bogor 16680, Indonesia

²⁾Forest Products Research Institute, Bogor 16680, Indonesia

³⁾Forest Research Institute, Lae, Papua New Guinea

Abstract

Three woods from plantation namely Wau beech (*Elmerrillia papuana*), Bintangur (*Calophyllum* sp) and Balsa (*Ochroma lagopus*), and three woods from natural forest namely Taun (*Pometia pinnata*), Garcinia (*Garcinia* sp) and Canarium (*Canarium* sp) were tested to subterranean termite regarding to Indonesian standard SNI 01.7207-2006, at the end of test wood weight loss was determined and then the wood was classified in to resistant class I or very resistant to class V or very poor resistant depending on the value of wood weight loss. The result showed that wood weight loss and resistant class of Wau beech were 7.27% and II, Bintangur were 4.77% and II, Balsa were 37.2% and V, Taun were 6.40% and II, Garcinia were 6.09% and II, and Canarium were 7.99% and III respectively, and these results were similar with PNG Publication.

Keywords: PNG wood, subterranean termite, weight loss, resistant class.

Introduction

Papua New Guinea (PNG) is one of the largest island nations in the Asia-Pacific region which produce a range of forest products, including furniture, plywood and prefabricated buildings for both domestic and export markets, and in 2009 exported about 1.8 million m³ logs with average price US\$ 82 per m³ (Massijaya et al. 2011). Furthermore it was explained that most of PNG's logs export go to Asian countries mainly 89% to China, followed by Japan, Korea, India, the Philippines, Taiwan, and Vietnam. In total, forestry contributes about 7% to PNG's gross domestic product plus millions of kina in taxes, landowner royalties, infrastructure development and work for more than 10,000 people.

Most of the trees harvested are converted into sawn timber for domestic consumption. There is a number of portable sawmills operating in the region, however records of their production and operational areas are often sketchy. Over the past two decades, the number of companies operating in forest and wood-based industries has declined from an early count of more than 40 to about 25. This decline has largely been a result of diminishing timber resources that are available to sustain these operations.

Plantation forest is one choice to accomplish wood supply from natural forest in the future, and PNG Forest Authority has scheme to enlarge the forest with planting mangium and other species. The wood resistance to termite attack from plantation forest and natural forest seems to be different because of tree age and presence of extractive. In this study, we compared termite resistance of three woods from plantation and three woods from natural forests from PNG.

Materials and Methods

Materials

Three small diameter trees from plantation were cut to wood samples for subterranean termite test, the woods were Wau beech (*Elmerrillia papuana*), Bintangur (*Calophyllum* sp), and Balsa (*Ochroma lagopus*). The three woods species extracted from natural forest were also prepared for the test, namely Taun (*Pometia pinnata*), Garcinia (*Garcinia* sp), and Canarium (*Canarium* sp). The wood specimens as wood samples sized for the test was 2.5 cm by 2.5 cm by 0.5 cm in length by width by thickness, respectively. The replication of wood samples for the test was three pieces, and all wood samples were from Lae area in Papua New Guinea.

Subterranean termite test

Wood specimens were placed in a 450- to 500-ml wide-mouth round glass jar with a bottom area of 25 to 30 cm², and 200 g of moist sand (7% moisture content under water holding capacity) and 200 healthy and active worker subterranean termites (*Coptotermes curvignathus* Holmgren) were placed in each jar. The glass jars were placed in a dark room for 4 weeks. Each week the bottles were weighed, and if the moisture content of the sand was reduced by 2 percent or more, water was added to reach the moisture content standard. At the end of the test wood weight loss percentage was determined, and then the resistance class of the wood was determined according to Indonesian Standard as shown in Table 1 (SNI 2006).

Table 1.—Resistance class against subterranean termite (SNI 01.7207-2006).

Sample condition	Weight loss (%)	Resistant class
Very resistant	<3.52	I
Resistant	3.52–7.50	II
Moderate	7.50–10.96	III
Poor	10.96–18.94	IV
Very poor	>18.94	V

On the other hand Eddowes (1977) published commercial timber of PNG with classified into class 1 to class 4 as described in Table 2 for comparison.

Table 2. Resistance class of PNG wood (Eddowes 1977)

Class	Description
1. Very Durable	Suitable for long term use in structures exposed to the weather, and in contact with the ground
2. Durable	Suitable for use in the ground and for unprotected exterior use under normal conditions
3. Moderately Durable	Suitable for protected exterior work and for interior use. Not suitable for use in contact with the ground
4. Non-Durable	Not suitable for exterior use unless treated with preservative

Results and Discussions

After four weeks period of subterranean termite test in laboratory, weight loss percentage average and resistant class of each wood species are shown in Table 3, and regarding to Hadjib *et al.* (2011) its specific gravity is also shown.

Table 3. Weight loss and resistant class of each wood species.

No	Wood	WL (%)	SNI Class	SG*)	PNG Class**)
1	Wau beech	7.27	II	0.35	2
2	Bintangur	4.77	II	0.51	3
3	Balsa	37.2	V	0.12	4
4	Taun	6.40	II	0.49	3
5	Garcinia	6.09	II	0.60	3
6	Canarium	7.99	III	0.38	4

*) Hadjib *et al.* (2011); **) Eddowes (1977).

From Table 3 can be explained that wood weight loss percentage and its resistant class of each wood species as followed Wau beech were 7.27% and II, Bintangur were 4.77% and II, Balsa were 37.2% and V, Taun were 6.40% and II, Garcinia were 6.09% and II, and Canarium were 7.99% and III respectively. Balsa wood with very low density has very poor resistant or resistant class V to subterranean termite attack, as Arango *et al.* (2006) mentioned based on their analysis of six hardwood species, which indicated a significant inverse association between percentage of mass lost and specific gravity; in other words, wood with a higher specific gravity has more resistance to *Reticulitermes flavipes* termites. Seng (1990) mentioned also that Balsa wood (*Ochroma bicolor* Rowlee) from Indonesia had very poor resistant to biodeterioration or had resistant class V.

If we compare the results of resistant class by Indonesian standard, the woods are classified into class II to V, or durable to very poor durable. On the other hand, referring to PNG Publication (Eddowes 1977) these woods are classified to durability class 2 or durable to class 4 or non-durable. Both results seem similar, which is indicated wood with class II from Indonesian standard became class 2-3 by PNG Publication, and the other woods with class III-V from Indonesian standard became class 4 by PNG Publication.

Canarium wood with specific gravity of 0.38 had moderate resistant or resistant class III to subterranean termite attack, and this result was similar with Seng (1990) which did research with Indonesian canarium wood. Both wood species namely Balsa and Canarium are supposed be treated with preservation prior to be used for any purpose. The other four wood species namely Wau beech, Bintangur, Taun, and Garcinia had resistant to subterranean termite attack or resistant class II, these species can be used without treated prior to use for any purpose except if it is placed in the very severe attack of termites.

Conclusion

The result showed that wood weight loss and resistant class of Wau beech was 7.27% and II, Bintangur was 4.77% and II, Balsa was 37.2% and V, Taun was 6.40% and II, Garcinia was

6.09% and II, and Canarium was 7.99% and III respectively, and these results were similar with PNG Publication. Balsa and Canarium are supposed be treated with preservation prior to use for any purpose, but the other four wood species can be used without any treatment if they are used in the ordinary area.

Acknowledgement

The authors would like highly appreciate (1) International Tropical Timber Organization (ITTO in Japan) and Common Fund for Commodity (CFC in Netherland) which gave sponsorship for conducting the research, (2) Bogor Agricultural University and Forest Products Research Institute in Indonesia for supporting the research.

References

- Arango, R.A., F. Green, K. Hintz, P.K. Lebow and R.B. Miller 2006 Natural durability of tropical and native woods against termite damage by *Reticulitermes flavipes* (Kollar). *Int. Biodeterior. Biodegrad.* 57,146–150.
- Eddowes, P.J. 1977 Commercial timbers of Papua-New Guinea. Forest Industries Council of Papua New Guinea. Port Moresby.
- Hadjib, N., M.Y. Massijaya, Y.S. Hadi, M.A. Massijaya and M.M. Niangu 2011 Identify suitable Papua New Guinea wood species and evaluate mechanical properties. Project Report of ITTO-CFC on Utilization of small diameter logs from sustainable sources for biocomposite products.
- Massijaya, M.Y., Y.S. Hadi, M.A. Massijaya and F. Agaru 2011 The market for Papua New Guinea wood and bio-composite products. Project Report of ITTO-CFC on Utilization of small diameter logs from sustainable sources for biocomposite products.
- Seng, O.D. 1990 Specific gravity of Indonesian woods and its significance for practical use. Communication No. 13. Forest Products Research and Development Centre, Ministry of Forestry, Bogor, Indonesia.
- SNI (Standar Nasional Indonesia, *Indonesian National Standard*) SNI 01.7207-2006. 2006. Wood and wood products resistance test to wood destroying organisms. National Standardization Bureau.

Resistance of three small diameter logs to subterranean termite attack

N Hadjib¹), MY Massijaya²), YS Hadi²), D Hermawan²)

¹)Forest Products Research Institute, Bogor 16680, Indonesia

²)Bogor Agricultural University, Bogor 16680, Indonesia

Abstract

Three woods species from plantation namely cempaka hutan (*Elmerillia ovalis* (Miq.) Dandy), rubberwood (*Hevea brasiliensis* Muell. Arg) and manglid (*Manglietia glauca* Blume.) were tested to subterranean termite regarding to Indonesian standard SNI 01.7207-2006, at the end of the test wood weight loss and feeding rate were determined and then the woods were classified in to resistant class I or very resistant until class V or very poor resistant depending on the value of wood weight loss. The result showed that wood weight loss, resistant class and feeding rate of cempaka hutan were 19.4%, V and 126 μ /day/termite, rubberwood were 19.5%, V and 129 μ /day/termite, and manglid were 2.1%, I and 14 μ /day/termite respectively. Cempaka hutan and rubberwood need preservation treatment prior to be used, and the three wood species has good prospectous utilization.

Keywords: Small diameter logs, subterranean termite, weight loss, feeding rate, resistant class.

Introduction

Logs supply in 2008 reach 32 million m³ and 77% was from plantation or man made forest (Ministry of Forestry 2009). Most of them had small diameter and young wood which had a lot of juvenile wood and the wood had inferior in physical-mechanical properties and lower durability as well. The plantation forests are spread out in the whole country to support wood industries of plywood, sawmill, particleboard, medium density fiberboard, and other wood processing.

The plantation forests are managed by government in the production forest and called industrial plantation forest, some of them are managed by government enterprise and called Perhutani or Inhutani, but in small areas people also are planting forest as community forest or private forest. Most of them are planting fast growing species, e.g. cempaka hutan (*Elmerillia ovalis* (Miq.) Dandy), rubberwood (*Hevea brasileinsis* Muell. Arg), manglid (*Manglietia glauca* Blume.) sengon (*Paraserianthes falcataria*), mangium (*Acacia mangium*) and other species. Suprapti and Krisdianto (2006) mentioned that wood from community forest is mostly small diameter log which is resulting juvenile wood, a lot of knots, low specific gravity, and low durability.

The durability of plantation wood is affected by wood species, tree site, age, silviculture system, part of the tree, and other factors. The purpose of study was to determine resistance of three woods namely cempaka hutan, rubberwood, and manglid, and these woods were from small diameter logs or less than 30 cm diameter.

Materials and Methods

Materials

Three small diameter logs were cut to wood samples for subterranean termite test, the woods were cempaka hutan, rubberwood, and manglid. The wood specimens as wood samples sized for the test was 2.5 cm by 2.5 cm by 0.5 cm in length by width by thickness, respectively. The replication of wood samples for the test was three pieces, and all wood samples were from around Bogor Indonesia.

Subterranean termite test

Wood specimens were placed in a 450- to 500-ml wide-mouth round glass jar with a bottom area of 25 to 30 cm², and 200 g of moist sand (7% moisture content under water holding capacity) and 200 healthy and active worker subterranean termites (*Coptotermes curvignathus* Holmgren) were placed in each jar. The glass jars were placed in a dark room for 4 weeks. Each week the bottles were weighed, and if the moisture content of the sand was reduced by 2 percent or more, water was added to reach the moisture content standard. At the end of the test wood weight loss percentage was determined, and then the resistance class of the wood was determined according to Indonesian Standard as shown in Table 1 (SNI 2006). Whereas Feeding rate was determined by mass loss per day per termite.

Table 1.—Resistance class against subterranean termite (SNI 01.7207-2006).

Sample condition	Weight loss (%)	Resistant class
Very resistant	<3.52	I
Resistant	3.52–7.50	II
Moderate	7.50–10.96	III
Poor	10.96–18.94	IV
Very poor	>18.94	V

Results and Discussions

After four weeks period of subterranean termite test in laboratory, weight loss percentage average and resistant class of each wood species are shown in Table 2.

Table 2. Weight loss and resistant class of each wood species.

No	Wood	WL (%)	SNI Class	Feeding Rate (μ/d/termite)
1	Cempaka hutan	19.4	V	126
2	Rubberwood	19.5	V	129
3	Manglid	2.1	I	14



Figure 1. Rubberwood attacked by subterranean termite

From Table 2 can be explained that wood weight loss percentage of cempaka and rubberwood were similar and both of them belonged to resistant class V or very poor resistant, but manglid wood had weight loss much lower than the other species, i.e. 2.1% compared to 19%, and the wood belonged to class I or very resistant. Furthermore, feeding rate of cempaka and rubberwood were similar 126 and 129 μ /day/termite, and these value were much higher compared to manglid wood which had feeding rate of 14 μ /day/termite. Both weight loss percentage and feeding rate had similarity values, namely high weight loss percentage had high feeding rate.

Specific gravity, alcohol benzen solubility and strength class of those species are shown at Table 3. Alcohol-benzen solubility indicated extractive content in the wood which is consisting of carbohydrat, tannin, gum and pigment, and if the content is high especially carbohydrates will be resulting more food for bio-deterioration agents including termite and fungi (Anonymous 2001). Alcohol benzen solubility in cempaka hutan wood was higher than the other, and also the wood had lower specific gravity compared the other, as Arango *et al.* (2006) stated based on their analysis of six hardwood species, which indicated a significant inverse association between percentage of mass lost and specific gravity; in other words, wood with a higher specific gravity has more resistance to *Reticulitermes flavipes* Kollar termites. On the other aspect, cempaka hutan wood has specific texture and can be made for fancy veneer.

Table 3. Specific gravity, alcohol benzene solubility and strength class of each wood species.

No	Wood species	Specific gravity	Alc-benzen solubility, %	Strength class (I-V)
1	Cempaka hutan	0.34	11.86*	IV
2	Rubberwood	0.58	4.58**	II-III
3	Manglid	0.44	4.21***	IV

Note : * :Anonymous (2004); ** : Anonymous (2001); *** : Anonymous (2010)

Rubberwood had high density but the containing of attractively extractive for termite, blue stain and also *Schyzophyllum spp* fungi, even the wood has good strength but susceptible attacked by bio-deterioration agents. Rubberwood and cempaka hutan wood need recommended

preservation treatment prior to be used, e.g. propylactic treatment to prevent blue stain attack. Manglid wood has medium specific gravity and high durability, and also it is easy to be manufactured, has special smell, redish color, and it is suitable for building materials, furniture, mebeuler, cabinet, wood panel, door-window frame, fancy veneer and other purposes (Anonymous 2010). The three wood species has very prospectous to be utilized for building materials, furniture, and also for plywood manufacturing especially for fancy veneer.

Conclusions

The result showed that wood weight loss, resistant class and feeding rate of cempaka were 19.4%, V and 126 μ /day/termite, rubberwood were 19.5%, V and 129 μ /day/termite, and manglid were 2.1%, I and 14 μ /day/termite respectively. Cempaka hutan and rubberwood need preservation treatment prior to be used, and the three wood species has good prospectous utilization.

Acknowledgement

The authors would like highly appreciate (1) International Tropical Timber Organization (ITTO in Japan) and Common Fund for Commodity (CFC in Netherland) which gave sponsorship for conducting the research, (2) Bogor Agricultural University and Forest Products Research Institute in Indonesia for supporting the research.

References

- Anonymous, 2001. Kajian pemanfaatan kayu karet sebagai bahan baku alternatif industri pengolahan kayu (*Study of rubberwood utilization as alternative raw material for wood processing industry*). Collaborative report Ministry of Forestry and Ministry of Industry and Trade.
- Anonymous, 2004. Indonesian Wood Atlas. Vol III. Forest Research and Development Agency, Ministry of Forestry, Indonesia.
- Anonymous. 2010. Laporan Teknis Hasil Penelitian (*Technical report of research results*). Forest Products Research Institute. Not published.
- Arango, R. A., F. Green, K. Hintz, P. K. Lebow, and R. B. Miller. 2006. Natural durability of tropical and native woods against termite damage by *Reticulitermes flavipes* (Kollar). *Int. Biodeterior. Biodegrad.* 57: 146–150.
- Ministry of Forestry (MOF). 2009. Forestry statistics of Indonesia 2008.
- SNI (Standar Nasional Indonesia, *Indonesian National Standard*) SNI 01.7207-2006. 2006. Wood and wood products resistance test to wood destroying organisms. National Standardization Bureau. Jakarta. Indonesia.
- Suprpti S and Krisdianto. 2006. The resistance of four plantation wood-species against several wood destroying fungi. *Journal of Forest Products Research.* Vol. 24 (4): (267-274)

Application of liquid smoke to control termite infestation in oil palm plantation on peat land

Yuliati Indrayani

Faculty of Forestry, Tanjungpura University Jl. Imam Bonjol, Pontianak 78124, Indonesia.

Abstract

Oil palm is one of the most important commodity in Indonesia which was contributed in the field of economic growth either occupation broadening. During its growth, oil palm is susceptible to pest infestation. Termite is one of the destructive insects to oil palm plantation on peat land. So far, the methods to combat termite infestation mainly use chemical that was hazardous to human being and environmental. The research target of this study is to develop bio-termiticide of liquid smoke to eliminate termite infestation in oil palm plantation on peat land. Utilization of liquid smoke as bio-termiticide to termite control is one of the interesting technology to develop. By using of liquid smoke as the material to control termite activity, it is expected no pollution because of chemicals. The purpose of this research are: (1) to produce liquid smoke product from empty fruit bunch with condensation process at temperature of 450°C, (2) to evaluate the activity of liquid smoke to termite on oil palm plantation on peat land, (3) to evaluate the application method of liquid smoke in the field i.e. spraying method and baiting method. Result of application liquid smoke in the field revealed that spraying more appropriate method than baiting. Spraying method resulting protection degree of 40-70 which is mean that re-infestation of termite in oil palm plantation could be occur in the next 2-3 month. While, baiting method have 0 protection degree since re-infestation of termites occurring after one month application.

Key words: liquid smoke, bio-termiticide, oil palm, peat land

Introduction

Oil palm might play an important role in economic in Indonesia and constitute one of the most priority commodity in term of increasing devise (US\$ 8.87 billion in 2007 and increasing to US\$ 12.38 billion in 2008). At present, Indonesia is categorized as the biggest producer country of crude palm oil (CPO) in the world after replace Malaysia in 2006 (elibrary.mb.ipb.ac.id). Besides, plantation and industry of oil palm constitute significant contribution in term of occupation and supplying raw material for industry and development of region.

In its growth, oil palm is susceptible to pest attack. Termite is one of the most important pest insect to oil palm plantation on peat land. Dominated termite species attacking oil palm plantation on peat land is *Coptotermes* sp. Termite infestation in plantation resulting physical destroying and interruption rotting system. As rotting system was interrupted, mineral and water supply would be obstructed and plantations were susceptible to diseases.

So far, methods to combat termite infestations mainly use a chemical that was hazardous to human and environment. Most of termiticide produced to protect building especially wooden part. Therefore, mostly termiticide having permanently residue in the soil and difficult to

decompose and causes environment pollution. Those termiticide un-suitable be used to plantation.

Biological control using biological agent such as entomopathogenic fungi, nematode, virus and bacteria to elimination subterranean termite have been done by (Khan *et.al.* 1991, Suzuki 1991, Milner 1996, John *et.al.* 1996, dan Pearce 1997). Utilizations of extractives from various wood species and plants to control termite infestation have been also investigated by (Ohmura *et.al.* 1997, Syafii, 1996). Applications of baiting technology to eliminate the termite colony have been studied also by (Indrayani *et.al.*, 2007).

Another alternative to combat termite pest could be done by using friendly material such as liquid smoke. Liquid smoke is a mix suspension made from colloid dispersion of wood smoke in the water, producing with condensate of smoke from ignition those woods (Maga 1987). The former research result shows that liquid smoke from various wood species potential as the natural preservative as anti-bacteria and anti-fungi. In Indonesia, utilization of liquid smoke from empty fruit bunch to elimination termite infestation in oil palm plantation is still few investigated.

The purpose of this study is to develop bio-termiticide liquid smoke from empty fruit bunch to combat termite infestation in oil palm plantation on peat land. Utilization of liquid smoke as natural termiticide to eliminate termite infestation is a properly and interesting technology to develop. By using of liquid smoke as the material to control termite activity, it is expected no pollution because of chemicals.

Materials and Methods

Producing of liquid smoke

Liquid smoke was produce by putting empty fruit bunch into reactor than condenser equipment was setting. Electrode was turn on with temperature of 450°C. Condensation process was done for 90 minutes. Smokes which come out from reactor than transfer to cold tube via the pipe and into that tube we adding cold water by pump. Dew in liquid smoke type was placed in the bottle; whereas smokes that un-condensate was throw away via the pipe of smoke residue.

Data collection of oil palm tree infested by termite

In oil palm plantation we collect the data of tree infested by termite. The evidence of the existence of subterranean termite infestation in oil palm plantation was proofing by the presence of active termite tube. Infestation of termite could be occurred from the soil around the tree, trunk, until the point of the tree. The infested trees were noted as sample. The arrangement of location of treatment consist of four trees every section and between locations separated by two or more row of tree.

Application of liquid smoke at infested oil palm tree

Liquid smoke was prepared in two concentrations of 25% and 30%. Application of liquid smoke in the field was done by spraying technique. Amount of 5 liter liquid smoke was put inside sprayer and directly syringed to all part of the infested tree. Treatment was replicate for four

trees. Observation was done once monthly for four months to evaluate the activity of liquid smoke to termite.

Data Collection

Data collected in this research was criteria such as protection level of liquid smoke to termite. Protection level was determined by re-infestation of termite after application. Observation of protection level was determined by scoring system as describe in Table 1.

Activity of liquid smoke was determining based on period of re-infestation. Investigated was successful if the termites do not attack within minimal two months (protection level up to 70).

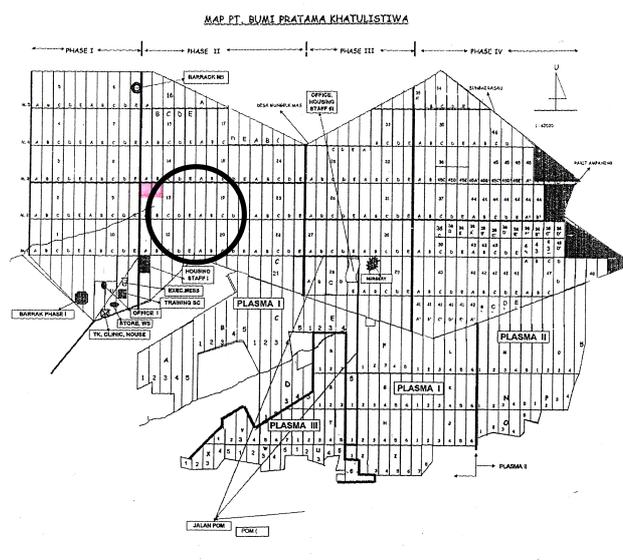
Table 1. Classification of protection level

Infestation condition	Protection level
0 – 1 month infested	0
> 1 – 2 month infested	40
> 2 – 3 month infested	70
> 3 – 4 month infested	90
> 4 month infested	100

Results and Discussions

Data collection of oil palm tree infested by termite

Eight oil palm trees was use as the sample, which were four trees sprayed with liquid smoke with concentration of 25% and the rest sprayed with concentration of 30%. Application of liquid smoke to infested tree was amount 5 liter for each. The sample was the infested tree and had been chosen randomly. The sample was located in Phase II, Block 13 A and Block 13 B as describe in Figure 1. Block 13 A for samples with concentration liquid smoke of 25%, while Block 13 B for samples with concentration liquid smoke of 30%.



**Figure 1. Location of sample in PT. Bumi Pratama Khatulistiwa / BPK (in the circle)
 Application of liquid smoke at infested oil palm tree**

Recapitulation of observation of application liquid smoke at infested oil palm is shown in Table 2. As shown in Table 2, application of liquid smoke with concentration of 30% resulting high activity of liquid smoke to termite which was indicated that termite infestation did not occur after one month application in a half of tree sample. Application of liquid smoke in this research could restrain termite infestation until four months. But this results not maximum since climate factor such as rain could not avoid during this research.

Table 2. Observation of liquid smoke at oil palm

Sample	Re-infestation of termite at month				
	0-1	1-2	2-3	3-4	>4
Concentration 25%					
123/1	O	O	O	O	O
104/2	O	X	X	X	X
96/1	O	O	X	X	X
81/2	X	X	X	X	X
Concentration 30%					
113/8	O	X	X	X	X
112/8	X	X	X	X	X
105/13	O	O	O	O	O
90/2	X	X	X	X	X

Note:

X: Do not attack

O: Attack

If the results compare with protection level, application of liquid smoke at two concentration of 25% and 30% have protection level of 90 that was mean that oil palm tree spare from termite infestation until four months. Former research by using liquid smoke with concentration of 15% amount 2 liter for each tree resulting protection level of 40 – 70 (Indrayani, 2011: unpublished data).

Climate factor such as rain influence the study in the field. In case of application of liquid smoke in this study, rainy could affect leach of liquid smoke applied to the tree. This could be activity of liquid smoke to termite at oil palm tree does not maximal. Therefore, we suggest that application of liquid smoke much better be done in dry season.

Conclusions

In general, similar results are shown for both concentration of 25% and 30% in term that application of liquid smoke in this research could restrain termite infestation until four months. Climate factor such as rain might influence the study in the field, since rainy could affect leachability of liquid smoke applied to the tree. It is suggest the application of liquid smoke much better is done in dry season. These results suggest the possibility of liquid smoke as an alternative natural preservative to combat termite infestation.

References

- Indrayani, Y., T. Yoshimura, and Y. Imamura. 2007. A novel control strategy for dry-wood termite *Incisitermes minor* infestation using a bait system. *Journal of Wood Science* 20 (3): 147-155.
- Jones, W.E, Grace, J.K and Tamashiro, M. 1996. Virulence of seven isolates of *beauveria* and *Metharhizium anisopliae* to *Coptotermes formosanus* (Isoptera : Rhinotermitidae). *Biological Control* 25 (2): 481-487.
- Khan K. Jyaraj and Goipalan, M. 1991. Mycopathogenes for biological control of *Odontotermes brunneus* (Hagen). *J. Biol. Control* 5(1), 32-35.
- Milner, R.J. Staples, J.A. and Lenz, M. 1996. Option for termite management using the insect pathogenic fungus *Metarhizium anisopliae*. The Int. Res. Group on Wood Preservation, Rosenheim, 7-11 June 1999, IRG/WP/10324.
- Ohmura, W., Seiji Ohara, and Atsushi Kato. 1997. Synthesis of Triterpenoid Saponins and Their Antitermitic Activities. *The Japan Wood Research Society*, vol. 43, No.10: 869-874.
- Pearce MJ. 1997. *Termite: biology and management*. New York: CAB International Publisher.
- Syafii, W. 1996. Extractives and its influence to wood durability. *Forest Product Technology Journal*, Vol. 9 No.2. Faculty of Forestry. IPB. Bogor.

Session 2

Physiology and Biochemistry

Isolation and identification cellulolytic bacteria from the termite *Coptotermes curvignathus* Holmgren and *Macrotermes gilvus* Hagen from secondary forest in West Kalimantan Indonesia

Farah Diba^{1*}, Siti Khotimah² and Utin Febriyana²

¹Forestry Faculty, Tanjungpura University, Indonesia

²Life Science and Mathematics Faculty, Tanjungpura University, Indonesia

*Corresponding author: farahdiba1611@gmail.com

Abstract

It is known that intestinal microorganism play important roles in wood degradation of diet components of the termites. The major gut bacteria of the worker caste of lower and higher of Indonesian termites were isolated and identified. The termites used were *Coptotermes curvignathus* Holmgren and *Macrotermes gilvus* Hagen. All species were facultative anaerobes or strict aerobes. A correlation appears to exist between the major gut bacterium and the family to which the termite belongs. The major bacterium from the lower termites *Coptotermes curvignathus* Holmgren (family Rhinotermitidae) was *Enterobacter* and *Flavobacterium* and from higher termites *Macrotermes gilvus* Hagen (family Termitidae) was *Sporocytophaga* and *Staphylococcus*.

Key words: intestinal microorganisms, gut bacteria, *Coptotermes curvignathus*, *Macrotermes gilvus*

Introduction

Termites are one of the few arthropods that exclusively thrive on dead plant matter and efficiently decompose lignocelluloses. A dense and diverse microbial community in the gut of termites is essential for the efficient decomposition, and thus is expected to be an attractive model for efficient utilization of biomass resources. Termites are important decomposers specializing in the degradation of recalcitrant components of plant residues through their association with symbiotic gut microorganisms. Termites are roughly divided into six families of lower termites and a single family of higher termites (Shinzato *et al* 2005). The bacterial community structures in higher and lower termite guts have been investigated (Sugimoto *et al* 1998; Lefebvre *et al* 2009; Lilburn 2001; Yamada 2007). However, such detailed investigations have been performed for only limited number of species, and more information from other termite species is needed to understand better the mechanism and evolution of digestive symbiosis in the termite gut.

The lower wood-feeding termites *Coptotermes curvignathus* Holmgren (family Rhinotermitidae) and higher fungus growing termites *Macrotermes gilvus* Hagen (family Termitidae) are one of the more notorious insect pest and an economically important termites species across the world, because of its large colony size, capacity to penetrate a variety of materials and ability to consume a wide range of wood types. Clarifying the microbial community structure in the gut of *Coptotermes curvignathus* Holmgren and *Macrotermes gilvus* Hagen would be useful not only

from the point of the view of microbial ecology but also from that of various possible applications. The development of termiticides that take effect by disturbing intestinal microorganisms is one of the applications to be considered. Although various insecticides effective in getting rid of termites have been developed and applied, the majority of them include organophosphorus compounds, which are harmful not only to termites but also to other organisms including human. In view of safety, chemicals able to beat the target microbes specifically is valuable to be develop and cataloguing the intestinal microbes in termites and a comparative analysis among them might make it possible to define the microbial lineages indispensable for their wood-feeding life style. Such microbes are possible target for termite control. In the present study we characterized the bacterial in gut of lower and higher termites, i.e. *Macrotermes gilvus* Hagen and *Coptotermes curvignathus* Holmgren from secondary forest that is the most termite species in West Kalimantan Province Indonesia.

Materials and methods

Termites were collected from the secondary forest in West Kalimantan Indonesia. The termites were collected from the tree *Acacia mangium* Willd, *Gmelina arborea* and *Hevea brasiliensis* Muell. All collected termites were transferred to the termites rearing room. The termites kept in a dark room at room temperature with 60% humidity. New slightly wet filter papers were supplied daily for one week. The isolation and identification of bacteria was conducted in the microbiology laboratory in Faculty of Life Sciences and Mathematics Tanjungpura University Pontianak West Kalimantan Indonesia.

Ten worker termites were surface sterilized with 70% ethanol and then washed in sterile distilled water. Under sterile conditions, the entire guts were removed from the abdomen by using a sterilized fine forceps and mixed with 10 mL NaCl 0.85%. Then 0.5 mL of the suspension was mixed with 4.5 mL medium 1 which contained 5 g L⁻¹ *Carboxymethylcellulose* (CMC) and 0.2 g L⁻¹ yeast extracts. The mixture was incubated at 30°C for 48 hour. Then the culture was spread on nutrient agar and pure colonies were obtained by several subsequent culturing and plating.

Identification of bacteria uses the key books of determination of Bergey's Manual of Determinative Bacteriology (John *et al* 1999). After several sub culturing, pure cultures of bacteria were obtained and the identification with macroscopic, microscopic and biochemistry characterization were performed. Macroscopic observations include colony morphology such as shape, surface, the edge and colour of bacterial colonies that grow on CMC-agar medium in petri dishes. Microscopic observations made by seeing cell morphology and colour with gram stain under a microscope with a magnification of 100 times. The biochemistry characterization consists of motility test; catalase enzyme test; oxidize test; citrate acid test; urease test; fermentation test: glucose, sucrose, and lactose; carbohydrate fermentation test; indole test and OF test (anaerobic test). The medium 1 for OF test contained 2.0 g L⁻¹ peptone, 5 g L⁻¹ NaCl, 0.3 g L⁻¹ KH₂PO₄, 3 g Agar and 3 mL 1% Bromthymol blue. All materials were dissolved together and pH was adjusted to 7.1. Five mL of medium 1 was added to each 13 cm diameter test tube and sterilized at 121°C for 20 min. After autoclaving, 0.5 mL filter sterilized glucose was added to each test tube. Two test tubes were inoculated with each bacterial isolate and one test tube was covered with a layer of sterile melted vaseline. The tubes were incubated at 30°C for 18-24 h. The Biolog reader was used for identification of bacteria by using the right kit base on the

Gram stain result. The pure cultures of bacteria were inoculated into the biolog broth and the turbidity of the inoculums was adjusted according to the Biolog protocol. One hundred and forty five microliter of the inoculums was pipette into each well of the 96 well micro plates and incubated for 4-6 or 16-24 h depending on the growth of bacteria and the ability of Biolog reader to analyze the results.

Results and Discussion

The major bacterium from the lower termites *Coptotermes curvignathus* Holmgren (family Rhinotermitidae) was *Enterobacter* and *Flavobacterium* and from higher termites *Macrotermes gilvus* Hagen (family Termitidae) was *Sporocystophaga* and *Staphylococcus*. The density of gut bacteria colony in lower termites *Coptotermes curvignathus* Holmgren was 50.2×10^8 CFU/ml and in higher termites *Macrotermes gilvus* Hagen was 54.1×10^8 CFU/ml. Cellulolytic bacteria has an important role in biosfer to recycle the cellulose. This bacteria is important to some fermentation process in industry (Kamara, *et al.* 2007). Termites are among the most important lignocellulose-digesting insects and possess a variety of symbiotic microorganisms in their hindgut, including bacteria, archaea and eukary (Konig 2006). There are many kinds of bacteria with different functions in termite gut that have been isolated and identified. They were hemicelluloses-degrading bacteria (Schafer *et al* 1996), lignolytic bacteria (Borji *et al* 2003), cellulolytic bacteria (Wenzel *et al* 2002), aromatic degrading bacteria (Harazono *et al* 2003), and nitrogen-fixing bacteria (Frohlich *et al* 2007). The first investigations for isolation of cellulolytic bacteria facultative anaerobes come from Adams and Boopathy (2005), who in their study identified some facultative anaerobes as *Enterobacter* families from the termite *Coptotermes formosanus* Shiraki. The bacterial characteristic from lower termites *Coptotermes curvignathus* Holmgren (family Rhinotermitidae) and from higher termites *Macrotermes gilvus* Hagen (family Termitidae) was shown in Table 1.

Table 1. Characteristic of gut bacteria from lower termites *Coptotermes curvignathus* Holmgren and higher termites *Macrotermes gilvus* Hagen

Test	Isolate 1	Isolate 2	Isolate 3	Isolate 4
Gram stain (24 h)	-	+	-	-
Cell Morphology	Basil	Coccus	Basil	Coccus
Motility	+	-	-	+
Catalase	+	+	+	+
Oxidase (24 h)	+	-	+	+
Glucose	+	+	+	+
Sucrose	-	-	-	+
Lactose	-	-	-	+
Manitol	-	+	+	-
Sucrose	+	+	+	+
Maltose	-	-	-	-
Indole	-	-	-	-
OF	-	-	-	-
Oxygen Need	Aerobes	Anaerobes Facultative	Aerobes	Anaerobes Facultative
Citrate Acid	+	+	+	+
Urease	+	-	-	+

Remarks : + : Positive reaction ; - : Negative reaction (John *et al*, 1994)¹⁰

Isolate 1 and isolate 2 gut bacteria from *Macrotermes gilvus* Hagen

Isolate 3 and isolate 4 gut bacteria from *Coptotermes curvignathus* Holmgren

The bacterial cells from isolate 1 were gram-negative, with basil cell morphology, aerobes and motile. Based on the Bergey's manual and biolog reader, isolate 1 from gut *Macrotermes gilvus* Hagen was identified as *Sporocytophaga*. This kind of bacteria has not been identified elsewhere from the termite *Macrotermes gilvus* Hagen. *Sporocytophaga* was a common cellulolytic soil bacterium which can degrade cellulose (Lembeck and Colmer 1967). *Sporocytophaga* was cellulose-decomposing micro-organisms which have the ability to hydrolyse cellulose to the constituent sugars, either cellobiose or glucose in the following two steps: (1) hydrolysis to glucose, succeeded by (2) oxidation or fermentation of this sugar to the final metabolic products (Sijpesteijn and Fahraeus 1968). This bacteria can degrade cellulose, cellobiose, glucose, mannose and as a source of carbon and energy. The morphology of colony of *Sporocytophaga* has soft filament in white until yellow colour. Chemoorganotrophic and used oxygen on transfer electron so this bacteria was aerobes. This optimum growth for *Sporocytophaga* bacteria was on pH 6.5-7.5 and temperature 30⁰C.

The bacterial cells from isolate 2 were gram-positive, with Coccus cell morphology, anaerobes facultative and non-motile. Based on the Bergey's manual and biolog reader, isolate 2 from gut *Macrotermes gilvus* Hagen was identified as *Staphylococcus*. *Staphylococcus* is the most abundant bacteria in higher termite gut (Breznak 1982; Konig 2006). *Staphylococcus* also found in higher termite *Nasutitermes* (Borji *et al* 2003). The optimum growth for these bacteria was on pH 6-8 and temperature 30-37⁰C. The bacterial cells from isolate 3 were gram-negative, with basil cell morphology, aerobes and non-motile. Based on the Bergey's manual and biolog reader, isolate 3 from gut *Coptotermes curvignathus* Holmgren was identified as *Flavobacterium*. Some bacteria in lower termites were gram-negative and *Flavobacterium* was abundant bacteria in lower termite gut (Dolan 2001). A correlation appears to exist between the major gut bacterium and the family to which the termite belongs. Eutick *et al* (1978) said that the *Flavobacterium* also found from the lower termites *Mastotermes darwiniensis* (family Mastotermitidae).

The bacterial cells from isolate 4 were gram-negative, with Coccus cell morphology, anaerobes facultative and motile. Based on the Bergey's manual and biolog reader, isolate 4 from gut *Coptotermes curvignathus* was identified as *Enterobacter*. These bacteria also found in gut of termites of four families Rhinotermitidae, i.e. *Heterotermes ferox*, *Coptotermes acinaciformis*, *Schedorhinotermes intermedius* and *Coptotermes lacteus*. Bacteria *Enterobacter agglomerans* from termites *Coptotermes formosanus* carried out a mixed acid type of glucose fermentation and mediated nitrogen fixation (Potrikus and Breznak 1977). *Enterobacter* is able to assimilate different phenolic compound (Deschamps *et al* 1980) and has lignocellulolytic activity (Borji *et al* 2003). Another researcher found *Enterobacter cloacae* and *Enterobacter aerogene* from *Coptotermes formosanus*. The ability to degrade hemicelluloses has also been demonstrated by *Enterobacter* which was isolated from the termite *Mastotermes darwiniensis* Froggatt and *Zootermopsis angusticollis* Hagen (Adams and Boopathy 2005). *Enterobacter cloacae* and *Enterobacter aerogene* also found from termite *Mastotermes darwiniensis* and *Nasutitermes nigriceps* and these bacteria were able to degrade lignin monomers (Kuhnigk *et al* 1994).

The ability of termite bacteria to digest lignocellulose compounds has been published. Acinetobacters, Bacillus cereus and Enterobacters have been reported to be able to degrade 34-62% cellulose and 14-32% hemicellulose and in addition Enterobacters are also able to degrade 18-39% lignin (Konig 2006), Acinetobacters are classified under the aerobic group with the capability to degrade caffeic acid and syringic acid (lignin monomers) and Enterobacters are grouped among bacteria with the capability of modifying lignin monomers and other aromatic compounds (Kuhnigk *et al* 1994; Varma *et al* 1994). Enterobacters are also able to assimilate different phenolic compounds considered as lignin related simple monomers (Deschamps *et al* 1980)

In this study all bacterial isolates were able to grown on CMC and cellobiose media indicating their cellulolytic capability. Other researcher found some cellulolytic bacteria from the gut of termite *Zootermopsis angusticollis* also able to grown on CMC medium (Wenzel *et al* 2002). Enterobacter bacteria have been investigated and the capability of these bacteria is clear to perform a series of anaerobic reactions such as O-demethylation and decarboxylation (Kuhnigk 1994). The main difference between higher and lower termites is that flagellates (protozoa) are present in the gut of only lower termites, whereas no protozoa were found in higher termites gut (Varma *et al* 1994). It is also known that higher termites decompose cellulose by using their own enzymes (Ohkuma 2003). The diversity of termite gut communities is extraordinarily high and the function of each group of symbionts is poorly known.

Conclusions

The present study resulted two cellulolytic bacteria from intestinal gut of higher termites *Macrotermes gilvus* Hagen i.e *Sporocytophaga*, *Staphylococcus* and two cellulolytic bacteria from lower termites *Coptotermes curvignathus* Holmgren i.e *Flavobacterium* and *Enterobacter*. This bacteria can degradation the cellulose, *Sporocytophaga* and *Flavobacterium* was aerobes meanwhile *Staphylococcus* and *Enterobacter* was anaerobes facultatif.

Acknowledgements

The authors are grateful to Ministry of National Education Indonesia, Directorate of Research and Development Community, Directorate General of Higher Education (DP2M DIKTI) for funding this research.

References

- Adams, L and R. Boopathy 2005 Isolation and Characterization of Enteric Bacteria from the Hindgut of Formosan Termites. *J. Bioresour. Technol.* **96**, 1592-1598.
- Borji, M., S. Rahimi, G. Ghorbani, J. Van Yoosefi and H. Fazaeli 2003 Isolation and Identification of Some Bacteria From Termites Gut Capable in Degrading Straw Lignin and Polycacaeharides, *J. Facul. Vet. Met.*, University Tehran **58**, 249-256.
- Breznak, J.A 1982 Intestinal Microbiota of Termites and Other Xylophagous Insects. *J. Ann. Rev. Microbio.* **36**, 323-343.
- Deschamps, A.M., G. Mahoudeau and J.M. Lebeault 1980 Fast Degradation of Kraft Lignin by Bacteria. *Eur. J. Applied Microbiol. Biotechnol.* **9**,45-51.

Dolan, M., F 2001 Specification of Termite Gut Protist: The Role of Bacterial Symbiont, J., *Int Microbiol* **4**, 203-208.

Eutick, M. L., R. W., O'Brien, and M., Slaytor 1978 Bacteria From The Gut of Australian Termites, J., *Insect Physiol.* **22**,1377-1380.

Frohlich, J. C. Koustiane, P. Kampf, R. Roselle-Mora, M. Valens, M. Berchtold, T. Kuhnigk, H. Hertel, D.K. Maheshwari and H. Konig 2007 Occurrence of Rhizobia in the Gut of the Higher Termites *Nasutitermes nigriceps*. *System.Appl.Microbiol.* **30**,68-74.

Harazono, K., N. Yamashita, N. Shinzato, H. Watanabe, T. Fukatsu and R. Kurane 2003 Isolation and Characterization of Aromatics-degrading Microorganisms from the Gut of the Lower *Coptotermes formosanus*. *J. Biosci. Biotechnol. Biochem.* **67**, 889-892.

John, G.H., R.K. Noel, H.A.S. Peter, T.S. James and T.W. Stanly 1999 Bergey's Manual of Determinative Bacteriology. USA. Williams and Wilkins.

Kamara, S. D., D.R. Saadah, G. dan Shabarni 2007 Degradasi Enzimatik Selulosa Dari Batang Pohon Pisang Untuk Produksi Glukosa Dengan Bantuan Aktifitas Selulolitik *Trichoderma viride*, *J. Mikrobiol Sains Universitas Padjadjaran Bandung*.

Konig, H. 2006 Bacillus Species in the Intestine of Termites and Other Soil Invertebrates. *J. Applied Microbiol.* **101**, 620-627.

Kuhnigk, T., E. Borst, A. Ritter, P. Kampf, A. Graf, H. Hertel and H. Konig 1994 Degradation of Lignin Monomers by the Hindgut Flora of Xylophagous Termites. *J. Syst. Applied Microbiol.* **17**, 76-85.

Lefebvre, T., E. Miambi, A. Pando, M. Diouf, C. Rouland-Lefeuvre 2009 Gut-specific Actinobacterial Community Structure and Diversity Associated with the Wood-feeding Termite Species, *Nasutitermes corniger* (Motschulsky) Described by Nested PCR-DGGE Analysis. *Insectes Sociaux* **56**,269–276.

Lembeck, W.J. and A. R. Colmer 1967 Effect of Herbicides on Cellulose Decomposition by *Sporocytophaga myxococcoides*. *Applied Microbiology* **15**(2), 300-303.

Lilburn, T.G. 2001 Nitrogen Fixation by Symbiotic and Free-living Spirochetes. *Science* **292**, 2495–2498.

Ohkuma, M. 2003 Termite Symbiotic Systems : Efficient Bio-recycling of Lignocellulose. *J. Applied Microb. Biotechnol.* **61**, 1-9.

Potrikus, C.J. and J.A. Breznak 1977 Nitrogen-Fixing *Enterobacter agglomerans* Isolated from Guts of Wood-Eating Termites. *Applied and Environmental Microbiology* **33**(2), 392-399.

Schafer, A., R. Konrad, P. Kuhnigk, P. Kampf, H. Hertel and H. Konig 1996 Hemicellulose-degrading Bacteria and Yeasts from the Termite Gut. *J. Applied Bacteriol.* **80**, 471-478.

Shinzato N, M. Muramatsu, T. Matsui, and Y. Watanabe 2005 Molecular Phylogenetic Diversity of the Bacterial Community in the Gut of Termites *Coptotermes formosanus*. *Biosci. Biotechnol. Biochem* **69** (6), 1145-1155.

Sijpesteijn, A.K. and G. Fahraeus 1968 Adaptation of *Sporocytophaga myxococcoides* to Sugars. *Journal of General Microbiology* **3** (2), 224-235

Sugimoto A, T. Inoue, I. Tayasu, L. Miller, S. Takeichi, and T. Abe 1998 Methane and Hydrogen Production in a Termites-Symbiont System. *Ecol. Res.* **13**, 241-257.

Wenzel, M., I. Schonig, M. Berchtold, P. Kampfer and H. Konig 2002 Aerobic and Facultatively Anaerobic Cellulolytic Bacteria from the Gut of Termite *Zootermopsis angusticolis*. *J. Applied Microbiol.* **92**, 32-40.

Varma, A., B. Krishna Kolli, J. Paul, S. Saxena and H. Konig 1994 Lignocellulose Degradation by Microorganisms from Termite Hills and Termite Guts: A Survey on the Present State of Art. *J. FEMS Microbiol. Rev.* **15**, 9-28.

Yamada, A. 2007 Evolutionary Trend of Phylogenetic Diversity of Nitrogen Fixation Genes in the Gut Community of Wood-feeding Termites. *Mol. Ecol.* **16**, 3768–3777.

Characterization of cellulase activities in wood-feeding termites of different evolutionary levels

Zhi-qiang Li, Wen-hui Zeng, Qiu-jian Li, Bing-rong Liu, Jun-hong Zhong

Guangdong entomological institute, Guangzhou 510260, China

Abstract

For three wood-feeding termites from phylogenetically different lineages, *Cryptotermes domesticus*, *Coptotermes formosanus*, and *Ahmaditermes sinuosus*, the cellulase activities and distributions of workers (or pseudoergate) were studied. The results showed that the cellulase activity of whole filter paper of the higher termite *A. sinuosus* was markedly higher than that of the both lower termites, and *Co. formosanus* had the highest whole activity of endo- β -1,4-glucanase. For the whole activities of β -glucosidase, there was no significant difference among the three species of termites. In terms of distributions of cellulolytic activities in the gut, the primary site of EG activities was presented in the hindgut of both the lower termites unlike in the midgut of the higher termite, and the primary site of BG activities was restricted to the midgut in the lower termites unlike to the head/foregut in the higher termite. The functions of the gut segments became further differentiated gradually in evolutionary process, and the digesting role of the midgut became more outstanding in the higher termite. The results suggested that characters of cellulase activities could reflect the phylogeny of wood-feeding termites to a certain extent. For the endogenous cellulases, the main site of EG activities was concentrated on the midgut from the lower termite to the higher termite; on the contrary, the main site of BG activities converged on the head/foregut.

Key words: Termites, wood-feeding, cellulase activity, endo- β -1,4-glucanase, β -glucosidases

Introduction

In nature, xylophagous insects have evolved the strategies to derive the energy needs from celluloses with cellulases, in which termites are the most efficient decomposers of celluloses (Watanabe and Tokuda 2010). Termites are important decomposers in tropical ecosystems (Noble et al. 2009), and Cellulase enzymes and Cellulase genes in the digestive systems of termites may have potentials for cellulosic ethanol production by biological process (Li et al. 2009).

Termites have specialized cellulose-digesting systems (Tokuda et al. 2007; Zhou et al. 2007). Various cellulases are involved in degradation of celluloses in termites and their symbionts. The related investigation of cellulase activities in termites have been an important research field at present. The three main types of cellulases are endo- β -1,4-glucanases (EGs; EC 3.2.1.4), cellobiohydrolases (CBHs; EC 3.2.1.91) and β -glucosidases (BGs; EC 3.2.1.21). Pattern and character of cellulases in the termites and their symbionts have been described extensively in the past decades (Watanabe and Tokuda 2010; Willis et al. 2010), and cellulase activities and their distributions in the digestive systems were different among diverse termites. Cellulase activity levels in wood-feeding termites are far higher than those found in the fungus-growers and the soil-feeders (Tokuda et al. 2004; Lo et al. 2011). Recently, distributions of diverse cellulase

activities in each gut segment of termites were mostly studied (Willis et al. 2010). In addition, differences of total cellulase activities were compared among lower termites (Mo et al. 2004; Lu et al. 2010), and EG expression among different castes of *Hodotermopsis sjostedti* (family Termopsidae) and *Nasutitermes takasagoensis* (family Termitidae) has been reported (Fujita et al. 2008).

Now, there are approximately 2,600 described termites species around the world (Kambhampati and Eggleton 2000), and the comprehensive studies of phylogenetic and taxonomic relationships among termite groups have been published (Donovan et al. 2000; Legendre et al. 2008). However, the cellulase activities of only around 16 termite species were reported (Lu et al. 2010; Lo et al. 2011). To provide further information about the relationship between cellulolytic activities and evolutionary statuses in wood-feeding termites from phylogenetically different lineages, here, *Cryptotermes domesticus* (Haviland) (Isoptera: Kalotermitidae), *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), *Ahmaditermes sinuosus* (Tsai et Chen) (Isoptera: Termitidae) were used to compare the cellulase activities and their distribution, and to discuss the evolution of cellulose digestion in wood-feeding termites.

Materials and methods

Cr. domesticus were collected from two laboratory-maintained colonies. Three *Co. formosanus* colonies and two *A. sinuosus* colonies were collected directly in Guangdong Province of China. The caste of termites used for experiments were healthy adult workers, but pseudoworkers for *Cr. domesticus*. The workers or pseudoworkers were directly put into liquid nitrogen before enzyme extraction.

To prepare enzyme extracts, the workers or pseudoworkers were washed with precooling 0.09% normal saline. Fifteen sets per termite colony of head (including salivary glands) and whole guts were dissected from termites, and each set was divided into head/foregut, midgut, and hindgut. The three sections and other five bodies of worker per colony were collected respectively in tubes and homogenized by using vitreous pestle in 500 μ L of 0.1 M sodium acetate buffer (SAB) (pH 5.6) on ice. The tubes were centrifuged at 12,000 rpm for 15 min at 4 °C, and the supernatants were brought to volume of 500 μ L by adding SAB and used as the enzyme extract. The same volume of SAB was used as the control.

Filter paper degrading activity (FPA). Circular filter paper after high temperature sterilization was put into the microtube with 120 μ L SAB (pH 5.6), and the crude enzyme (12 μ L) was incubated with the filter paper at 37 °C for 60 min. Based on dinitrosalicylic acid (DNS) method (Eveleigh et al., 2009), the glucose production was detected colorimetrically at 540nm, using glucose as a standard. The protein content of the sample was determined spectrophotometrically at 660nm according to the Coomassie Brilliant Blue G-250 method (Lott et al. 1983), using bovine serum as a standard. The activities of both Endo- β -1,4-glucanase and β -glucosidase activity were determined using 120 μ L of 1% sodium carboxymethylcellulose and 120 μ L of 1% salicin as the substrates, respectively. One unit (U) of enzyme activity was defined as the amount of enzyme capable of releasing one μ mol reducing sugar per minute. Specific activity was express as units per mg protein.

The data were analyzed by one-way analysis of variance with least significant difference post-hoc tests (LSD) with SPSS 17.0 for Windows Software.

Results and discussion

Differences of cellulase activity in the wood-feeding termites

The data (Table 1) of cellulase activities of whole body in termite workers (or pseudoworkers) showed that the BG activities were not significantly different among three wood-feeding termites, but *Co. formosanus* was significantly high in EG activities. EG activity was principal component in all three wood-feeding termites.

Distribution of EG and BG activities in the gut

According to the data from the hindgut (Table 1), it was suggested that flagellate-harboring termites possessed higher percentage of intestinal microbial EG and BG than *A. sinuosus*. In addition, activities of both EG and BG were no significant difference in midgut among three wood-feeding termites, and neither were BG activities in hindgut. *Co. formosanus* had the highest activity of EG in head/foregut and hindgut, and *A. sinuosus* had the highest activity of BG in head/foregut.

For endogenous endoglucanase and β -glucosidase, EG activities were no significant difference between in the head/foregut and midgut of *Cr. domesticus*, and so were *Co. formosanus*. However, the higher termite *A. sinuosus* had the higher EG activity in midgut. By contrast, the higher BG activity in *A. sinuosus* was in the head/foregut. So, the main position of EG activity tended to the midgut with evolution from the lower form into a higher form of wood-feeding termites, but BG activity to the head/foregut.

Table 1 EG and BG activities of different segments in the termite workers.

Cellulase	Species	Whole body activity	Cellulase activity in each gut segment		
			Head/Foregut	Midgut	Hindgut
EGs	<i>Cr. domesticus</i>	0.347±0.028b	0.152±0.016b B	0.185±0.073a B	0.499±0.020b A
	<i>Co. formosanus</i>	0.780±0.047a	0.363±0.016a B	0.295±0.120a B	0.818±0.126a A
	<i>A. sinuosus</i>	0.525±0.122b	0.214±0.075b C	0.399±0.085a A	0.289±0.079b B
BGs	<i>Cr. domesticus</i>	0.116±0.007a	0.080±0.005b C	0.160±0.006a A	0.116±0.007a B
	<i>Co. formosanus</i>	0.426±0.057a	0.153±0.068b A	0.314±0.107a A	0.200±0.033a A
	<i>A. sinuosus</i>	0.747±0.470a	0.363±0.033a A	0.305±0.162a B	0.289±0.103a C

Mean±S.E. with different small letter means significant difference in the same species and the same column, while different capital letter means significant difference in the same line at the 0.05 probability level.

Filter paper assay

Filter paper degradation is directly related to digestibility of naturally occurring cellulose. According to the data of FPA (Table 2), the most evolved species *A. sinuosus* had the highest activity of FPA in the whole body of three wood-feeding termites, but there were not significantly different among FPA of three termites in each gut segment. Regarding distributions of FPA in guts of the termites, *A. sinuosus* showed significantly more different FPAs in three gut segments than the lower termites tested. Furthermore, the midgut was the main segment of FPA in *A. sinuosus*.

Table 2 FPA of different segments and whole body in the termite workers

Species	Filter paper assay			
	Whole Body	Head/Foregut	Midgut	Hindgut
<i>Cr. domesticus</i>	0.167±0.036 b	0.139±0.055aA	0.130±0.057aA	0.155±0.072aA
<i>Co. formosanus</i>	0.241±0.031ab	0.131±0.027aB	0.107±0.017aB	0.229±0.022aA
<i>A. sinuosus</i>	0.417±0.102a	0.213±0.087aC	0.326±0.157aA	0.288±0.051aB

For cellulase activity assay, the data are often difficult to compare among studies. Multinomial factors are very important to measure the cellulase activity, such as temperature, substrate, detection method and so on (Tokuda et al. 2005; Willis et al. 2010). The relative expression levels of cellulase genes do not correspond with their activity, which could be affected by different regulators (Fujita et al. 2008). In addition, for the workers of higher termites, the cellulose-digesting division of labour might indirectly influence the determination results (Fujita et al. 2008).

The present study showed that the hindgut was the primary site of cellulose digestion in lower termites, which was consistent with the report of Tokuda et al. (2005). As for endogenous termite cellulases, Tokuda et al. (2004) proposed that the expression of the endogenous cellulase genes has shifted from the salivary glands of lower termites to the midgut of higher termites. In terms of the dynamic shift in wood-feeding termites, our results showed that activities of EG and FPA were most highly concentrated on the midgut of higher termite than that of lower termites, which supported the previous studies of EG (Mo et al. 2004; Tokuda et al. 2004, 2005; Fujita et al. 2008; Tokuda et al. 2009; Lo et al. 2011), but the dynamic change of BG was contrary to the related report (Fujita et al. 2008; Tokuda et al. 2009). The higher termite *A. sinuosus* had the highest degrading activity of FP and the highest percentage value in complete cellulases. However, the previous study showed that the higher termite *Nasutitermes takasagoensis* was markedly lower using microcrystalline cellulose (MCC) as substrate than that of flagellate-harboured termites (Tokuda et al. 2005). For this, Tokuda et al. (2005) considered the cellulase activity of *N. takasagoensis* was likely to be similar to its requirement for energy metabolism. The cellulase activity of *Co. formosanus* has been intensively studied on the different substrates (Willis et al. 2010). *Co. formosanus* had the higher activity of EG and BG among wood-feeding termites (Tokuda et al. 2005; Lu et al. 2010). The present study showed that *Co. formosanus* had the highest degrading activities of EG in the three termites, and its BG activity was not significant difference with species of family Kalotermitidae which was consistent with the report of Mo et al. (2004).

Conclusions

Termites play an important role in degradation of cellulosic materials in nature. Termites have different feeding groups. Wood-feeding termites have evolved an efficient cellulose-decomposing system. As for the comparison of cellulase activities in the wood-feeding termites, the evolved wood-feeding termites such as the termites of the Rhinotermitidae and Termitidae may have higher BG activity in the head/foregut, and the cellulase activity of whole filter paper of higher termite may be the higher than that of lower termites. Moreover, it was suggested that characters of cellulase activities could be relevant to evolutionary levels of wood-feeding termites. For the endogenous cellulases, the main site of EG activities was concentrated on the midgut from the lower termite to the higher termite; on the contrary, the main site of BG activities converged on the head/foregut.

References

- Donovan, S., D. Jones, W. Sands and P. Eggleton 2000 Morphological phylogenetics of termites (Isoptera). *Biological Journal of the Linnean Society* **70**(3), 467-513.
- Eveleigh, D.E., M. Mandels, R. Andreotti and C. Roche 2009 Measurement of saccharifying cellulase. *Biotechnology for Biofuels* **2**(1), 1-8.
- Fujita, A., T. Miura and T. Matsumoto 2008 Differences in cellulose digestive systems among castes in two termite lineages. *Physiological Entomology* **33**(1), 73-82.
- Gusakov, A.V., T.N. Salanovich, A.I. Antonov, B.B. Ustinov, O.N. Okunev, R. Burlingame, M. Emalfarb, M. Baez and A.P. Sinitsyn 2007 Design of highly efficient cellulase mixtures for enzymatic hydrolysis of cellulose. *Biotechnology and bioengineering* **97**(5), 1028-1038.
- Kambharnpati, S. and P. Eggleton 2000 Taxonomy and phylogeny of termites. In: Abe T, Bignell DE, Higashi M (eds) *Termites: evolution, sociality, symbioses, ecology*, pp.1-23. Kluwer academic publishers, Dordrecht.
- Legendre, F., M.F. Whiting, C. Bordereau, E.M. Canello, T.A. Evans and P. Grandcolas 2008 The phylogeny of termites (Dictyoptera: Isoptera) based on mitochondrial and nuclear markers: implications for the evolution of the worker and pseudergate castes, and foraging behaviors. *Molecular phylogenetics and evolution* **48**(2), 615-627.
- Li, X, H. Yang, B. Roy, D. Wang, W. Yue, L. Jiang, E.Y. Park and Y. Miao 2009 The most stirring technology in future: Cellulase enzyme and biomass utilization. *African Journal of Biotechnology* **8**(11), 2418-2422.
- Lo, N., G. Tokuda and H. Watanabe 2011 Evolution and function of endogenous termite cellulases. In: D.E. Bignell et al. (eds.), *Biology of Termites: a modern synthesis*, pp.51-67. Springer Science+Business Media, London New York.
- Lott, J, V.A. Stephan and Jr. K. Pritchard 1983 Evaluation of the Coomassie Brilliant Blue G-250 method for urinary protein. *Clinical chemistry* **29**(11), 1946-1950.

Lu, J., T. Deng, J. Li and J. Mo 2010 Activities of some lignocelluloses-degrading enzymes in workers of five common termites (Isoptera). *Sociobiology* **55**(3), 749-762.

Mo, J., T. Yang, X. Song and J. Cheng 2004 Cellulase activity in five species of important termites in China. *Applied Entomology and Zoology* **39**, 635–641.

Noble, J.C., W.J. Miller, W.G. Whitford and G.H. Pfitzner 2009 The significance of termites as decomposers in contrasting grassland communities of semi-arid eastern Australia. *Journal of arid environments* **73**(1), 113-119.

Rubin, E.M. 2008. Genomics of cellulosic biofuels. *Nature* **454**(7206), 841-845.

Scrivener, A. and M.Slaytor 1994 Properties of the endogenous cellulase from *Panesthia cribrata* saussure and purification of major endo- β -1, 4-glucanase components. *Insect biochemistry and molecular biology* **24**(3), 223-231.

Todaka, N., T. Inoue, K. Saita, M. Ohkuma, C.A. Nalepa, M. Lenz, T. Kudo and S. Moriya 2010 Phylogenetic analysis of cellulolytic enzyme genes from representative lineages of termites and a related cockroach. *PLoS One* **5**(1), e8636.

Tokuda, G., N. Lo and H. Watanabe 2005 Marked variations in patterns of cellulase activity against crystalline- vs. carboxymethyl-cellulose in the digestive systems of diverse, wood-feeding termites. *Physiological Entomology* **30**(4), 372-380.

Tokuda, G, N. Lo, H. Watanabe, G. Arakawa, T. Matsumoto and H. Noda 2004 Major alteration of the expression site of endogenous cellulases in members of an apical termite lineage. *Molecular Ecology* **13**(10), 3219-3228.

Watanabe, H. and G. Tokuda 2010 Cellulolytic systems in insects. *Annual review of entomology* **55**, 609-632.

Willis, J.D., C. Oppert and J.L. Jurat-Fuentes 2010 Methods for discovery and characterization of cellulolytic enzymes from insects. *Insect Science* **17**(3), 184-198.

cDNA cloning and bioinformatics analysis of a novel endogenous cellulase from termite, *Reticulitermes flaviceps* (Isoptera; Rhinotermitidae)

Wenhui Zeng, Ruixian Liu, Zhiqiang Li, Bingrong Liu, Qiujian Li and Junhong Zhong

Guangdong Entomological Institute, Guangzhou 510260, China

Abstract

A novel endogenous β -glucosidase (Rf-BGaseI) belonging to the glycoside hydrolase family (GHF) 1 from termite, *Reticulitermes flaviceps* had been obtained by degenerate PCR and RACE. The Rf-BGaseI gene spans 1691 bp and code for 495 amino acid residues which showed 95% amino acid sequence identity to the *Reticulitermes flavipes*. The bioinformatics analysis results suggested that a potential leucine-rich nuclear export signals is at pos.430 amino acid residue, a signal peptide cleavage site maybe exist between pos. 17 and 18 amino acid residue, and there are 25 potential phosphorylation sites.

Key words: β -glucosidase, cDNA cloning, *Reticulitermes flaviceps*

Introduction

Termites digest 74-99% of the cellulose in wood via a collaboration of endogenous and symbiont-derived lignocellulase enzymes (Scharf et al. 2010, Arakawa et al. 2009, Lee et al. 2004). The enzymatic hydrolysis of lignocelluloses is supposed to be one of the most promising ways to produce renewable energy (Todaka et al. 2010). The biologically mediated degradation of cellulose mainly requires β -1, 4-endoglucanase (EC 3.3.1.4, EG), β -glucosidase (EC 3.2.1.21, BG) and exoglucanases (EC 3.2.1.91, CBH) (Zhou et al. 2007).

To date, the endogenous BG of termites was classified into GHF1 (Inoue et al. 2005, Ni et al. 2005). Meanwhile, for lower termites, BG genes have been sequenced and functionally investigated from termites *Neotermes koshunensis*, *Cryptotermes secundus*, *Coptotermes formosanus*, *Reticulitermes speratus* and *Reticulitermes flavipes* (Todaka et al. 2010, Zhang et al. 2010, Tokuda et al. 2009, Wheeler et al. 2007).

In past researches, cellulase activity of in lower termites were relatively higher, especially for xylophagous termites *Reticulitermes* and *Coptotermes* (Tokuda et al. 2004). Previously, we performed a lot of comparative studies of lignocellulase activity and gut distribution patterns in several termite genera (unpublished data). It was showed that one lab rearing colony of *R. flaviceps* exhibited higher BG activity than other field *R. flaviceps* colonies.

In this paper, we reported a full-length cDNA cloning of a novel gene encoding endogenous BG from *R. flaviceps* for the first time. These data provide novel basic information on termite cellulases, as well as biomass-to- bioethanol applications.

Materials and methods

Termites

Termites used in this study were *R. flaviceps* which were maintained in lab rearing for 15 years. For total RNA extraction, just workers of *R. flaviceps* were selected and kept alive until use.

Total RNA isolation and degenerate RT-PCR

Total RNA was isolated from whole tissues. Using the oligo-dT as the reverse transcription primer, cDNA was synthesized from total RNA. Based on the conserve positions of cellulases mRNA sequences of *Reticulitermes flavipes*, *Nasutitermes takasagoensis*, *Odontotermes formosanus*, *Neotermes Koshunensis* and *Coptotermes formosanus*, we designed degenerate primers according to iCODEHOP program. The segments of cellulases genes which obtained through degenerate PCR then cloned to the T-vector (TaKaRa, Dalian) and sequencing.

RACE (rapid amplification of cDNA ends) of β -glucosidase

The 5'- and 3'-ends of cDNA were amplified by 5' and 3' RACE methods using the GIBCOBRL and Invitrogen RACE kit manual as references. Based on the nucleotide sequences of degenerate PCR products, gene specific primers were designed for 3'-Race and 5'-Race. Then, race products cloned to the T-vector (TaKaRa, Dalian) and sequencing. The DNA segments were assembled by biology software DNASTAR 7.0.

Bioinformatics analysis

Homology searches of both nucleic acid and amino acid sequences of *R. flaviceps* BG cDNA were performed using the BLAST program provided by the National Center for Biotechnology Information website (NCBI, <http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>), and Multiple alignment and Distance tree of the homology researches were performed through NCBI online program (<http://www.ncbi.nlm.nih.gov/blast/treeview> and <http://www.ncbi.nlm.nih.gov/tools/cobalt/cobalt.cgi>). The annotations of functional units in proteins were according to the Conserved Domain Database of NCBI (<http://www.ncbi.nlm.nih.gov/cdd>). Hydropathy analysis, prediction of the transmembrane domains and signal peptide cleavage sites were performed using the CBS Prediction Servers (<http://www.cbs.dtu.dk/services>).

Results and discussion

Degenerate PCR amplification and RACE of *R. flaviceps* β -glucosidase cDNA

We designed degenerate primers on the basis of the highly conserved partial amino acid sequences of several species of termites. The deduced amino acid sequences of the following species were considered: *N. takasagoensis* (Genbank Accession No.BAI50023.1, BAI50022.1, BAI50024.1, BAI50021.1, BAI50018.1, and BAI50019.1); *O. formosanus* (Genbank Accession No.ADD92156.1); *R. flavipes* (Genbank Accession No.ADK12988.1); *C. formosanus* (Genbank Accession No.ADB23476.1). Sequencing the fragments of degenerate RT-PCR of the CDNA from the whole *R. flaviceps* tissues revealed that the deduced amino acid sequence of these fragment showed a high sequence identity with the reported GHF1 enzymes, suggesting that it is

a partial sequence of BG of *R. flaviceps*. Finally, the full-length cDNA of this gene with total 1691 bp was determined by the 5'- and 3'-RACE and designated as "Rf-BGaseGZ". The full-length cDNA contained an open reading frame (ORF) of 1488 bp long encoded a 495 amino acid. The translation initiation codon ATG was found in cDNA nucleotide position from 45 to 47 and determination codon TAA from 1530 to 1532. In the 3'-terminal region, a putative polyadenylation signal sequence AATAAA and a poly (A+) tail were found, which indicated that the Rf-BGaseI was derived from eukaryotic organisms.

Bioinformatics analysis of the β -glucosidase "Rf-BGaseGZ" from *R. flaviceps*

A cDNA homology search indicated that Rf-BGaseGZ nucleotide sequence is most closely related to *R. flavipes* BG mRNA (Genbank Accession No. HM152540.1) (Identities=1638 / 1687 97%). The second closely related nucleotide sequence is the *C. formosanus* BG mRNA (Genbank Accession NO. GQ911585.1) (Identities= 1483 / 1700 89%). The mRNA of Rf-BGaseGZ is around 100 bp shorter than that of *C. formosanus* BG, and the shortage positions mostly located at 5'-end. Meanwhile, BG mgNtBG2 mRNA from *N. takasagoensis* (Genbank Accession NO.GQ911585.1) shared 85% (Identities = 1435/1706) identities with the Rf-BGaseGZ mRNA, while they have almost the same length nucleotide sequence.

The amino acid sequence of predicted mature Rf-BGaseGZ was compared with sequences currently available in protein databases using the NCBI online multiple alignment program. It shares 95%, 86%, 81% identity with the BG protein sequences of *R. flavipes*, *C. formosanus* and of *N. takasagoensis*, and shared 54% identity with *Tenebrio molitor* origin BG. Meanwhile, its putative conserved domain (pos. 24aa ~ pos. 489aa) had been detected to belong to the GHF1. The discrepancies of amino acid completely in the region of GHF 1 conserved domain (Fig 1).

A phylogenetic analysis using the amino acid sequences of highly homology BG (Identity > 50%) revealed that the BG of these insects are roughly distributed into five clusters. Termites BGs could roughly be divided in one cluster, and BGs from higher termites could be referred to one sub cluster, except for two BGs from *O formosanus* and *C. formosanus* respectively (Fig 2).

Hydropathy, transmembrane domains and signal peptide cleavage sites of Rf-BGaseGZ had been predicted. There is a leucine-rich nuclear export signals (NES) located at the pos.430 amino acid residue (Fig. 3). Secondly, a signal peptide cleavage site between pos. 17 and 18 amino acid residue had been found (Fig. 4). The identifiable NES and signal peptide cleavage site suggested that the mature protein maybe secreted. Generic phosphorylation sites analysis results showed Rf-BGaseGZ have 12 serine, 4 threonine and 9 tyrosine phosphorylation sites (Fig 5), which suggested the existent of post-translational modifications of protein. Furthermore, transmembrane helices in proteins TMHMM result showed Rf-BGaseGZ is probably outside the membrane (Fig. 6).

Conclusions

The full-length cDNA of a novel endogenous β -glucosidase (Rf-BGaseI) belonging to the glycoside hydrolase family (GHF) 1 from termite, *Reticulitermes flaviceps*, had been cloned.

The Rf-EGaseGZ gene coded for 495 amino acid residues, and was mostly related to *Reticulitermes flavipes* BG (95% amino acid sequence identity). Referring to the available database, this is the first BG full-length cDNA sequence from *Reticulitermes flaviceps*. The next work will be the fusion expression of the Rf-EGaseGZ in *E.coli* expression system.

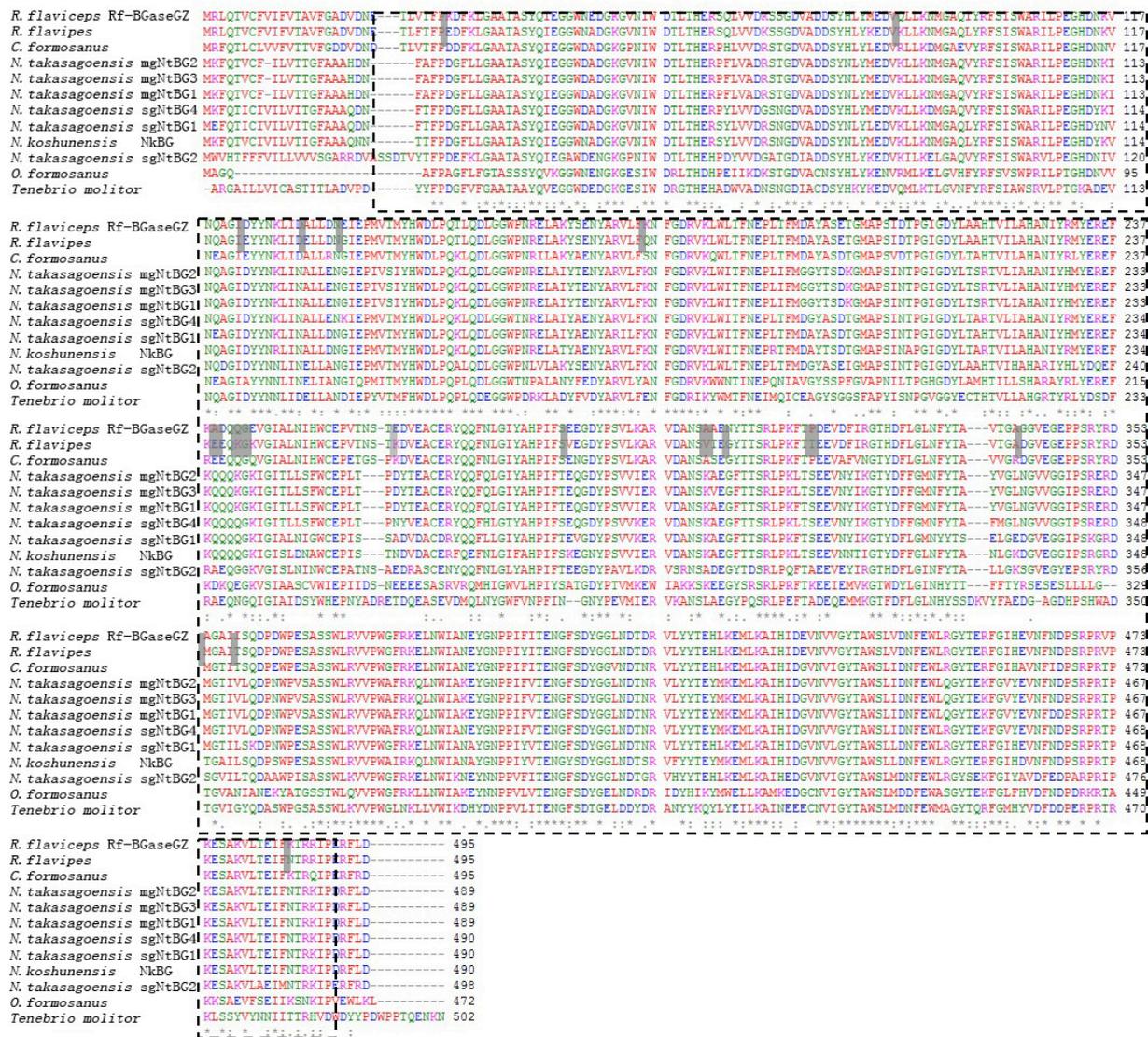


Fig. 1 Amino acid sequences alignment among Rf-BGaseGZ, *R.flavipes* BG, *C.formosanus* BG, mgNtBG1~3, sgNtBG1~2, sgNtBG4, *O formosanus* BG and *Tenebrio molitor* BG. The asterisks denote the conserved amino acid positions. The complete conserved domain of GHF1 was inside the black dotted box. The mutation positions between Rf-BGaseGZ and *R. flavipes* BG were marked in grey boxes.

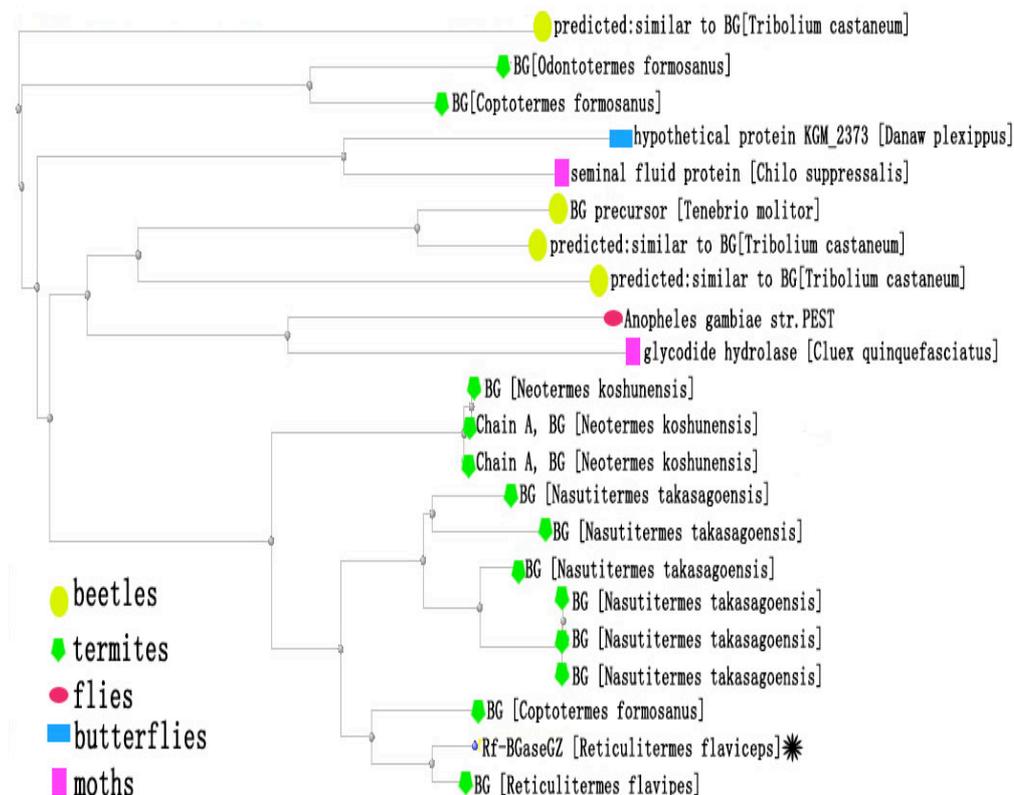


Fig. 2 Phylogeny of Rf-BGaseGZ related highly homology β -glucosidase (Identity > 50%). This is an un-rooted tree inferred using “Fast Minium Evolution” method; the parameter of max sequence difference was 0.85 and distance was Grishin (protein).

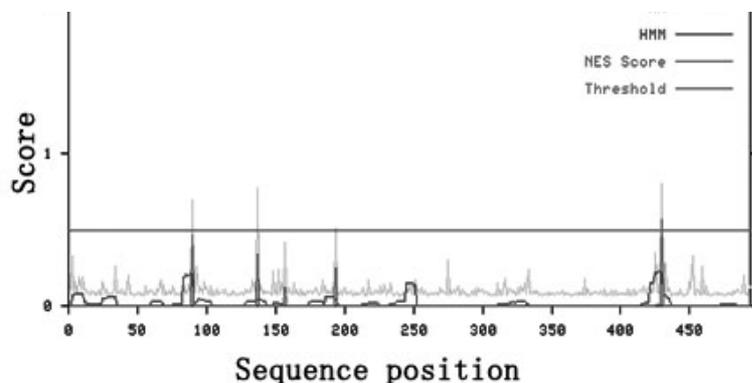


Fig. 3 Nuclear export signals prediction of Rf-BGaseGZ. NetNES 1.1 server predicts leucine-rich nuclear export signals (NES) in eukaryotic proteins using a combination of neural networks (NN) and hidden Markov models (HMM). <http://www.cbs.dtu.dk/services/NetNES/>

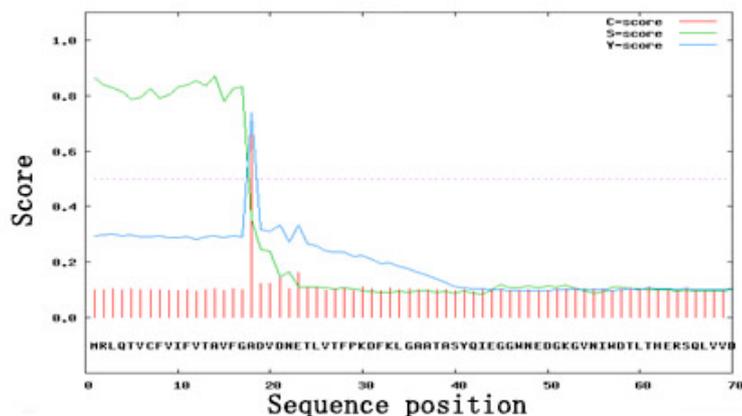


Fig. 4 Signal peptides cleavage sites prediction of Rf-BGaseGZ. SignalP 4.0 online program was used to analyze the signal peptide cleavage sites. <http://www.cbs.dtu.dk/services/SignalP/>

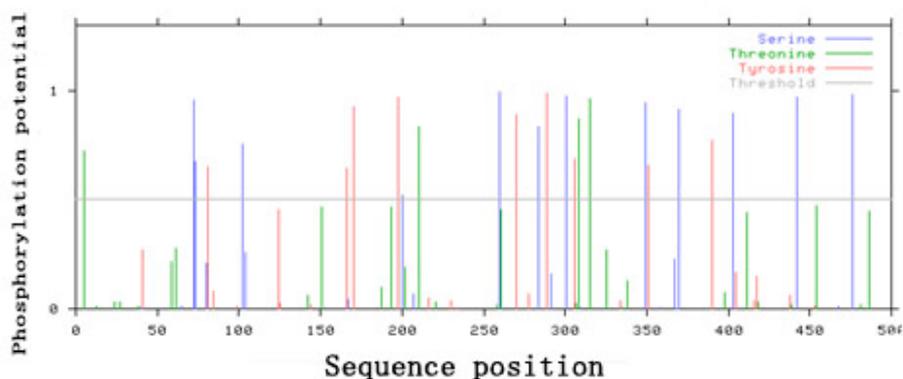


Fig. 5 Phosphorylation sites prediction of Rf-BGaseGZ. The NetPhos 2.0 server produces neural network program was used to predict for serine, threonine and tyrosine phosphorylation sites. <http://www.cbs.dtu.dk/services/NetPhos/>

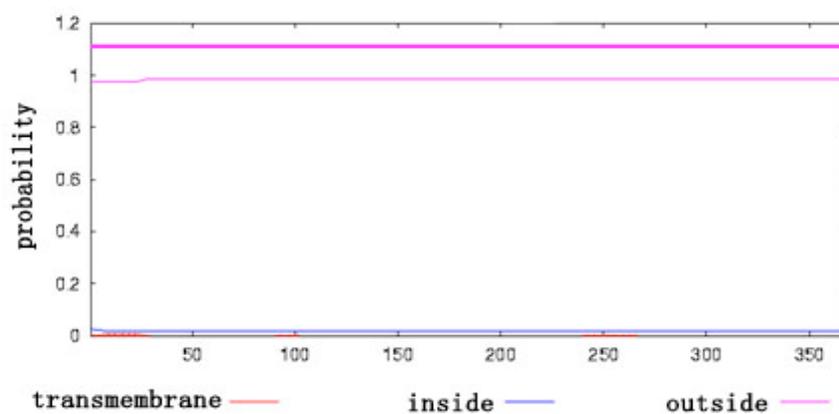


Fig. 6 Prediction of transmembrane helices in Rf-BGaseGZ. Transmembrane helices program was the TMHMM Server v. 2.0. <http://www.cbs.dtu.dk/services/TMHMM/>

References

- Arakawa, G., H. Watanabe, H. Yamasaki, H. Maekawa and G. Tokuda 2009 Purification and molecular cloning of xylanases from the wood-feeding termite, *Coptotermes formosanus* Shiraki. *Biosci. Biotechnol. Biochem.* **73**, 710-718.
- Inoue, T., S. Moriya, M. Ohkuma, and T. Kudo 2005 Molecular cloning and characterization of a cellulase gene from a symbiotic protist of the lower termite, *Coptotermes formosanus*. *Gene* **349**, 67-75.
- Lee, S. J., S. R. Kim, H. J. Yoon, I. K., and R. B. Jin 2004 cDNA cloning, expression, and enzymatic activity of a cellulase from the mulberry longicorn beetle, *Apriona germari*. *Comparative Biochemistry and Physiology Part B*, 107-116.
- Ni, J. F., M. Takehara and H. Watanabe 2005 Heterologous Overexpression of a Mutant Termite Cellulase Gene in *Escherichia coli* by DNA Shuffling of Four Orthologous Parental cDNAs. *Biosci. Biotechnol. Biochem.* **69**, 1711-1720.
- Scarf, M. E., E. S. Kovaleva, Jadhao, S. and D. G. Boucias 2010 Functional and translational analyses of a beta-glucosidase gene (glycosyl hydrolase family 1) isolated from the gut of the lower termite *Reticulitermes flavipes*. *Insect Biochem. Mol. Biol.*, 611-620.
- Todaka, N., C. M. Lopez, T. Inoue, K. Saita, J. Maruyama, M. Arioka, K. Kitamoto, T. Kudo and S. Moriya 2010 Heterologous expression and characterization of an endoglucanase from a symbiotic protist of the lower termite, *Reticulitermes speratus*. *Appl. Biochem. Biotechnol* **160**, 1168-1178.
- Tokuda, G., N. Lo, H. Watanabe, G. Arakawa, T. Matsumoto and H. Noda 2004 Major alteration of the expression site of endogenous cellulases in members of an apical termite lineage. *Mol. Ecol.* **13**, 3219-3228.
- Tokuda, G., M. Miyagi, H. Makiya, H. Watanabe and G. Arakawa 2009 Digestive beta-glucosidases from the wood-feeding higher termite, *Nasutitermes takasagoensis*: intestinal distribution, molecular characterization, and alteration in sites of expression. *Insect Biochem. Mol. Biol.* **39**, 931-937.
- Wheeler, M. M., X. Zhou, M. E. Scharf and F. M. Oi 2007 Molecular and biochemical markers for monitoring dynamic shifts of cellulolytic protozoa in *Reticulitermes flavipes*. *Insect Biochem. Mol. Biol.* **37**, 1366-1374.
- Zhang, D. H., A. R. Lax, J. M. Bland, J. J. Yu and W. C. Nierman 2010 Hydrolysis of filter-paper cellulose to glucose by two recombinant endogenous glycosyl hydrolases of *Coptotermes formosanus*. *Insect Science* **17**, 245-252.
- Zhou, X., J. A. Smith, F. M. Oi, P. G. Koehler, G. W. Bennett and M. E. Scharf 2007 Correlation of cellulase gene expression and cellulolytic activity throughout the gut of the termite *Reticulitermes flavipes*. *Gene* **395**, 29-39.

Session 3

Evaluation of bio-compounds and microbial agents

Preliminary research results on the ability of termite exocrine gland secretion resisting pathogenic microorganisms

Ta Kim Chinh

Biotechnology section, Union of Science & Technology Vietnam (VUSTA), Research Center
Production of Biological Products(CBR), Vietnam. Email: takimchinhebr@gmail.com

Abstract

In the nature, there are many pathogenic microorganism species affecting termites, two of which are *Metarhizium anisopliae* and *Baeuveria bassiana*. In the surviving process of a termite society, the development of the exocrine gland helps to produce a substance to check the development of the pathogenic microorganisms. This article described some experiments showing the self-protection ability of termites and this provides a base for biological control of termites.

Introduction

In the nature, subterranean and dampwood termite species live hidden in wet and dark underground conditions, constantly being in contact with the pathogenic microorganism, but their society still is always surviving and developing.

The fungal garden of the Termitidae family is rich in nutrients but only one fungus cultivated by the termites can grow here. Then, what substance and ability help the termites to protect themselves against the lethal fungi and microorganisms causing diseases to them?

Research results showed that the exocrine glands of the termite soldiers play a very important role against their enemies and diseases. In a number of *Coptotermes* and *Rhinotermes* species, the exocrine gland are developed into a large mass occupying almost the whole front thorax and abdomen sections. In the majority of other species only the mandibular and pharyngeal glands are developed. The secretion of different species differs in colour, taste and resistance to pathogenic microorganism. In the war time, Vietnamese soldiers who suffered from malaria had the experience in selecting a number of fungal gardens of some termite species diluted in water to have an antibiotic drug against the disease. At the 11th International Congress –IUSSI 1990 held in India, J.A.L., Watson (Australia) also had remarked on the inhibition of growth of *Metarhizium anisopliae* inside the termite colony, but the cause was unknown by the author.

For those reasons, the study on the ability of termite exocrine gland secretion resisting to pathogenic fungi was carried out by the authors in order to seek a possibility of using the microorganism in biological control for termites.

Materials and methods

1. Materials.

*The termite species using in the experiment were *Macrotermes barneyi* Light, the subterranean species and *Coptotermes ceylonicus* Hav. as the wetwood termite species.

*The tested pathogenic microorganisms were of *Metarhizium anisopliae*(Mestch.) Sorokin and *Baeuveria bassiana* (Bals.)Vuil.

2. Methods.

*Description of the location and morphology of the exocrine glands of termite is based on the cross section slice at mandibular base and length wise slice of the frontal gland.

*The resistance of the termite's exocrine secretion to the pathogenic entomogenous fungi

Metarhizium anisopliae and *Baeuveria bassiana* is tested through the observation of the antiseptic circle on malt agar medium.

Results and discussion

1.Description of the exocrine gland.

a.Coptotermes ceylonicus soldiers

The frontal gland runs from the front spit along the whole head through the thorax and develops into a big sac occupying almost the whole abdomen. The wall of the gland is made of secretion cells with rather small nucleus. The end of secretion cells tents to turn to the secretion sac. The secretion is a milk-white acidic fluid wwith pH=2.5-3 and its odour is a hot.

The shapes of the termite's gland differ in different species of the genus *Coptotermes*. That is one of good taxonomical features usually paid attention by taxonomists.

b.Macrotermes barneyi soldiers

Macrotermes barneyi have not a frontal gland as *Coptotermes* but their mandibular and hypopharyngeal gland are well developed. Hypopharyngeal gland has a big secretion sac and pours into the mouth with mandibular and gland.

The small soldier's mandibular gland more developed than that of big soldier. Secretion cell have big nuclei and their secretion ducts pour into secretion sac. When taking care of the colony, the termite uses the mandibular muscle to press the secretion sac to release the secretion. The termite uses the end of the mandibular to smear the secretion to the fungal garden or on the queen's body. When the termite workers feed the soldiers by their mouth, they also receive part of the secretion to maintain their colony. The secretion *M.barneyi*, *Globitermes sulphureus*, *Hypotermes sumatrensis* and *Odontotermes angustignathus* is green, sulfur-yellow, purplish brown and coffee-milk in colour, repectively.



Figure 1. Cross section slice of hypopharyngeal gland (Hy)



Figure 2. Mandibular gland (m) muscle, (l) gland (d) direct ejection

2. Testing the resistance of exocrine secretion of *M.barneyi* soldier to entomogenous fungi *M.anisopliae* and *Baeuveria bassiana*.

Experiment on the resistance of the secretion was realized by measuring the diameter (d,mm) of the antiseptic cricle. The results are as follows:

Termite Entofungi	Fr.gland secretion (D,mm) <i>C.ceylonicus</i>				Hy.gland secretion (D,mm) <i>M.barrneyi</i>			
	1	2	3	Mean	1	2	3	Mean
<i>M.anisopliae</i>	0.50	0.85	0.70	0.68	0.87	0.92	0.86	0.88
<i>B.bassiana</i>	0.49	0.65	0.65	0.65	0.91	0.87	0.89	0.89

Remark: Through the experiment we find out that the exocrine secretion of both two termite species given all positive results expressed by the fungus tree circles. The diffusion degree of the secretion of *M.barneyi* proved stronger than that of *C.ceylonicus*.

Discussion: The anatomy of the exocrine gland has been studied and described by A. Queendey, M. Ampion but nowhere in the literature the chemical composition of the secretion and its function of various glands are mentioned.

We selected two strains of fungi *M.anisopliae* Ma5 and *Baeuveria bassiana* Bb1 able to adapt exocrine secretions of the termite. From two strains of this fungus has successfully produced two biologic medication to prevent termite damage crops are: Vimetarzimm96DP and Biobaue5% WW

This paper presented research results about the additional function of the exocrine gland. This secretion possesses the resistant ability against pathogenic entomogenous fungi.

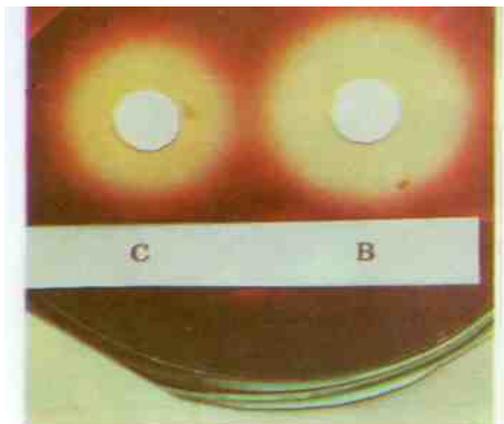


Figure 3: Antiseptic of the secretion gland
B- *Macrotermes barneyi*
C- *Coptotermes ceylonicus*



Figure 4: Antiseptic of the secretion of *M. barneyi* oldier's mandibular gland free of *M. anisoplae*

Conclusions

In order to kill the termite by infection with microorganism a prerequisite principle is to select the strains adaptable to the environment containing the termite exocrine secretion.

References

- Queendey, A. 1982 Modifications structurales de la cuticule des glandes tegumentaires des termites. *Bull. de la Soc. Zool. de France* **107**, 465.
- Watson, J.Q.L. 1990 Biological control of termites in Australia. 11th International Congress-IUSSI 1990, India, 632-633.
- Kham, N.D. and V.V. Tuyen 1985 Termites and termite control. Agricul. Public. House, Vietnam.
- Hanel, N. and J.A. Watson 1982 Preliminary field tests on the use of *Metarhizium anisoplia* for control of *Nasutitermes exitiosus* Hill (Termitidae). *Bull. Entom. Research* **75**, 305-313.

Termite feeding deterrents produced by a brown rot fungus *Fibroporia radiculosa*

Shota Nishizawa¹, Akiko Nakagawa-Izumi¹, Shuichi Doi¹, Tsuyoshi Yoshimura², Sakae Horisawa³

¹University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan.

²Kyoto University, Uji, Kyoto 611-0011, Japan.

³Kochi University of Technology, Kami, Kochi 782-8502, Japan.

Abstract

Brown rotted wood stakes without termite-feeding were collected from a termite-infested field. Isolated fungi from the stakes were identified as *Fibroporia radiculosa* from their genome information. Deterrent feeding was observed on non-dried specimens while stimulated feeding was observed on dried specimens prepared from the stakes in no-choice feeding tests with *Reticulitermes speratus*. The n-hexane extract of the decayed stakes showed the deterrent effect against feeding of the termite.

Key words: *Fibroporia radiculosa*, brown rot, feeding deterrents, n-hexane extract

Introduction

There are some papers on the interaction between termites and decayed woods. Pine wood blocks infected with a brown rot fungus *Gloeophyllum trabeum* congregated *Reticulitermes flavipes*, *R. virginicus* and *Nasutitermes columbicus* (Esenther *et al.* 1961). Matsumura *et al.* (1968) revealed that the attractant in the wood decayed by *G. trabeum* was (Z,Z,E)-3-6-8 dodecatrien-1-ol. Cornelius *et al.* (2002) showed that the sawdust infected with *Marasmiellus troyanus* was significantly preferred by *Coptotermes formosanus*.

On the other hand, there are a few papers on the feeding deterrence in relation to wood decay fungi. Amburgey and Beal (1977) reported that white-rotted southern yellow pine stakes were not preferred compared with sound ones. Grace *et al.* (1992) showed that filter paper treated with siderophore isolated from *G. trabeum* suppressed feeding by *C. formosanus*. There is no report on the constituent related to antagonistic interaction except siderophore.

The objective of this study is to elucidate substances in the feeding deterrence of termite produced by a wood decay fungus, *Fibroporia radiculosa*.

Materials and methods

Termite: Colonies of *Reticulitermes speratus* were collected from the Living Sphere Simulation Field, RISH, Kyoto University sited at Kagoshima prefecture. They were kept in plastic containers in the laboratory until use for experiments.

Stakes tested: Pine sapwood stakes covered with white- to yellow- colored hyphae without termite damage were collected from the field mentioned above. Stakes without fungal and

termite damage were also collected from the same field. The stakes were put into polythene bags and stored in a cool place to prevent drying.

Identification of wood decay fungi isolated from decayed stakes: To isolate wood decay fungi from the decayed stakes, small pieces of decay parts of the stakes were sampled and put on potato dextrose agar (PDA) plates containing 100 ppm of benlate and 50 ppm of tetracycline hydrochloride to prevent mold and bacterial growth. The isolation procedure was conducted at 20°C. Identifications of the fungi were conducted based on the nucleotide sequence of ITS region of ribosomal DNA.

Feeding tests of specimens prepared from the stake: The fresh specimens sized about 10mm by 10 mm by 20 mm prepared from the stake to conduct preliminarily a choice feeding test on a sand medium.

The non-dried small specimens sized 5 mm by 5 mm by 5 mm prepared from the stakes were exposed to *R. speratus* in a no choice feeding test. Control specimens were prepared from the stakes without fungal and termite damage. A no-choice feeding test was conducted as follows. Each specimen placed on a plastic saucer on a hard plaster covered with wet sand in a 100 ml plastic cup. The cup had a 10 mm-diameter hole at the bottom for supplying water through a plaster layer from a moistened paper pad spread in a test chamber. One hundred workers of *R. speratus* were introduced into each cup. The chambers were maintained in an incubator at 27°C. After the exposure, the specimens were taken out, oven-dried at 60°C for 48 h and weighed, and mass losses of the specimens were calculated from the difference of dry weight before and after the exposure. Weights before exposure were calculated from their moisture contents and wet weights before the test. Three replications were conducted per each group.

The air-dried and oven-dried at 60°C for 48 h specimens were also exposed to the termite same as the above.

Feeding test of paper discs immersed with *n*-hexane extracts obtained from decayed stakes: The wood meal prepared from the stake decayed by *F. radiculosa* was extracted with *n*-hexane for 3 d at room temperature. Yielded extracts were immersed into 8mm diam. paper disc

(ADOVANTEC TOYO, Tokyo, thick type) at the same retention in decayed wood. The paper discs were subjected to no-choice feeding tests same as wood specimens.



Fig.1: Feeding behavior of termites to the specimens prepared from a wood stakes on day 3 in the choice feeding test. Upper specimens were prepared from sound part and lower specimens were prepared from decayed part of the wood stake, respectively.

Feeding test of the paper-

chromatographed *n*-hexane extracts: The *n*-hexane extracts were fractionated by paper chromatography using a filter paper for paper chromatography (ADVANTEC TOYO, Tokyo)

sized 20 mm by 120mm with *n*-hexane and ethyl acetate (1:3) as developing solvent. After the chromatography, the papers divided into 9 pieces having 10 mm length were subjected to the no-choice feeding test.

Results and discussion

Feeding test of stakes: Fig. 1 shows the feeding behavior of termites to the specimens prepared from the decayed stakes on day 3 in the feeding test. At the initial stage of the test, termites aggregated around the specimens prepared from the decayed stakes. However, after 3 days exposure, most of the termites aggregated only around the specimens prepared from sound ones and kept away from the specimens prepared from decayed ones.

Fig. 2 shows mass losses of the specimens by termites in the no-choice feeding test of non-dried specimens prepared from the stakes. The mass losses of the specimens prepared from the decayed stakes No.1 and 3 were significantly less than that of control. No significant difference was observed in case of between the specimens prepared from the decayed stake No.2 and from control. This difference might be caused by the difference of decay degree. However, after air-drying or oven-drying, the feeding deterrence of the specimens completely disappeared (Fig.3). The same phenomena were observed in no choice tests using samples decayed under the laboratory condition using the isolated fungus This means that factors act as feeding deterrents against termites were possibly vaporized or changed during drying. It was also presumable that the decayed stakes contain some attractants because termites aggregate specimens prepared from

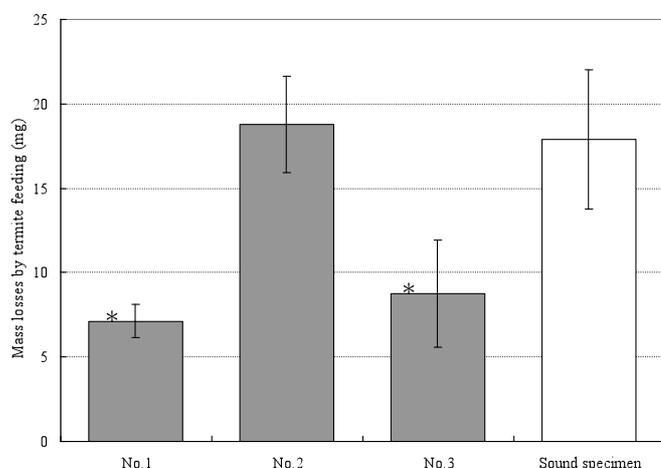


Fig.2: Mass losses of the specimens by termites in the no-choice feeding test of non-dried specimens prepared from the stakes. Specimen of no.1-3 was prepared from 3 different decayed stakes, respectively. Error bars indicate standard deviations. Asterisks indicate significantly differences from control ($P<0.05$).

significantly less than that of paper discs immersed with *n*-hexane extract from a sound stake or only with a solvent (data not shown). It is showed that some feeding deterrents were

contained in the *n*-hexane extract from the decayed stakes. The mortality of the termites after the exposure to the paper discs immersed with the *n*-hexane extract from the decayed stakes was

decayed stakes in the initial stage of the feeding test.

Identification of the isolated basidiomycete from stakes: All the fungi isolated from the stakes were identified as a brown rot basidiomycete *Fibroporia radiculosa* (Peck) Parmasto. This result was supported by the paper description about morphological characteristics reported by Lombard and Gilbertson (1965).

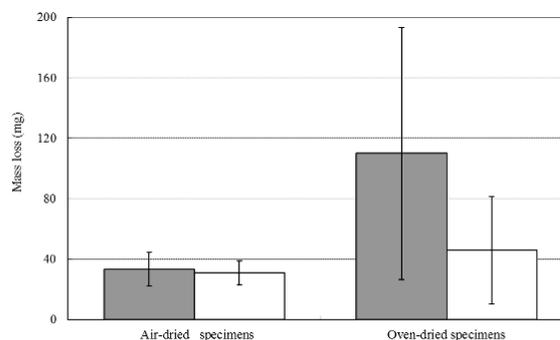
Feeding test of paper discs

immersed with *n*-hexane fraction:

The mass loss of the paper discs immersed with the *n*-hexane extract from the decayed stake was

significantly higher than that of the termites under the starvation condition for the same period. This means that the *n*-hexane extract also has potential to kill termites.

Fig. 3: Mass losses of the specimens by termites in the no-choice feeding test of the dried specimens prepared from the stakes. Closed bars indicate the mass loss of the specimens prepared from decayed stakes and open bars indicate the mass loss of the specimens prepared from sound ones, respectively.



Feeding test of the paper-chromatographed *n*-hexane extracts:

To fractionate the deterrents, paper chromatography of the extracts from the decayed stake was conducted. The mass losses of each divided portion of the developed filter paper were determined after termite exposure. The upper portion of developed filter paper was suppressed termite feeding compared to the other portions or control filter paper (data not shown). It was clarified that the feeding deterrents that *F. radiculosa* produce during wood decay is contained in this fraction.

Conclusion

The non-dried specimens prepared from wood stakes decayed by the brown rot fungus *Fibroporia radiculosa* suppressed termite feeding comparing with the specimens prepared from sound ones. On the other hand, no difference is observed between the decayed specimens dried at room temperature or 60°C and the sound specimens. There are any termite feeding deterrents present in wood decayed by *F. radiculosa*, and that the deterrent effect is lost by drying.

The deterrents are possibly highly-volatile constituents because they were separated by paper chromatography with comparatively low polar solvent system. In future study, it is expected to clarify chemical structures those arrest termite feeding.

Acknowledgements

This work was supported by Grant-in-Aid for Scientific Research (21580200).

References

- Amburgey, T. L., and R.H. Beal 1977 White Rot Inhibits Termite Attack. *Sociobiology* **3**,35-38.
- Cornelius, M. L., D.J. Daigle, W.J. Connick, M. Tellez, K.S. Williams and M.P. Lovisa 2002 Interactions between the Formosan Subterranean Termite (Isoptera: Rhinotermitidae) and Wood Decay Fungi. *Proceedings of the 4th International Conference on Urban Pests*, Charleston, SC, USA, 319–324pp.
- Esenther, G.R., J.E. Allen, J.E. Casida and R.D. Shenefelt 1961 Termite Attractant from Fungus-Infected Wood. *Science* **134**, 50.

Grace, J.K., B.S. Goodell, W.E. Jones, V. Chandhoke and J. Jellison 1992 Evidence for Inhibition of Termite (Isoptera: Rhinotermitidae) Feeding by Extracellular Metabolites of a Wood Decay Fungus. *Proceedings of the Hawaiian Entomological Society* **31**, 249–252.

Lombard, F.F. and R.L. Gilbertson 1965 Studies on Some Western Porias with Negative or Weak Oxidase Reactions. *Mycologia* **57**, 43-76.

Matsumura, F., H.C. Coppel and A. Tai 1968 Isolation and Identification of Termite Trail-Following Pheromone. *Nature* **219**, 963-964.

Toxicity of volatile oil of *Corymbia citriodora* (Hook.) K.D. Hill & L.A.S Johnson against subterranean termites *Coptotermes vastator* Light and drywood termites *Cryptotermes dudleyi* Banks

Irma I. Palanginan and Carlos M. Garcia

Forest Products Research and Development Institute
Department of Science and Technology, Los Baños, Laguna - Philippines

Abstract

The toxicity of plantation grown lemon-scented gum's [*Corymbia citriodora* (Hook.)K.D. Hill & L.A.S. Johnson] crude volatile leaf oil and its methanol- and hexane fractions were evaluated against subterranean termites, *Coptotermes vastator* Light and drywood termites, *Cryptotermes dudleyi* Banks. The test solutions were topically applied on the thoracic region of the test insects. Mortality of test insects within a 24-hr period was the basis in determining toxicity of volatile oil. Generally, there was increased mortality of test insects as the concentration of crude volatile oil and its fractions was increased. Higher mortality rates were observed on insects topically applied with either methanol or hexane fraction than with crude volatile oil. The hexane fraction shows potential anti-termite property and to confirm this, further investigation on its efficacy against Philippine termites must be done by applying it on wood samples.

Keywords: *pesticidal plants, C. citriodora, volatile oil, Coptotermes, Cryptotermes*

Introduction

The application of synthetic chemicals as preventive or remedial treatment has been proven effective in preventing termite attack in wooden components of houses and buildings. However, the widespread utilization of these chemicals has raised considerable concerns among environmentalists because of risks these compounds posed to man and his environment. Thus, other sources of compounds like plant extracts are being investigated as alternative control measures against termite attack.

Studies showed that macerated bark of antiaris and ki pahit extracted with n-hexane, ethyl acetate and methanol caused 82.0 - 100% mortality of *Coptotermes curvignathus* (Priantno 2010). The efficacy of the extracts varied with the method of volatile oil extraction and plant species. Bark and leaf extract of *Carbera odolum* was highly toxic to *Coptotermes* sp. (Tarmadi et al. 2007), while seed extract of *C. manghas* L. at 10% (w/v) concentration killed 100% of *C. gestroi* (Tarmadi et al. 2010).

The termiticidal property of the essential oils of clove (*Eugenia caryophyllum* Tumberg, cubeb pepper (*Piper cubebai* L) and lemon grass (*Cymbopogon winterianus* Jowitt) against *C. gestroi* varied with the plant species (Ismayati et al. 2010). Results showed that clove oil- and cubeb pepper oil-treated paper discs at 10% concentration (v/v) completely decimated the termites compared to the 76% kill with lemon grass treatment. Clove oil exhibited the highest potential anti-termite property since it killed all test insects in 10 days compared to 14 days in cubeb pepper-treated paper disc. Likewise, distilled eugenol derived from Indonesian clove oil was proven highly effective as shown by 100% mortality of *C. gestroi* in 10 days by forced feeding

in treated filter paper (Setiawan et al. 2010). On the other hand, fractionated eugenol at 5.0% killed all termites as early as 4 days after treatment.

Crude volatile leaf oils from *Eucalyptus camaldulensis*, *E. tereticornis* and *E. grandis* were also effective against the subterranean termites *Microcerotermes losbañosensis* Oshima, drywood termites *Cryptotermes dudleyi* Banks and the powder-post beetles *Dinoderus minutus* L. (Giron et al. 2009). It is possible that other species of *Eucalyptus* also contain active compounds that are toxic to termites. Thus, this study was conducted to determine the efficacy of the volatile leaf oil of *Corymbia (Eucalyptus) citriodora* against two species of Philippine termites.

Materials and methods

Extraction of volatile oil. Crude volatile oil was obtained from leaves of *C. citriodora* by steam distillation process. The volatile oil was further fractionated by passing through the silica gel column using methanol and hexane as solvents to obtain the polar and non-polar fraction, respectively. The crude volatile oil, methanol- and hexane fraction were used as treatments to screen their toxicity to Philippine termites.

Test insects. Workers of subterranean termites *C. vastator* Light and drywood termites *C. dudleyi* Banks were used as test insects for the toxicity test. Test insects were collected by gently splitting cultured wood materials infested with termites. The collected termites were kept in a paper-lined aluminium tray and conditioned for 24 hrs prior to the test.

Toxicity test. Crude volatile oil and its derived fractions were applied at 0.2 ul to the thoracic region of test insect using a manually operated micro-applicator. Preliminary screening was first conducted by topical application of 20 workers with one replicate using 10%, 25%, 50%, 75% and 100% doses of crude volatile oil and its fractions. Further toxicity test was conducted by bracketing concentrations that could kill 50% of the test insects. The succeeding toxicity tests were conducted using the same number of test insects replicated thrice. A standard chemical (deltamethrin) and untreated groups were included for comparison. The degree of toxicity of volatile oils to termites was classified according to the following rating:

% Termite Mortality	Degree of Toxicity
0	No mortality, Not toxic
1 -25	Slightly toxic
26 – 50	Moderately toxic
51 -75	Highly toxic
76 -100	Very toxic

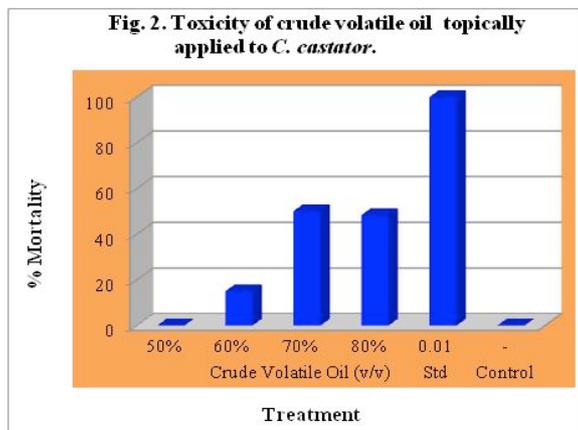
Statistical Design. The experiment was laid-out following the Completely Randomized Design. Differences in the toxicity among treatments were compared using the F-Analysis of Variance, while treatment means were analyzed following the Duncan's Multiple Range Test (DMRT).

Results and discussion

The preliminary toxicity test showed that concentrations between 50% to 75% of the volatile oils and its fractions approximately provided the doses that killed 50% of test populations of *C. vastator* and *C. dudleyi*. Serial dilutions of 50% - 80% concentrations were prepared for the final toxicity test.

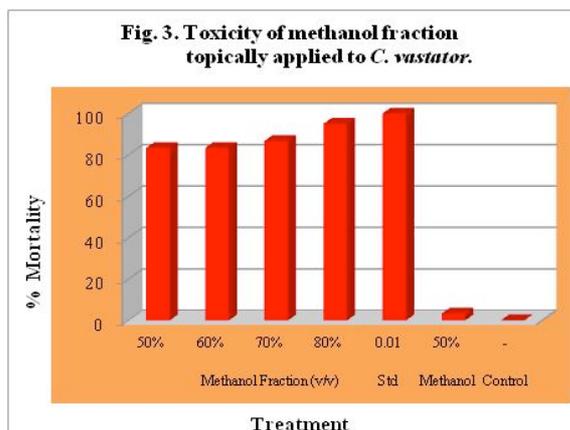
Toxicity to milk termites, *C. vastator*

There were significant differences on the toxicity of crude volatile oil and its fractions to *C. vastator* (p-value = <0.0001). The 70% crude volatile oil inflicted the highest mortality of 50.0% (Fig. 2). Its toxicity was comparable with the 48.3% kill caused by 80% concentration. The

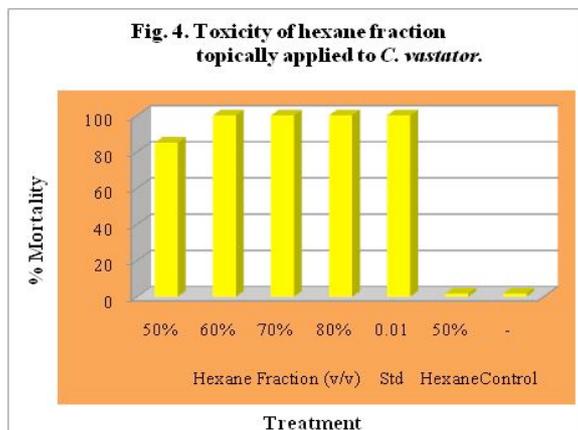


lowest concentrations of 50% and 60% were the least toxic and caused only 0 - 15.0% kill on test insects. Some of the treated insects appeared dead, but later recovered and survived. Deltamethrin was very toxic and no doses of crude volatile oil were comparably fatal to cause 100% kill. All insects in the untreated group remained alive, suggesting that the test populations used were healthy and active.

The lethal effect of methanol fraction was not significantly different among the concentrations tested (p-value <0.0001). However, only the highest concentration of 80% was very toxic as deltamethrin, which killed 100% of *C. vastator* (Fig. 3). Insect mortality 0 in methanol solvent was very low and did not differ from zero kill in the untreated group. Methanol solvent at 50% concentration contributed only 3.3% mortality suggesting that it was not toxic to the test insects.



Similarly, all concentrations of the hexane fraction, except the 50% dose, were very toxic as the standard chemical and completely wiped out the test insects. The 50% dose only gave 85.0% mortality (Fig. 4). Hexane alone was not toxic and affected only 1.7% of *C. vastator*.

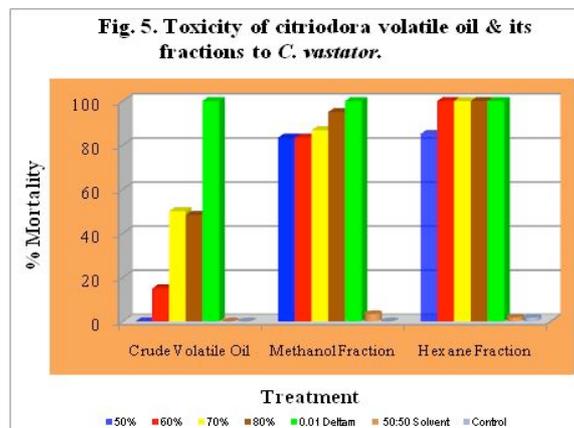


Results showed that methanol and hexane fractions were highly toxic to *C. vastator* than the crude volatile oil (Fig. 5). The higher mortalities of termites might be due to the presence of greater concentration of toxic components in the fractionated forms than in crude volatile oil. The active component might have reached the target site as the treated *C. vastator* died

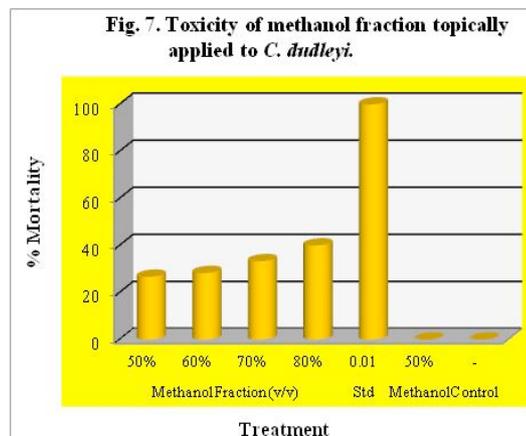
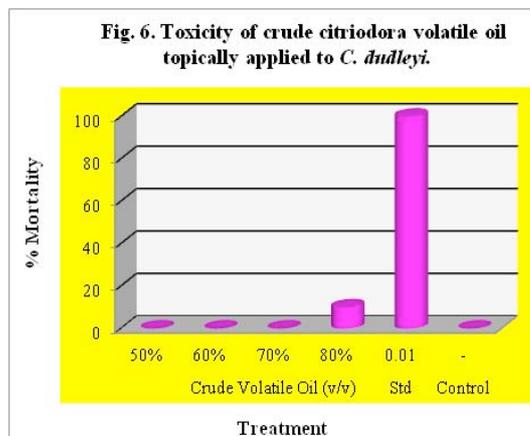
instantly after getting the fractionated solution on their thoracic regions.

Toxicity to drywood termites, *C. dudleyi*

There were significant differences on the toxicity of treatments applied to *C. dudleyi* (p-value <0.0001). Only the 80% concentration of crude volatile oil was slightly toxic to *C. dudleyi* as reflected by the very low 10.0% kill (Fig. 6). The lower concentrations (50, 60 and 70%) did not affect the test insects, suggesting that the crude volatile oil was either not toxic or only slightly toxic to *C. dudleyi*. In contrast, 0.01% deltamethrin was highly toxic and inflicted 100% mortality when applied. No mortality of test insect in the untreated group was noted.



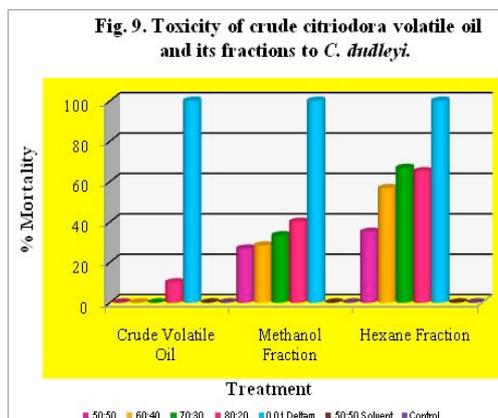
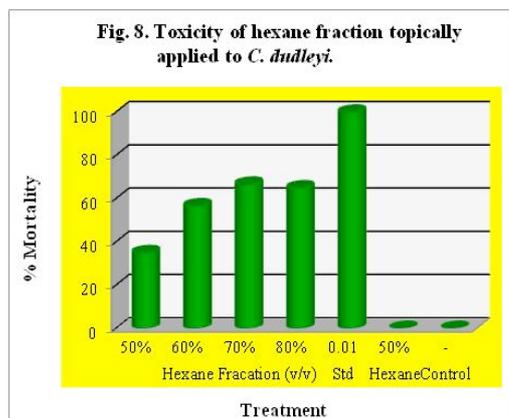
On the other hand, the methanol fraction was moderately toxic and affected 26.7 to 40.0% of *C. dudleyi* (Fig. 7). The lethal effects of 50%, 60% and 70% concentrations were not significantly different in causing mortality that ranged from 26.7 to 33.3% (p-value <0.0001). Although 40.0% mortality was observed in 80% hexane fraction, it was not very toxic as 0.01 deltamethrin that killed 100% of the test insects. Methanol solvent was not toxic as reflected by zero mortality. No mortality of test insect was recorded in the untreated group.



The toxicity of hexane fraction to *C. dudleyi* rose with increase in concentration (Fig. 8). The higher concentrations (60%, 70% and 80%) were highly toxic to the test insects with kills ranging from 56.7% to 66.7%. The lowest concentration of 50% was the least toxic with only 35.0% kill. However, all dosages of hexane fractions were not significantly toxic as 0.01 deltamethrin, which eliminated all test insects (p-value <0.0001). Hexane solvent at 50% v/v was not lethal and no test insects died as in the untreated group.

Methanol or hexane might be a good solvent for separating active components with termiticidal property that made the fractions more toxic to test insects than the crude volatile oil (Fig. 9). The methanol- and hexane fractions caused mortality in *C. dudleyi* that ranged from 26.7 to 40.0% and 35.0 to 65.0%, respectively compared with the 0 to 10.0% kill in crude volatile oil. Toxicity depends on the dosage and the volume of toxic compound and the 80% crude volatile oil was not

sufficient to exert a toxic effect on the critical target site in the *C. dudleyi*. This conformed with the preliminary screening result that the highest concentration of crude volatile oil (100%) caused 85.0 to 100% kill in *C. dudleyi*.



Conclusions and recommendation

1. The toxicity of crude volatile leaf oil of *C. citriodora* to subterranean termites *C. vastator* and drywood termites *C. dudleyi* varies with the types of solvents used in the extraction process. However, increasing the dosage of the volatile oil has a corresponding increase in toxicity to test insects.
2. Crude volatile leaf oil is slightly to non-toxic to *C. vastator* as reflected in a remarkably lower mortality compared with the standard chemical.
3. Methanol and hexane fractions, regardless of concentration, are highly toxic to *C. vastator*. However, only the dosages of 80% methanol fraction and 60 to 80% hexane fraction are comparably lethal as the standard chemical.
4. Crude volatile leaf oil is not toxic to *C. dudleyi* as shown by 0% to 10.0% mortality, while methanol- and hexane fractions are slightly to moderately toxic.
5. Mortalities in *C. vastator* and *C. dudleyi* topically applied with water or solvent are very low (0 to 3.3%), suggesting that the insects used in the test are healthy and active.
6. Hexane fraction has potential termiticidal property and its efficacy against Philippine termites should be further investigated to confirm the results of this study.

References

- Giron, M.Y., M. SP. Dionglay, C.M. Garcia and M.R. San Pablo 2008 Evaluation of the bioactivity of volatile leaf oil of three *Eucalyptus* species against wood-destroying organisms. Terminal Report. FPRDI, Los Baños, Laguna. 4031. Philippines. 34pp.
- Ismayati, M., K.H. Setiawan, D. Tarmadi and S. Yusuf 2010 Laboratory evaluation of Indonesia's essential oil against the subterranean termite *Coptotermes gestroi*. Proceedings of the 7th Conference of the Pacific Rim Termite Research Group. Singapore. 32-35 pp.
- Setiawan, K.H., M. Ismayati, D. Tarmadi and S. Yusuf 2010 Termiticidal activity of eugenol derived from Indonesian clove leaf oil (*Eugenia caryophyllata* Tumberg) against subterranean termites *Coptotermes gestroi* Wasmann. Proceedings of the 7th Conference of the Pacific Rim Termite Research Group. Singapore. 37-41pp.

Tarmadi, D., A.H. Prianto, I. Guswenrivo, T. Kartika and S. Yusuf 2007 Pengaruh ekstrak bintaro (*Carbera odollam* Gaertn) dan kecubung (*Brugmansia candida* Pers) terhadap rayap tanah *Coptotermes* sp. *Jurnal Ilmu dan Teknologi Kayu Tropis* 5(1), 38-42.

Tarmadi, D., M. Ismayati, M., K.H. Setiawan and S. Yusuf 2010 Antitermite activity of *Carbera manghas* L. seed extract. Proceedings of the 7th Conference of the Pacific Rim Termite Research Group. Singapore. 28-31 pp.

Prianto, A.H. 2010 The effectiveness of antiaris and ki pahit bark against subterranean termite *Coptotermes curvignathus* through maceration and soxhlet methods. Proceedings of the 7th Conference of the Pacific Rim Termite Research Group. Singapore. 24-27 pp.

Evaluation of the effects of *Metarhizium* (M1) for the control of subterranean termite *Coptotermes formosanus*

Nguyen Thi Phuong Thao, Nguyen Minh Duc, Vo Thu Hien and Dao Thi Lanh

Institute for Termite Control and Works Protection, Chua Boc, Dong Da District, Ha Noi City, Vietnamese

Abstract

From 9 samples *Metarhizium* (M1, M2, M3, M4, M5, M6, M7, M8, M9) collected, selected and preserved at the Institute for Termite Control and Works Protection, M1 strain was identified as *Metarhizium anisopliae*, with similar level to *Metarhizium anisopliae* FJ755244.1 strain was more than 99%. Effect of *Metarhizium anisopliae* (M1) on *Coptotermes formosanus* was the highest, with the value of LC_{50} was 8.9×10^5 spores ml^{-1} and LT_{50} was 3.7 days at 10^5 spores ml^{-1} . *Metarhizium anisopliae* (M1) strain has great potential to control *Coptotermes formosanus*.

Keywords: *Metarhizium anisopliae*, entomopathogenic fungi, pathogenicity, *Coptotermes formosanus*

Introduction

Coptotermes formosanus is a dangerous termite species for buildings and crops in many tropical countries including Vietnam. They intrude and destroy major wood and other cellulose products.

Entomopathogenic fungus *Metarhizium anisopliae* was highly effect to control termites. In some before researches, we were isolated 23 *Metarhizium* samples. From 23 fungi isolates, we selected 9 strains that were highly effective to control *Coptotermes*. The purpose of this study is to select and preserve the best effect strain and it was identified and determined the LC_{50} and LT_{50} to make a standard for preserving this strains.

Bioassay of entomopathogenic fungus *Metarhizium anisopliae* as biocontrol for subterranean termites *Coptotermes* have been conducted in *in-vitro* and *in-vivo*. The results suggest that *Metarhizium anisopliae* is the most effective fungus to control *Coptotermes* (Trinh Van Hanh et al, 2001). Recently, studying on biological insecticides to control *Coptotermes* are becoming of great interest to reduce chemical insecticides uses (Sukartana et al., 2000). *Metarhizium anisopliae* is one of several natural agents for controlling a broad range of insects by direct penetration of the host cuticle (Trinh Van Hanh 2001, Pik-Kheng H 2009, Sannasi A 1969, Myles TG 2002).

Materials and Methods

Source of termites:

Termites were collected from a residential district by wood boxes. The termite cultures were maintained at $25^{\circ}C$ ($\pm 2^{\circ}C$) temperature and 75-80% R.H.

***Metarhizium anisopliae* strains:**

Nine *Metarhizium anisopliae* strains (M1, M2, M3, M4, M5, M6, M7, M8, M9) were obtained from Institute for Termite Control and Works Protection.

Preparation of plate cultures and conidial suspensions

Cultures of all the nine isolates were maintained on Sabouraud Dextrose Agar supplemented with 1% yeast extract (SDAY) at 6.5 pH and grown at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ temperature in the dark. Conidial suspensions were prepared from 14 day SDAY plate cultures by scraping the fungal surface with a sterile surgical blade. Conidial suspensions were harvested and serially diluted in a solution of sterile distilled water with 0.02% Tween 80 to achieve 1×10^8 , 1×10^7 , 1×10^6 , 1×10^5 , 1×10^4 spores ml^{-1} .

Relative pathogenicity bioassay and Dosage mortality test

Three replicates of 100 termites (95 workers and 5 soliders) each were placed on standard plate cultures of fungal isolate for 2 hours exposure. A separate culture plate was used for each replicate.

After exposure, each group of 100 termites was carefully removed from the culture plate and placed in a plastic petri dish lined with a moistened Whatman No.1 filter paper. Termites were incubated at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ temperature in the dark.

0.5 ml aliquot of the appropriate suspension (or control suspension solution) was applied by micropipette to the dorsal surface of each termite. Conidial suspensions were prepared as previously stated. Control termites were sprayed with 0.02% solution of Tween 80 in sterile distilled water. The resulting mortality of termites was recorded at an interval of 24h up to 8 days.

Morphological Classification and Identification

Morphological classification and identification of *Metarhizium anisopliae* were carried out at Institute for Termite Control and Works Protection associate and Institute of Microbiology and Biotechnology, Vietnamese.

Results and Discussion

Effect of *Metarhizium* in the control *Coptotermes formosanus*

The initial screening for pathogenicity of nine samples indicated that all of them are virulent, affecting significantly mortality level (79-100%) by the third day. Biological control of *Metarhizium* strains were divided into three clearly groups. The highest group (group 1) was M1, mortality rate of termite was 100% after 3 days. The second group was strong effective group, consist of M2, M3, M4, M5, M7, M8, M9. Third group was normal effective group, M6 (Table 1).

Table 1. Effect of nine *Metarhizium* samples on the mortality of *C. formosanus* at different time interval

<i>Metarhizium</i> Strains	Corrected Mortality (%)				
	Days				
	1	2	3	4	5
M1	14.7	77.3	100		
M2	11.3	59.3	79.7	100	
M3	11.7	53.3	81.3	100	
M4	12	66.7	88.7	100	
M5	10	61.3	83.7	100	
M6	8	64	81	93.7	100
M7	12	70	86.3	100	
M8	10	66	91.7	100	
M9	14	68	80	100	

Morphological Classification of *Metarhizium anisopliae* (M1)

The morphological characteristics of M1 was presented in Table 2. This result showed that M1 strain had special morphological characteristics of *Metarhizium anisopliae*.

Table 2. Morphological characteristics of *Metarhizium anisopliae* (M1)

<i>Metarhizium anisopliae</i> (M1)	
Color of colony	
Top	Yellow mycelia mat with thick green conidia on the colony
Bottom	Yellowish orange, dark orange in the centre
Diameter of colony	6.22±0.09 cm
Mycelia texture	Thick, cottony and adpressed
Colony Shape Mean	Round
Conidia shape	Cylinder
Measure	(6.02- 7.30) x (2-2.5) µm
No. of days to sporulation	7

DNA Analysis

M1 strains was identified as *Metarhizium anisopliae* by DNA analysis on nucleotide sequences of the ITS1, 5.8 rDNA, ITS2, and 28S rDNA (D1/D2). The DNA analysis result of M1 was compared with *Metarhizium anisopliae* _FJ755244.1 strains on * NCBI. The result of DNA

analysis of M1 showed that M1 was similar with *Metarhizium anisopliae* _FJ755244.1 strains, more 99% (Table 3).

Table 3. *Metarhizium anisopliae* (M1) was indentified by bio-molecular techniques

Strain	Nearest species	Compared strain	Similar level	Size (base)
M1	<i>Metarhizium anisopliae</i>	FJ755244.1	99.14%	1032/1041

*NCBI: National Center for Biotechnology Information, USA.

TGTGATTATACCTTTAATTGTTGCTTCGGCGGGACTTCGCGCCCGCCGGGGACCCAA
ACCTTCTGAATTTTTTAATAAGTATCTTCTGAGTGGTTAAAAAATGAATCAAACCT
TTCAACGGATCTCTTGGTTCTGGCATCGATGAAGAACGCAGCGAAATGCGATAAGT
AATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACATTGCGCCCGT
CAGTATTCTGGCGGGCATGCCTGTTTCGAGCGTCATTACGCCCTCAAGTCCCTGTG
GACTTGGTGTGGGGATCGGCGAGGCTGGTTTTCCAGCACAGCCGTCCCTTAAATTA
ATTGGCGGTCTCGCCGTGGCCCTCCTCTGCGCAGTAGTAAAACACTCGCAACAGGA
GCCCCGCGCGGTCCACTGCCGTAACCCCAACTTTTTATAGTTGACCTCGAATC
AGGTAGGACTACCCGCTGAACTTAAGCATATCAATAAGCGGAGGAAAAGAAACCA
ACAGGGATTGCCCCAGTAACGGCGAGTGAAGCGGCAACAGCTCAAATTTGAAATCT
GGTCCCCAGGGCCCGAGTTGTAATTTGCACAGGATGCTTTTGGTGAGGTGCCTTCCG
AGTTCCTGGAACGGGACGCCATAGAGGGTGAGAGCCCCGTCTGGTTGGATACCGA
GCCTCTGTAAAGCTCCTTCGACGAGTCGAGTAGTTTGGGAATGCTGCTCTAAATGGG
AGGTATATGTCTTCTAAAGCTAAATATTGGCCAGAGACCGATAGCGCACAAAGTAGA
GTGATCGAAAGATGAAAAGCACTTTGAAAAGAGGGTTAAATAGTACGTGAAATTGT
TGAAAGGGAAGCACTTATGACCAGACTTGGCCCCGGTGAATCATCCAGCGTTCCC
GCTGGTGCACCTTGCCGGGGTTCAGGCCAGCATCAGTTCGCTCCGGGGGATAAAGG
CTTTGGGTAATGTGGCTCCCTCGGGAGTGTATAGCCCATTGCGTAATACCCTGTGG
CGGCTGAGGTTTCGCGC.

Median lethal concentration (LC₅₀)

Results of the probit – transformed mortality data obtained from applying known conidia suspensions have been used to determine the LC₅₀ for M1. LC₅₀ value of M1 strain was 8.9x10⁵ spores ml⁻¹. The data presented in Table 4 shows LC₅₀ value of M1.

Table 4. Median lethal concentration (LC₅₀) of *Metarhizium anisopliae* (M1)

<i>Metarhizium anisopliae</i> Strain	Heterogeneity (X ²)	LC ₅₀ (spores ml ⁻¹)	95% Fiducial limits (spores ml ⁻¹)
M1	5.08	8.9x10 ⁵	1.7x10 ⁵ – 2.7x10 ⁶

Median lethal time (LT₅₀)

Variation in LT₅₀ values at different concentrations were evident (Table 5). LT₅₀ values decreased with increase in concentrations. At 10⁸ spores ml⁻¹, low LT₅₀ value was 2.8 days and at 10⁵ spores ml⁻¹, LT₅₀ was 3.7 days. Dipendra Singha et al (2011), the median lethal time

(LT₅₀) of *Metarhizium anisopliae* strain (IARI) at 10⁵ spores ml⁻¹ was 2 days and LT₅₀ of *Metarhizium anisopliae* strain (PDBC) at 10⁵ spores ml⁻¹ was 3 days when they tested the effect of these strains on 30 termites, *Microtermes obesi* Holmgren. Trinh Van Hanh et al (2001), LT₅₀ of *Metarhizium anisopliae* ranged from 2.5 to 2.7 days at 10⁷ spores ml⁻¹ when they researched bio-control effect of *Metarhizium anisopliae* on *Coptotermes formosanus*.

Table 5. Median lethal time (LT₅₀) of *Metarhizium anisopliae* (M1)

<i>Metarhizium anisopliae</i> Strain	Concentration (spores ml ⁻¹)	LT ₅₀ (days)	LT ₉₀ (days)
M1	10 ⁸	2.8	3.6
	10 ⁷	2.9	3.8
	10 ⁶	3.5	4.9
	10 ⁵	3.7	5.4
	10 ⁴	4.5	6.1

Conclusion

Laboratory bioassay studies were carried with 9 *Metarhizium* samples (M1, M2, M3, M4, M5, M6, M7, M8, M9) against *Coptotermes formosanus*. In the highest mortality rate, with 100% at the third day, M1 strain was the best effective strain to control *Coptotermes formosanus*. M1 was identified as *Metarhizium anisopliae*, with similar level to *Metarhizium anisopliae* _FJ755244.1 strain, more 99 %. Effect of *Metarhizium anisopliae* (M1) strain on *Coptotermes formosanus* was the highest, with the value of LC₅₀ was 8.9x10⁵ spores ml⁻¹ and LT₅₀ was 3.7 days at 10⁵ spores ml⁻¹. The LT₅₀ value ranged from 2.8 to 3.6 days at 10⁸ spores ml⁻¹. Field applications of M1 also produced promising results.

References

- Abbott, W. S. 1925 A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology* **18**, 265-267.
- Ahmed, S., M.R. Ashraf, M.A. Hussain and M.A. Riaz 2009 Pathogenicity of isolates of *Metarhizium anisopliae* from Gujranwala(Pakistan) against *Coptotermes heimi* (Wasmann) (Isoptera: Rhinotermitidae). *Int J Agric Biol* **11**, 707–711.
- Dong, C., J. Zhang, H. Huang, Chen, W. and Y. Hu 2009 Pathogenicity of new China variety of *Metarhizium anisopliae* (*Manisopliae* var.dcjhium) to subterranean termite *Odontotermes formosanus*. *Microbiol Res* **164**, 27–35
- Finney, D.J. 1971 Probit analysis, III edn. Cambridge University Press, Cambridge, UK, 1–333 pp.
- Ignoffo, C.M. 1992 Environmental factors affecting persistence of entomopathogens. *Fla Entomol* **75**, 516–625.

Jones, W.E., K.J. Grace and M. Tamashiro 1996 Virulence of seven isolates of *Beauveria bassiana* and *Metarhizium anisopliae* to *Coptotermes formosanus* (Isoptera: Rhinotermitidae). *Environ Entomol* **25**(2), 481–487.

Khan, K.H., S. Jayaraj and M. Gopalan 1993 Muscardine fungi for the biological control of Agroforestry termite *Odontotermes obesus* (Rambur). *Insect Sci Appl* **14**(4), 529–535.

Milner, R.J., J.A. Staples and T.R.Hartly 1988 Occurrence of *Metarhizium anisopliae* in nest and feeding sites of Australian termite. *Mycol.Res.* **102**(92), 216-220.

Myles, T.G. 2002 Alarm, aggregation, and defense by *Reticulitermes flavipes* in response to a naturally occurring isolate of *Metarhizium anisopliae*. *Sociobiology* **40**, 243–255.

Pik-Kheng, H., J. Choon-Fah, K.J. Bong and R. Amartalingam 2009 Evaluation of *Metarhizium anisopliae* var. *anisopliae* (Deuteromycotina: Hyphomycete) isolates and their effects on subterranean termite *Coptotermes curvignathus* (Isoptera: Rhinotermitidae) American. *J. Agric. Biol. Sci.* **4**(4), 289–297.

Rosengans, R. and J. F.A. Traniello 1997 Part biology and Disease Transmission in damp wood termites (*Zoomtermopsis angusticollis*, Isoptera: Termopsidae) infected with the fungus *Metarhizium anisopliae* (Deuteromycotina: Hypomycetes). *Sociobiology* **30**(2), 435-439.

Trinh Van Hanh, Đinh Xuân Tuấn, Võ Thu Hiền and Trần Thu Huyền 2007 Nghiên cứu tuyển chọn một số chủng nấm *Metarhizium anisopliae* có hoạt lực cao để diệt mối *Coptotermes formosanus* hại công trình kiến trúc. *Tạp chí Nông nghiệp và phát triển nông thôn* **10,11**, 108-111.

Session 4

Systematics and Biodiversity

Diversity and distribution of termite species on oil palm plantation at the PTP Nusantara VIII Bogor, West Java-Indonesia

Arinana¹⁾, Noor Farikhah Haneda¹⁾, Tinto Punto Kahar²⁾

¹⁾Faculty of Forestry, Bogor Agricultural University, Darmaga-Bogor-Indonesia

Email: arinanaiskandaria@yahoo.co.id

²⁾Student of Faculty of Forestry, Bogor Agricultural University, Darmaga-Bogor-Indonesia

Abstract

Indonesia is one of the largest palm oil producers in the world. Today, palm oil plantations are expand to Java Island. However, there is no informations about termites species which attack palm oil plantations in Java. The study was held in Cikasungka plantation, Cigudeg Bogor-West Java-Indonesia owned by PTP Nusantara VIII. The presence of termites diversity is by identifying their attacks to palm oil stands and vicinity, by baiting a pine wood stakes (*Pinus merkusii*) with size (1.9 x 1.9 x 45.7) cm³ according to ASTM D 1758-96 2008 standard. The size of researched area is 1 hectare consisting of 4 blocks. The amount of planted wood as bait is 200 pieces. After one month, wood stakes taken and observed attacks. The key of termite identification is refer to Tho (1992) and Ahmad (1958). The results show that the entire palm oil tree (100%) in block 1 to block 4 were attacked by termites, with four species attacked in PTPN VIII Cikasungka, which are *Macrotermes gilvus*, *Coptotermes curvignathus*, *Nasutitermes javanicus*, dan *Capritermes mohri*. Whereas *Macrotermes gilvus* was dominating the distribution, and found in every block with large amount. This means that palm oil plantations on the island of Java, especially in West Java potentially attacked by termites.

Key words: oil palm plantation, subterranean termite, wood bait, *Pinus merkusii*, PTP Nusantara VIII, West Java-Indonesia

Introduction

Palm oil industry is one of the leading sectors of Indonesia and it gives high contribution to national non-oil exports. Most of the oil palm tree planted in Sumatera and Kalimantan. Termite infestation is a problem in oil palm plantations in Indonesia. The serious attack was firstly reported in the oil palm plantation PT Perkebunan Nusantara IV, Torgamba, North Sumatera (Sudartho *et al.* 1990). Termite had attacked 10 % of oil palm tree on 6-11 years old. Nowadays the case of termite attack getting higher on palm oil plantations, especially in land that converted from peat land and ex primary forest land. The opening of primary forest in peat lands to oil palm plantation often leaves wood stump scattered on the ground or in the soil thus creating habitat preferred by termites. In the first year termites will obtain an abundant food source, but in subsequent years after the termite colony grow, they have to find new food sources, including plants in the area. That is termites role as a very important pest.

Currently, the development of palm oil plantations starts to plant in Java, especially West Java. However, there is no information about the species of termites that attack palm oil plantations in Java. The problem of termite attack on palm oil plantations are not easy to control. Control efforts are often made too late after plant death. Therefore, information about the species of

termites that attack oil palm plantations is very useful in efforts to determine the method of control. So that, it is necessary to identify the species of termites that attack the oil palm plantations, particularly oil palm plantations in West Java.

Materials and methods

The study was conducted from June to October 2011 in PT. Perkebunan Nusantara VIII Cikasungka, District Cigudeg, Bogor, West Java. The identification of termites species was done in Wood Anatomy Laboratory, Department of Forest Product, Faculty of Forestry, Bogor Agricultural University. Pine wood (*Pinus merkusii* Jungh) with size (1.9 x 1.9 x 45.7) cm³ was used in this study, according to ASTM D 1758-96, 2008 standard. Identification of termite species refers to Tho (1992) and Ahmad (1958).

Distribution and diversity of termites attack to plam oil plantation

Observations of termite attack carried out directly on each oil palm tree. Observations made on the stem of the plant oil palm. If found the termites are still active, then the termites taken and put in a bottle collection that already contains alcohol 70%. The soldier caste of termites should be taken to the taxonomy study (identification).

Distribution and diversity of termites attack in the vicinity of oil palm plantation.

The initial detection of the termites presence in oil palm plantations used bait (stakes). The stakes are made from pine wood with sized (1.9 x 1.9 x 45.7) cm³ in air dry. The upper part of stakes painted with bright colors (red) to make easy during the observations. The installation of bait made in moist soil that is not disturbed by activities in the area. Each bait buried vertically into the soil as deep as 2/3 part of the length (approximately 30 cm), so the bait that is above the soil surface approximately as high as 15.7 cm. Bait timber shape and position in the field can be seen in Figure 1.

Observations of bait made every week and after one month, the bait extracted from the soil to observed the form of the damage and termite attack. Soldier castes of termite put in the bottle that contain 70% alcohol and then identify up to species. Furthermore, the infected bait cleaned and assessed on the damage by ASTM D 1758-96, 2008 standard.



Figure 1 Wood bait and its installation in the field

Results and discussion

Study site

The study was carried out in PT. Perkebunan Nusantara VIII Cikasungka, Cigudeg, Bogor, West Java-Indonesia. The total area of oil palm plantation was around 3960 hectares. The plantation is divided into 6 afdeling. The study was conducted at afdeling that has an area of approximately 706 hectares. The oil palm tree was initially planted in 2002 (211 ha), then 2003 (40 ha), 2004 (411 ha) and 2009 (44 ha). Oil palm tree planted in 9.14 m distance with triangle pattern, so there are 120-130 trees in 1 hectare. Fruit harvesting occurs every day and transferred to PT Perkebunan Nusantara Kertajaya Banten for processing. Oil palm plantations condition can be seen in Figure 2.



Figure 2 Condition of oil palm plantations in PTPN VIII Cikasungka.

Diversity of termites attack to palm oil plantation

An observation of termite attack was directly performed on oil palm plantations in afdeling 3. The trees were planted in 2006. The area of observation study was 1 ha and the amount of oil palm tree was 124 trees. The observation plot was divided into four block and each block was composed by 31 oil palm trees. Lay out the location of the study can be seen in Figure 3.

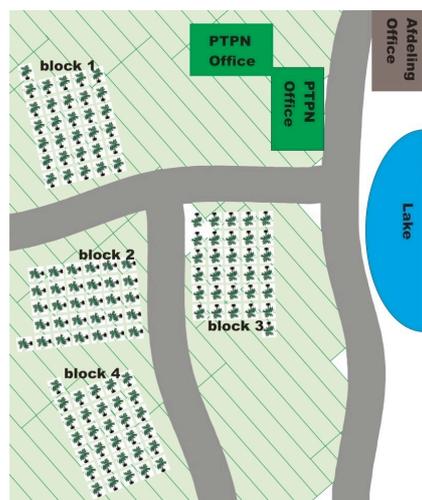


Figure 3 Layout of study site in PTPN VIII Cikasungka

The results showed that 124 oil palm trees (100%) have been infested by termites. This is strengthened by tunnels found on the palm oil trees. The infected component was the stem, fruit

bunches and flower (Figure 4). There is possibility that termites also attacking to root, but due to permit limitations, we're forbidden to observe the roots.

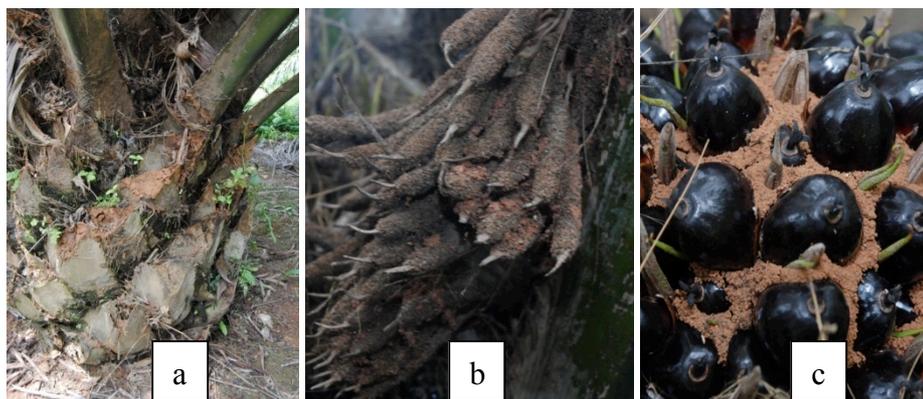


Figure 4 Palm oil component attacked by termites : stem (a),flower (b), and fruit (c).

From about 124 palm trees were observed, 46 plants have been found termites are still active while the rest found the tunnel only (78 tunnels). Based on the results of the identification of termites have been found, amounting to 87.0% or about 40 individual termites is *Macrotermes gilvus*. The rest is *Coptotermes curvignathus* (6.5% or 3 individual termites), *Nasutitermes javanicus* (4.3% or 2 individual termites) and *Capritermes mohri* (2.2% or 1 individual termite). The percentage of termites species attacked palm oil tree can be seen oin Figure 5.

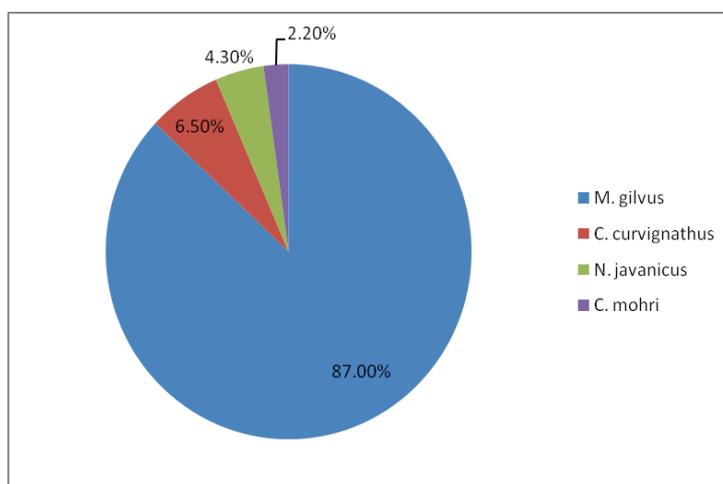


Figure 5 The diversity of termites attacked palm oil.

The distribution of termite attacked in palm oil

Subteranean termites spreads from block number 1 to block number 4 dominated by *M. gilvus* species, which found in every single block in the plantation. Meanwhile *C. curvignathus* and *N. javanicus* only found in block number 3, *C. mohri* only found in block 4. *M. gilvus* only found in

block 1 and 2. The amount of attacks and species spreads infest to palm oil plantation can be seen in Figure 6.

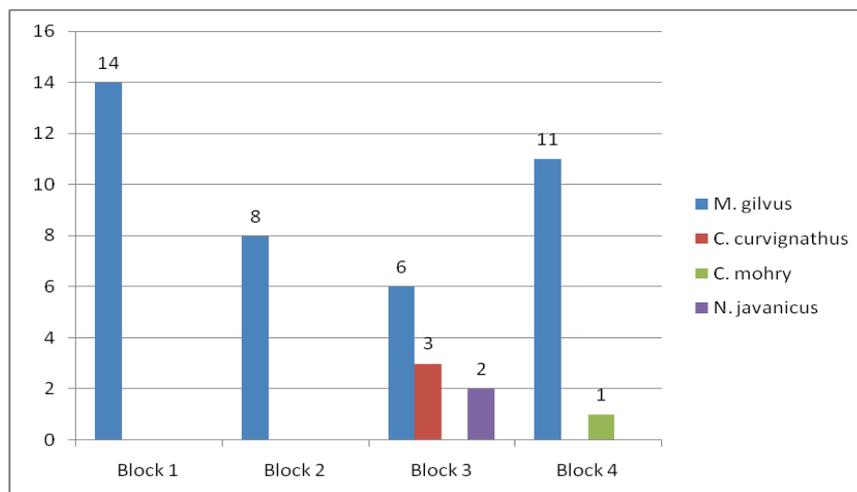


Figure 6 Amount and species of termites attacked palm oil in every block

Termites diversity in the vicinity of oil palm plantation

The amount of bait around the oil palm tree at the study site as much as 200 pieces, with the spread of each block 50 baits buried. The observation result in second week can be seen in Figure 7. In Figure 7, the wood bait was attack by termites, determine by tunnel presence along the wood bait on the soil surface.



Figure 7 Attacked wood bait by termites.

After one month, the baits were taken from the soil and observed forms of attack and identified the species of termites that attack. From 200 baits, a total of 100 (50%) bait attacked by termites. They were 13 baits in block 1(26%), 21 baits in block 2 (42%), 31 baits in block 3 (62%) and 35 baits in block 4 (70%). The percentage of attacked wood bait are 70% in block 4, followed by block 3 62%, then block 2 42% and block1 as the smallest 26%. High rate of attacks in block 4 due to its silence condition and quite far from crowd, so termites became easily to consume

wood baits. Meanwhile block 1 located near from office and road, so termites tend to dislike this condition. The percentage of attacked wood bait can be seen in Table 1.

Table 1 The percentage of in the vicinity of oil palm plantation

Block	Percentage of wood attacked (%)
1	26
2	42
3	62
4	70

The identification results, the termites that attacked wood bait were, *M. gilvus* attacked 55 baits (55.0%), *C. curvignathus* 44 baits (44.0%) and *C. mohri* only 1 bait (1.0%). The diversity of wood bait attack arrounds palm oil plantation can be seen in Figure 8.

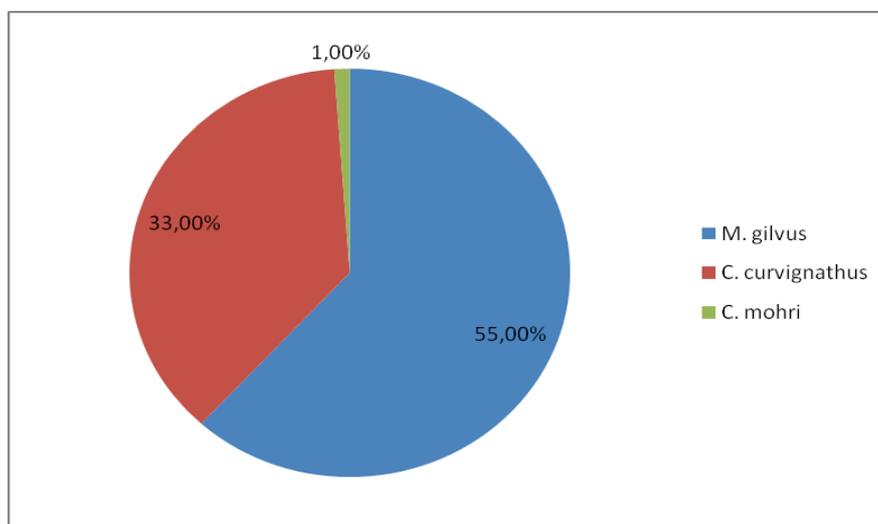


Figure 8 The diversity of termites attacked in the vicinity palm oil plantation

In comparison of termites species that attack palm oil tree, there were differences between amount of termites, which found 4 species attacked palmoil tree, and 3 species attacked wood bait. *N. javanicus* was not found in the bait. There were also differences between percentage of amount, where the affected wood bait by *C. curvignathus* was bigger than its attack to palm oil tree, wheter *M. Gilvus* still dominating. According to Nandika *et al.* (2003) *C. curvignathus* and *M. gilvus* have been reported seriously attacking palm oil plantation in many places.

Distribution of termites attack in the vicinity of oil palm plantation

The spread of termites arround palm oil tree in block 1 to block 4, as similar as in the palm oil tree, dominated by *M. gilvus*, which found in every single block. In the other hand, *C. curvignathus* was not found in block 1, and *C. mohri* only found in block 2. In Block 2 were found three species of those termites. The amount of attack and species of infected palm oil by termites can be seen in Figure 9.

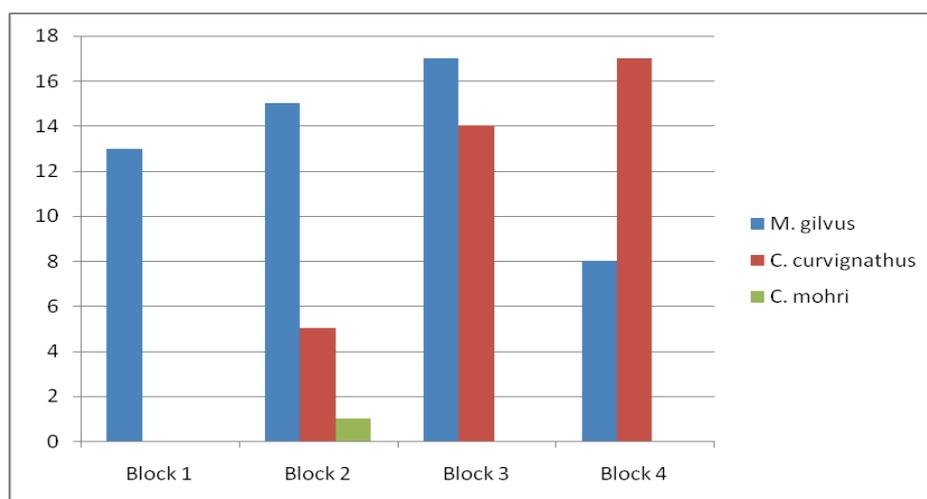


Figure 9 The amount of termites attack and species to palm oil tree and wood bait in every block located around palm oil plantation

The difference of termite attacks to palm oil tree with the attack on the wood bait, presumably because the location of wood bait is a waypath for termites in order to food hunting. Where palm oil tree is a food of termites. So, we have found different species of termites in the area between the locations of bait adjacent to attacked oil palm tree.

M. gilvus



C. curvignathus



C. mohri



N. javanicus



Termites found tend to be very dangerous for the survival of oil palm tree, because can decrease production and will eventually kill the tree. Therefore, it is necessary to prevent oil palm tree from termite infestation.

Conclusions

Based on result of the study, the entire palm oil tree (100%) in block 1 to block 4 were attacked by termites, with four species attacked in PTPN VIII Cikasungka, which are *Macrotermes gilvus*, *Coptotermes curvignathus*, *Nasutitermes javanicus*, dan *Capritermes mohri*. Whereas *Macrotermes gilvus* was dominating the distribution, and found in every block with large amount. In summary, preventive management is necessary to control termites, so they will not damage palm oil tree seriously.

References

Ahmad, M. 1958 Key to the Indomalayan Termites. Biologia. Vol 4. Department of Zoology University of the Panjab. Lahore [ASTM] American Society for Testing and Materials. 2008.

Standard Test Method of Evaluating Wood Preservatives by Field Test with Stakes. American Society for Testing and Materials. United States: ASTM D 1758-08.

Nandika, D., Y. Rismayadi, F. Diba 2003 Rayap: Biologi dan Pengendaliannya. Surakarta: Universitas Muhammadiyah Surakarta.

Sudharto, P.S., A. Sipayung and R.D. Chenon 1990 Termite, A New Problem on Oil Plantations in Indonesia. Seminar Nasional dan Diskusi Panel Perlindungan Tumbuhan Indonesia Barat. Medan 18-20 Oktober 1990.

Tho, Y.P. 1992 Termites of Peninsular Malaysia. Forest Research Institute Malaysia, Kepong, Kuala Lumpur.

Termite occurrence as pest of structures in Temenggor lake catchment area of northern Peninsular Malaysia.

Aiman Hanis J. and Abu Hassan A.

School of Biological Sciences, Universiti Sains Malaysia, 11800 Gelugor, Penang, Malaysia.

Abstract

A study was conducted to investigate the occurrence of termites as pest of building and structure in forest area developed for economic purposes in Temenggor Lake Catchment area (TLCA). Broad-surveys were carried out in seven locations by two surveyors, inspecting building and structures that may have been infested by termite. There were 17 species of termites found at which 15 of them were designated as pest species, infesting and damaging building and structures within the area. At least 3 species were recognized as the common pest in TLCA; *Globitermes sulphureus*, *Microtermes pakistanicus* and *Odontotermes sarawakensis*. Among these species, *Globitermes sulphureus* was the most important pest species.

Key words: termite, pest species, Temenggor Lake Catchment area, *Globitermes sulphureus*.

Introduction

Termites (Order: Blattodea) are eusocial insects that live together in large colonies of several hundred to several million individuals. Their occurrences in tropical rain forest are very high although they are not easily seen. They are the dominant arthropod decomposers of forest litter and other organic matters (Wood & Sand, 1978, Matsumoto & Abe, 1979). Approximately more than 2,600 species of termites in 281 genera are taxonomically known worldwide (Kambhampati & Eggleton, 2000). Common species of termites in Malaysia are *Coptotermes* spp., *Schedorhinotermes* spp., *Macrotermes* spp., *Microtermes* spp., *Odontotermes* spp. and *Globitermes sulphureus*.

The *Coptotermes* spp. was considered the most important pest species accountable for almost 85% of the total termite infestation on premises in Malaysia (Lee *et al.*, 2007). In studies conducted in Malaysian plantation forest, the *Coptotermes curvignathus* was found to be the key pest species (Aiman Hanis & Abu Hassan, 2011, Tho, 1974, Cheng *et al.*, 2008, Kirton *et al.*, 1999).

The Royal Belum State Park (RBSP) in Gerik, Perak covers a total of 290, 000 hectares of tropical rain forest that serves as water catchment area for the Temenggor Lake. It is divided into the lower and upper sections. The Upper Belum forest area stretches about 117,500 hectares of virgin forest while the lower part is known as the Temenggor Lake Catchments area (TLCA) mostly covered by secondary forest (Wong, 2003). This study was conducted in selected area within the lower section, the TLCA.

Through the North Corridor Economic Region (NCER), the government has initiated several development projects to promote RBSP as a strategic eco-tourism destination in the northern Peninsular Malaysia. In a natural rain forest that especially rich in termite species, disturbances within this area to give way to development of eco-tourism industry may cause some termite

species to thrive well in the new environment. These species may emerge as economically important pest in the area. Structures especially those made of wood and cellulosic materials are prone to termite infestation. Therefore, the objectives of this study are to investigate the occurrence of termite as the pest of structure in forest area developed for eco-tourism industry in hope to provide in-sight on future termite management plan in this area.

Materials and methods

Seven locations within the TLCA were chosen for this study. All of these locations are being disturbed by human activities and houses a number of concrete and wooden structures. Observation on the number of structures and severity of infestation were recorded. Sample collections of termites on infested structures in these disturbed areas within the forest were done using the broad-survey method by two surveyors. Structures were inspected thoroughly for any sign of active infestation. Termite specimens found were collected by hand-picking method with the use of soft forceps and preserved in universal bottles containing alcohol 70%. The samples were then be taken to the lab for species identification using key provided by Tho (1992), Thapa (1981) and Ahmad (1965).

Results and Discussion

A total of 17 species of termites from 10 genera were found during this study. Of these species, the two species of *Hospitalitermis* were not considered as pest. These termite genera feed on lichens (Chuah *et al.*, 1986, Tho, 1992), as such poses no threat to the structures. The other 15 species were found feeding and causing damage on the structures that they are designated as pest species.

Pulau Aman is a small island within the TLCA that had been cleared up for development. There are about 13 man-made structures on the island, mostly wooden. Eight of these structures were infested by termite. Three species of termite were found on these structures (Table 1). The species found infesting structures in Pulau Aman was mostly the *Globitermes sulphureus* which heavily infesting 7 out of the 13 structures and 1 co-infested by *Coptotermes* sp. accounting for about 87.5% of the total infestation. Two fairly large mounds of *Globitermes sulphureus* were found within the island.

Table 1: Termites found at Pulau Aman

Termite species	Location collected
<i>Globitermes sulphureus</i>	Wooden stairs, pillars, walls and frames
<i>Microtermes pakistanicus</i>	Tree and wooden stakes
<i>Coptotermes kalshoveni</i>	Wooden frames.

LAP water treatment facility (Pulau Banding) consists of water treatment facilities and staff quarters. The quarters were inspected and two out of the four houses were infested by termite. One of the houses (abandoned) was heavily infested by *Macrotermes* sp. The termite species found were given in Table 2.

Table 2: Termites found at LAP water treatment facility.

Termite species	Location collected
<i>Macrotermes gilvus</i>	Wooden frames within concrete house
<i>Microcerotermes serrula</i>	Arboreal nest on a tree, wooden storage room

Pulau Bendong is an island with resort facilities consisting of approximately 10 wooden structures, 1 zinc cabin and 3 concrete structures. Most of the wooden structures were infested by termite. However infestation sign found in most living quarters were non-active as treatment was said to have been conducted. However, the dining place was found to be by *Microcerotermes serrula* and at a platform next to it, *Coptotermes curvignathus* found infesting on one of the pillar. Only the praying room (surau) was heavily infested by *Havilanditermes* sp. and *Odontotermes* sp. Table 3 shows the species found infesting the structures in this island.

Table 3: Termites found at Pulau Bendong.

Termite species	Location collected
<i>Coptotermes curvignathus</i>	Wooden pillars
<i>Microcerotermes serrula</i>	Concrete wall leading to wooden pillar
<i>Havilanditermes proatripennis</i>	Wooden wall (surau)
<i>Hospitalitermes medioflavus</i>	Foraging in the open and on structures
<i>Odontotermes sarawakensis</i>	Tree stump, wooden pillar and wall (surau), wooden table

Pulau Mubaligh is an island with 9 wooden structures mostly still under construction. All of these structures were already being infested *Macrotermes* sp., *Microtermes* sp. and *Odontotermes* sp with *Microtermes pakistanicus* as the dominant pest species found on 5 different structures. There was a wooden on-ground platform found to be infested by 4 different species of termites; *Microtermes pakistanicus*, *Odontotermes oblongatus*, *Schedorhinotermes medioobscurus* and *Macrotermes gilvus* (Table 4).

Table 4: Termites found at Pulau Mubaligh.

Termite species	Location collected
<i>Microtermes pakistanicus</i>	Wooden on-ground platform, wooden bench, house frames.
<i>Odontotermes oblongatus</i>	wooden on-ground platform
<i>Schedorhinotermes medioobscurus</i>	wooden on-ground platform
<i>Macrotermes gilvus</i>	wooden on-ground platform
<i>Odontotermes grandiceps</i>	Wooden pillar, tree

Sungai Paloh Nature Camp located next to a fast-flowing stream. There was only one wooden structure (approximately 3×5 m²) present at which the condition of this structure was unusable. It was heavily infested by three species of termite (Table 5) and severely damaged.

Table 5: Termites found at Sungai Paloh Nature Camp.

Termite species	Location collected
<i>Odontotermes sarawakensis</i>	Wooden table legs, pillar, bench
<i>Schedorhinotermes medioobscurus</i>	Wooden pillars, roof
<i>Odontotermes javanicus</i>	Wooden pillar

Pulau Pertanian houses 9 wooden structures mostly living cabin and 1 concrete structure. Table 6 shows the pest species found in this island. Six of the wooden structures were infested by *Globitermes sulphureus* where 3 of these structures were heavily infested and 1 was co-infested by *Odontotermes sarawakensis*. Only one building was infested by *Nasutitermes* sp. *Microtermes pakistanicus* was also found feeding on a wooden stakes near to a living cabin During the inspection 3 relatively large mound (approximately 1m wide, 1m height each) of *Globitermes sulphureus* which explain the high infestation within the island.

Table 6: Termites found and their locations.

Termite species	Location collected
<i>Odontotermes sarawakensis</i>	Wooden wall
<i>Globitermes sulphureus</i>	Wooden pillars, wall, benches
<i>Microtermes pakistanicus</i>	On-ground wooden stake.
<i>Nasutitermes havilandi</i>	House frame, wall
<i>Nasutitermes matangensiformis</i>	Wooden wall in a living quaters

Pulau Tali Kail (Perhutanan) consists of numerous concrete and wooden structures such as living quarters, bathroom, dining hall, kitchen etc. Infestation of termites within this area was found to be low as it was well maintained by the management. Most of the mud-trails found on the structures were inactive. In-ground stations of Sentricon Baiting System were found a various spot within the island. Apart from that, most wooden structures are built above ground on concrete pillars which reduce the risk of termite infestation. However, some termite species especially Nasutitermitinae and *Microcerotermes* spp. were still able to built protective mud-trail to climb up the concrete pillars and walls. Three species were found on structures while the *Globitermes* sp. found infesting a tree near a structure (Table 7).

Table 7: Termites found and their locations.

Termite species	Location collected
<i>Globitermes sulphureus</i>	Tree trunk, mound
<i>Microcerotermes dubius</i>	Mud-trail on wooden wall
<i>Nasutitermes havilandi</i>	Mud-trail on concrete wall
<i>Hospitalitermes hospitalis</i>	Foraging in the open and on structures

It was common to find more than one species of termite infesting the same structure. Such cases occurred in five of the locations; Pulau Aman, Pulau Bendong, Pulau Mubaligh, Pulau Pertanian and Sungai Paloh Nature Camp. Lee *et al.* (2007) also reported that case of several termite species infesting premises at any one time is common in Malaysia. The most common pest species found infesting structures in TLCA were the *Globitermes sulphureus*, *Microtermes pakistanicus* and *Odontotermes sarawakensis*. These 3 species appear in at least 3 different locations. Among these three species, *G. sulphureus* was the most destructive species causing significant damages on wooden structures in locations where they present. Pulau Aman and Pulau Pertanian were found to be severely infested by this species.

This study suggests that local termite species in a natural forest area that was developed for eco-tourism and other economic activities were forced to feed on the structures and building as their

food sources were depleting. The species that thrive well in the new environment emerged as the key pest species infesting structures and building in such area.

Conclusions

There are 15 species of the 17 species of termites found during this study designated as pest species in Temenggor Lake Catchment area. The most common pest species found infesting structures in Tasik Temenggor were *Globitermes sulphureus*, *Microtermes pakistanicus* and *Odontotermes sarawakensis* with *Globitermes sulphureus* being the most important. Unlike in urban and sub-urban area where lower termites are important, most of the species found in this study are from the higher termite group. Therefore, there is a need for pest management industry to shift their focus on improving the available control methods on higher termite management.

References

- Ahmad Z 1965 Termites (Isoptera) of Thailand. *Bulletin of the American Museum of Natural History* **131**, Article 1.
- Aiman Hanis J. and A. Abu Hassan 2011 Termite incidence in an *Araucaria* plantation forest in Teluk Bahang, Penang. *Insects* **2**, 469-474.
- Cheng S., L.G. Kirton and S. Gurmit 2008 Termite attack on oil palm grown on peat soil: Identification of pest species and factors contributing to the problem. *The Planter* **84**, 659-670.
- Chuah, C.H., S.H. Goh and Y.P. Tho 1986 Soldier defense secretions of the Genus *Hospitalitermes* in Peninsular Malaysia. *Journal of Chemical Ecology* **12**, 701-712.
- Kambhampati S. and P. Eggleton 2000 Taxonomy and Phylogeny of Termites. In T. Abe, D. E. Bingell & M. Higashi (eds.). *Termites: Evolution, Sociality, Symbiosis, Ecology*. Kluwer Academic Publisher, Dordrecht, Netherlands, 1-23.
- Kirton, L.G., V.K. Brown and A. Azmi 1999 The pest status of the termite *Coptotermes curvignathus* in *Acacia mangium* plantation: Incidence, mode of attack and inherent predisposing factors. *J. Trop. Forest Sci.* **11**, 822-831.
- Lee C. Y., C. Vongkaluang and M. Lenz 2007 Challenges to Subterranean Termite Management of Multi-Genera Faunas in Southeast Asia and Australia. *Sociobiology* **50**, 213-221.
- Matsumoto, T. and T. Abe 1979 The role of termites in an equatorial rain forest ecosystem of west Malaysia. 2. Leaf litter consumption on the forest floor. *Oecologia* **38**, 261-274.
- Thapa R. S. 1981 Termites of Sabah. *Sabah Forest Record* **12**, 1-374.
- Tho, Y.P. 1974 The termite problem in plantation forestry in peninsular Malaysia. *Malaysian Forester* **37**, 278-283.
- Tho, Y.P. 1992 Termites of Peninsular Malaysia. *Malayan Forest Records* **36**.

Wong, S.L. 2003 The Royal Belum: Crowning glory of Peninsula. Perbadanan Taman Negeri Perak, 117pp.

Wood, T.G. and W.A. Sands 1978 The Role of Termites in Ecosystems. In *Production Ecology of Ants and Termites*; Brian, M.V., Ed.; Cambridge University Press, Cambridge(UK), 245-292.

Studies on termites (Isoptera) living in *Eucalyptus urophylla* S.T.Blade and *Acacia* spp. plantations in Vietnam

Nguyen Thi Bich Ngoc and Bui Thi Thuy

Forest Science Institute of Vietnam, Tuliem, Hanoi, Vietnam

Abstract

The article refers to discovery of 26 termite species live in *Eucalyptus urophylla* S.T.Blade and *Acacia* spp. plantations in Northeast, Northwest and Central Highlands of Vietnam. *Microtermes pakistanicus*, *Macrotermes barneyi* and *Macrotermes annandalei* are the most commonly species in 3 plantation regions. The number of species are richer in the first year plants, reduced in the second year plants and in the third year plants.

Key words: termites, eucalyptus, acacia, plantations

Introduction

In forestry sector, termites play an important role in metabolis cycle because they help decompose residues of plant to increase the humus for the soil; termites also are sources of food for many animals living in the forest. However, termites are a serious menace to seedling of plantation. The rate of killed eucalyptus and acacia seedling by termites in Canada, South America, Australia, South Africa, Taiwan and the Philippines is 34% to 50%, in some areas to 100% [9].

Eucalyptus spp. and *Acacia* spp. are planted with large area in Vietnam. During planting, eucalyptus and acacia species are harmed by many insects including termites. In Vietnam, some of the studies about the termites that damage the plant are conducted in the early years and the results are limited. The study about the termite species in *Eucalyptus* and *Acacia* plantations will create a scientific basis for termite control measures to protect plantation.

Materials and methods

Termite samples were collected from eucalyptus and acacia plantations in Northeast, Northwest and Central Highland of Vietnam in three years (2009-2011). The samples include of nymphs, workers, solders, reproductives and are preserved in alcohol 70⁰.

Analysing and determiting species of termite: Base on “The Fauna of Vietnam - Isoptera” 2007; the key of termite classification of Roolwal (1969), Ahmad (1965) and Thapa (1981).

Results and discussion

1. Composition of termite species in eucalyptus and acacia plantations

310 termite samples were collected in *Eucalyptus urophylla* S.T.Blade and *Acacia* spp. plantations in Northeast, Northwest and Central Highland region of Vietnam in three years (2009-2011), which determined 26 termite species belong to 11 genus, 2 families Termitidae (22 species) and Rhinotermitidae (4 species) (Table 1). This is the most complete list of termite

species composition in *Eucalyptus urophylla* S.T.Blade and *Acacia spp.* plantations in Vietnam up to now.

Table 1: List of termite species in the study regions

	Taxon	Northwest	Northeast	Central Highland
	Termitidae			
	Amitermitinae			
1	<i>Microcerotermes bugnioni</i> Holmgren 1911			+
	Macrotermitinae			
2	<i>Hypotermes makhamensis</i> Ahmad 1965	+	+	
3	* <i>Hypotermes obscuriceps</i> Wasman 1902	+	+	+
4	* <i>Hypotermes sumatrensis</i> Holmgren 1913	+	+	
5	<i>Odontotermes maesodensis</i> Ahmad 1965			+
6	* <i>Odontotermes angustignathus</i> Tsai et Chen 1963	+		+
7	<i>Odontotermes ceylonicus</i> Wasman 1902			+
8	* <i>Odontotermes hainanensis</i> Light 1924	+	+	+
9	<i>Odontotermes javanicus</i> Holmgren 1912			+
10	<i>Odontotermes yunnanensis</i> Tsai et Chen 1963	+	+	
11	<i>Macrotermes carbonarius</i> Hagen 1858			+
12	* <i>Macrotermes malaccensis</i> Haviland 1898	+		+
13	* <i>Macrotermes maesodensis</i> Ahmad 1965	+	+	
14	<i>Macrotermes gilvus</i> Hagen 1858			+
15	<i>Macrotermes annandalei</i> Silvestri 1914	+	+	+
16	<i>Macrotermes barneyi</i> Light 1924	+	+	
17	<i>Microtermes pakistanicus</i> Ahmad 1955	+	+	
	Termitinae			
18	* <i>Pericapritermes latignathus</i> Holmgren 1914	+		+
19	<i>Pericapritermes semarangi</i> Holmgren 1913		+	
20	<i>Pericapritermes nitobei</i> Shiraki 1909			+
21	* <i>Discuspiditermes garthwaitei</i> Gardner 1944	+		
	Nasutitermitinae			
22	<i>Lacessititermes albipes</i> Haviland 1898			+
	Rhinotermitidae			
	Coptotermitinae			
23	<i>Coptotermes formosanus</i> Shiraki 1909	+		
	Rhinotermitinae			
24	* <i>Schedorhinotermes javanicus</i> Kemner 1934	+		+
25	* <i>Schedorhinotermes medioobscurus</i> Holmgren 1914		+	
	Heterotermitinae			
26	* <i>Reticulitermes assamensis</i> Gardner 1944	+		

	Total	16	11	15
--	--------------	-----------	-----------	-----------

The Northeast has the most number of species (16 species, representing 61.5% of all species founded), followed by the Central Highlands (with 15 species, representing 57.6%) and the lowest number at the Northwest (there are 11 species, representing 42.3%).

Distribution characteristics of the termite species are different. Three species, include of *Hypotermes obscuriceps* Wasman; *Odontotermes hainanensis* Light and *Macrotermes annandalei* Silvestri are present in all of study areas. In contrast, the number of species of narrow distribution (only founded in each area) up to 11 species. Subfamily Macrotermitinae has the largest number of species, with *Odontotermes* and *Macrotermes* have 6 species.

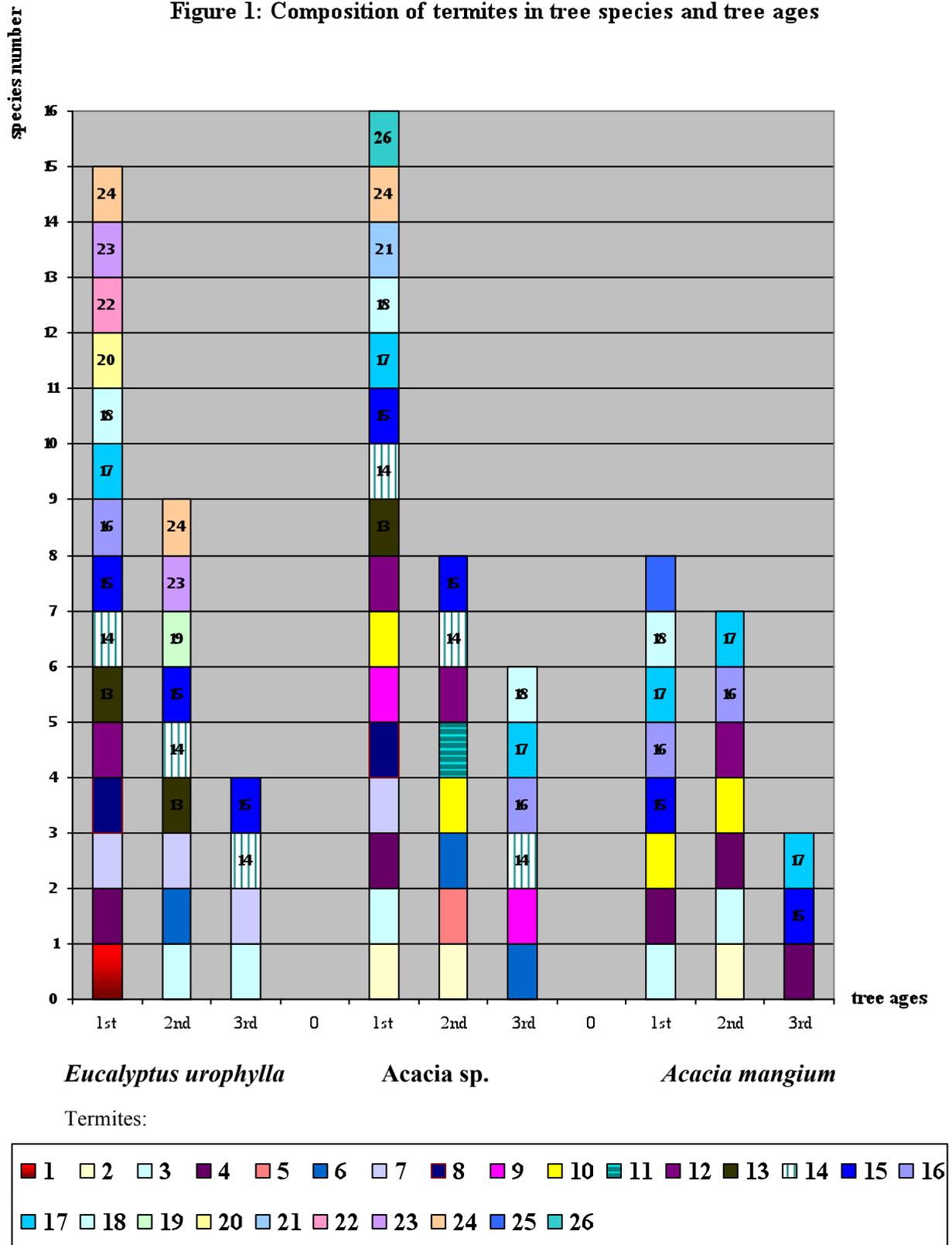
Comparing with the survey results were published, the number of termite species in the eucalyptus and acacia plantations are less than in other habitats. Comparing with the results of Nguyen Duc Kham (1976) showed that there are 61 termite species in the North of Vietnam (Nguyen Duc Kham 1976) the number of termite species in eucalyptus and acacia forests in Northeast and Northwest areas reach only 30%. In Central Highland, 48 species of termites in area with planted coffee, cocoa and rubber were founded (Nguyen Tan Vuong et al., 2007a; Nguyen Tan Vuong et al.2007b), and in eucalyptus and acacia forests are founded only 15 species (reaching 33%).

The species were first discovered in Thai Nguyen, Phu Tho, Bac Giang, Hoa Binh provinces (Northeast, Northwest of Vietnam) listed in Table 1 with the symbol (*) (Nguyen Duc Kham et al. 2007a; Nguyen Duc Kham et al. 2007b].

Termite species compositions in eucalyptus and acacia plantations are different and depend on the age of plantation. The results present in Figure 1. For example, *Coptotermes formosanus* (with No. 23 in Table 1) is only founded in *Eucalyptus urophylla* plantation with age 1-2 years; not founded in plantation *Acacia mangium* and *Acacia* hybrid. *Odontotermes yunnanensis* (No. 10, Table 1) only founded in *Acacia* forest. Species No. 8 (*Odontotermes hainanensis*) and No. 14 (*Macrotermes gilvus*) only available in eucalyptus and acacia hybrid plantations, not founded in *Acacia mangium* plantations.

Five termite species distribute widely in three regions and in all types of eucalyptus and acacia plantation, include No. 3 (*Hypotermes obscuriceps*), No. 4 (*Hypotermes sumatrensis*) No. 16 (*Macrotermes barneyi*) No. 17 (*Microtermes pakistanicus*) and specially No. 15 (*Macrotermes annandalei*). The number of termite species in eucalyptus and acacia plantations is similar (18 and 19 species).. The highest number of termite species in newly planted stage (first year), then decreased significantly at the 2nd year and 3rd year .

Figure 1: Composition of termites in tree species and tree ages



2 Common level of termites in *Eucalyptus* and *Acacia* plantations

Common level of a species is determined by the number of samples collected or percentage (%) of the sample species to the total number of samples collected in a habitat. Common level of termites in forest habitats *Eucalyptus urophylla*, *Acacia* hybrid, *Acacia mangium* plantations and other habitats are summarized in Table 2.

Table 2. Common level of termites in *Eucalyptus* and *Acacia* plantations

Species (in order of table 1)	eucalyptus urophylla		acacia hybrid		acacia mangium		other habitats	
	number	%	number	%	number	%	number	%
1	1	1						
2			14	10,4	2	2,7	2	9,1
3	4	5	4	2,9	2	2,7		
4	2	2	5	3,7	7	9,3	3	13,6
5			1	0,7				
6	2	2	4	2,9			2	9,1
7	3	4	3	2,2			2	9,1
8	1	1	2	1,5				
9			3	2,2				
10			15	11,2	6	8	4	18,2
11			2	1,5				
12	1	1	6	4,5	1	1,3		
13	5	6	12	8,9				
14	6	7	11	8,2			1	4,5
15	9	11	24	17,9	9	12	3	13,6
16	22	27,8	1	0,7	12	16		
17	12	15,2	18	13,4	33	44	4	18,2
18	2	2	3	2,2	1	1,3		
19	1	1						
20	1	1						
21			1	0,7				
22	2	2						
23	2	2						
24	3	4	2	1,5				
25					2	2,7	1	4,5
26			3	2,2				
Total	79	100	134	100	75	100	22	100

The results present in Table 2 show that in *Eucalyptus urophylla* plantations, No. 16 (*Macrotermes barneyi*) has the highest common level (accounting for 27.8%), followed by No.

17 (*Microtermes pakistanicus*) account for 15.2 % and No.15 (*Macrotermes annandalei*) account for 11%. In Acacia hybrid No. 15 presents more often (up 17.9%), followed by No.17 accounts for 13.4% and No.10 (*Odontotermes yunnanensis*) reaches 11.2%.

In *Acacia mangium* plantations, the most common species is No. 17 (44%), then followed by No. 16, No.15 and No. 10. It is notable that No. 10 is common in *Acacia* hybrid and *Acacia mangium* plantations, completely is absent in *Eucalyptus urophylla* plantations.

The species No.10, No. 15 and No. 17 are the most common in the eucalyptus and acacia plantations are also the most common species in other habitats. Maybe this is a feature to identify the major pests for eucalyptus and acacia plantations, and also to have appropriate control measures. Particularly, No.4 (*Hypotermes sumatrensis*) are abundant in other habitats, is also relatively common in *Acacia mangium* plantations, but are very little in the eucalyptus plantation (2%) and *Acacia* hybrid plantations (reaching 3.7 %).

Conclusions

1. 26 termite species belong to 11 genus, 2 families Termitidae (22 species) and Rhinotermitidae (4 species) is discovered in *Eucalyptus urophylla* S.T.Blade and *Acacia* spp. plantations in Northeast, Northwest and Central Highlands of Vietnam in three years (2009-2011).
2. The Northeast has the most number of species, followed by the Central Highlands and the lowest was Northwest. The number of species are richer in the first year plants, reduced in the second year plants and in the third year plants.
3. *Microtermes pakistanicus*, *Macrotermes barneyi* and *Macrotermes annandalei* are the most commonly species in 3 plantation regions. In the *Eucalyptus urophylla* plantations, *Macrotermes barneyi* has the highest level (up 27.8%), followed by *Microtermes pakistanicus* (15.2%) and *Macrotermes annandalei* (11%). In the *Acacia* spp. plantations, *Macrotermes annandalei* meets more often (up 17.9%), followed by *Microtermes pakistanicus* (13.4%) and *Odontotermes yunnanensis* (11.2%). In the *Acacia mangium* plantations, *Microtermes pakistanicus* has the highest level (44%), followed by *Macrotermes barneyi* and *Macrotermes annandalei*.

References

- Ahmad, M.1965 Termites of Thailand. Bull. Ame. Mus. Nat. Mis. **131**, 113.
- Nguyen Duc Kham 1976 *Termites in North Vietnam*. Science and Technology Publishing House in Hanoi, 83 -121 pp.
- Nguyen Duc Kham, Trinh Duc Hanh, Nguyen Tan Vuong, Nguyen Van Quang, Nguyen Thuy Hien, Le Van Nghien, Vu Van Trien, Vo Thu Hien, Ngo Truong Son 2007a *Fauna of Vietnam*, vol. **15**, Isoptera, Science and Technology Publisher, Hanoi: 303pp.
- Nguyen Tan Vuong Nguyen Thuy Hien, Nguyen Thi My, Ngo Truong Son, Nguyen Quoc Huy 2007b Composition of termites in the rubber, coffee and cocoa plantations in the Central Highland areas. *Journal of Agriculture and Rural Development* **11,12**, 151-153.

Roolwal, M.L. 1969 Measurement of termites (Isoptera) for taxonomic purpose.
J.Zool.Soc.Indian **21** (1), 9 - 66.

Thapa P. S. 1981 Termites of Sabah. *Sabah forest record* **12**, 374.

UNEP/FAO/ Global IPM Facility Expert Group on Termite Biology and Management
Workshop 2000.69 pp.

Biodiversity and distribution features of termites (Insecta: Isoptera) in Hanoi area, Vietnam

Nguyen Van Quang, Nguyen Hai Huyen, Ngo Kim Khue, Nguyen Thanh Huong,
VNU University of Science, VNU, 334 Nguyen Trai, Thanh Xuan, Hanoi, Vietnam

Trinh Van Hanh, Nguyen Thi My, Vo Thu Hien
Institute for Termite Control and Works Protection, Hanoi, Vietnam

Abstract

The biodiversity investigation of termites was carried out from 2008 to 2010 in 3 locations of Hanoi: Ba Vi forest, Xuan Mai hills and urban areas which were representative of the mountainous, hilly and plain areas of Hanoi respectively. A total of 57 species belonging to 3 families, 7 subfamilies and 19 genera were found. Among them, two genera *Coptotermes* and *Odontotermes* had the highest number of species (9 species for each), 9 genera (*Cryptotermes*, *Neotermes*, *Microtermes*, *Dicuspiditermes*, *Euhamitermes*, *Microcerotermes*, *Peribulbitermes*, *Havilanditermes* and *Ahmaditermes*) had the lowest number of species, each represented by only one species. Our survey result has found an addition of 9 species for the Vietnamese termite fauna. This study has provided the most sufficient checklist of termite species of Hanoi area. About the patterns of termite distribution in the studied area are as follows, 40 species (equal to 70.1% of total of species found in Hanoi) found in the mountainous area, 24 species (42.1%) in the hills, 13 species (22.8%) in the plain. We also noticed that Nasutitermitinae and Kalotermitinae (except *Cryptotermes domesticus*) and genus *Reticulitermes* were found only in the mountainous area, while Amitermitinae and Termitinae (except for *Dicuspiditermes*) distributed only in the hilly area.

Key words: biodiversity, termite, Hanoi, distribution of termite

Introduction

Termites are extremely important components of tropical ecosystems (Lee and Wood, 1971; Wood and Sands, 1978; Swift, 1979; Wilson, 1990), they are among the key decomposers in tropical terrestrial ecosystems (Bignell and Eggleton, 2000), and ecosystem engineers through their activities that help in improving soil structure and nutrient cycling (Jones et al., 1994; Levelle et al., 1997). Termites are also important food resources for many vertebrates, e.g. mammals, birds, reptiles, amphibians.

In contrast, some termite species cause significant economic damages. They are highly destructive pests that feed on wooden components of building in urban areas, and cause damage to living trees, many crop plants, power poles, railway sleepers, timber-in-service, both inside and outside of the buildings (William, 1994). Several termite species of the subfamily Macrotermitinae with hypogeal nests are harmful for dikes and dams in some countries (Nguyen Duc Kham, 1976).

The first report of termites from Hanoi area was by Nguyen Duc Kham (1976) based on the survey of termite fauna in northern Vietnam. In his study, 22 species, 10 genera and 3 families

were recorded. Bui et al. (2000) studied the termites that damage river dikes in Hanoi and found 6 termite species of two genera in the studied area. Bui et al. (2003) found an addition of 15 species for the termite fauna of Hanoi when studying the specimens collected from Ba Vi National Park. Nguyen Tan Vuong (2007) reported 6 termite species that damaged the housing in the ancient streets of Hanoi city. Although the termite taxa are well identified, no detailed studies have been done on features of species distribution in Hanoi. The present study will partially fill that gap of knowledge. The main aim of this study is to investigate the distributions of termite taxa in the different landscapes of Hanoi: mountainous, hill and plain area.

Materials and methods

Study location

Hanoi area is about 3,344.7 km² and situated by the banks of Red river between 20°53' N to 21°23' N latitude and 105°44' E to 106°02' E longitude. Hanoi is bounded by Thai Nguyen and Vinh Phuc provinces in the North, Ha Nam and Hoa Binh provinces in the South, Bac Giang, Bac Ninh, Hai Duong provinces in the East, Phu Tho province in the West.

The topography of Hanoi consists of mountainous, hilly and plain areas. The soil of Hanoi can be broadly classified into six groups, namely alluvial soils, rich soils, lateritic soils, sandy yellow clay, sandy loam soils, yellow loam soils.

Hanoi area has a warm humid subtropical climate with high precipitation. It represents the typical climate of northern Vietnam, where it is hot and humid during summer, and relatively cool and dry during winter. The summer, lasting from May to September, receives the majority of the annual rainfall. The winter is short, relatively dry, and mild, while the interval between winter and summer can bring light rains.

Our survey was conducted from 2008 to 2010 in three locations in Hanoi: Ba Vi forest, Xuan Mai hills and urban area, representative of mountainous, hilly and plain area of Hanoi respectively.

Ba Vi forest lies at an altitude of more than 600m and is located at about 55 km west of Hanoi centre. Its vegetation cover is natural forest. Xuan Mai is situated at 33 km Southwest of Hanoi centre and is plantations consist several kinds of trees e.g. pine, eucalyptus, acacias. The altitude of Xuan Mai is from 50m to 200m. Urban area of Hanoi where termite samples were implemented consists of residential quarters or villages, roads, river dikes in Hoang Mai, Hoan Kiem, Dong Da, Ba Dinh districts. This area is about 5m to 20m above sea level.

Termite sample and identification

For collecting termites in the Ba Vi forest and in Xuan Mai Hills, we applied the standardized protocol proposed by Jones and Eggleton (2000), consisting of 100 m long transects, divided into 20 successive quadrats of 5 m x 2 m. A maximum of one hour was spent in each quadrat searching for termites in all potential microhabitats, including wood, leaf litter, soil, visible nests and galleries up to a height of 2 m. Two belt transects were used for each study area.

Due to the heterogeneity of spatial distribution of termites and the complication of the habitats, besides collecting in the belt transects, we also applied the qualitative termite sample following the methodology by Nguyen Duc Kham (1976). Sampling termites was carried out randomly along a line transect of 500m to 1000m length. This protocol was also applied to survey termites in dikes and collect specimens in trees grown in urban Hanoi.

In the residential areas the termite samples were implemented in households, based on the method by Nguyen (1976). Each surveyed site consisted of 20 households. We did not use belt transect sample of termite during surveying in urban area of Hanoi.

The termite specimens were kept in small vials with alcohol of 75-80% and deposited at the laboratory of the Department of Invertebrate Zoology, Faculty of Biology, University of Science, VNU.

The morphological identification of termite was performed with the use of the documents: Measurement of Termites (Isoptera) for taxonomic purpose (Roonwal, 1989), Key to Malayan Termites (Ahmad, 1958); Termite (Isoptera) of Thailand (Ahmad, 1965); Termites of Sabah (East Malaysia) (Thapa, 1981); The Identification of Worker Castes of Termite Genera from Soils of Africa and the Middle East (Sands, 1998); Termites of Peninsular Malaysia (Tho, 1992); Fauna sinica (Insecta, Vol. 17, Isoptera) (Huang et al., 2000); Fauna Vietnamese – Termites (Isoptera), Vol.15, (Nguyen et al., 2007).

Species were also assigned to feeding groups based on *in situ* observations and gut content classification method by Donovan *et al.* (2001).

Results and discussion

Termite richness and composition

Analyzing 402 samples collected from study sites in Hanoi area we identified a total of 57 species of 19 genera, 7 Subfamilies (Kalotermitinae, Coptotermitinae, Heterotermitinae, Macrotermitinae, Termitinae, Amitermitinae and Nasutitermitinae), 3 families (Kalotermitidae, Rhinotermitidae and Termitidae) (Table 1). The family Termitidae had the highest number of species in the studied area (36 species, equal to 63.2% of the total species), while the Rhinotermitidae had 16 species (28%) and Kalotermitidae had 7 species (12.3%). There were 9 genera (47.3% of the total genera), namely *Cryptotermes*, *Neotermes*, *Microtermes*, *Dicuspiditermes*, *Euhamitermes*, *Microcerotermes*, *Peribulbitermes*, *Havilanditermes* and *Ahmaditermes*, represented by only 1 species each, 5 genera (26.5%), i.e. *Hypotermes*, *Pericapritermes*, *Procapritermes*, *Nasutitermes* and *Aciculoditermes*, each represented by 2 to 4 species, 3 genera (15.8%), *Glyptotermes*, *Macrotermes* and *Reticulitermes*, each with 5 to 7 species, and 2 genera (10.5%), *Coptotermes* and *Odontotermes* with 8 to 10 species.

In comparison with the study results by Nguyen et al. (2007) and Trinh et al. (2009), we have found additional 9 species to the Vietnamese termite fauna (*Reticulitermes setosus* Li et Xiao, *R. affinis* Hsia et Fan, *R. solidimandibulas* Li et Xiao, *Neotermes tuberosular* Xu et Han,

Glyptotermes montanus Kemner, *G. ceylonicus* (Holmgren), *G. longnanensis* Gao et Zhu, *Procapritermes mushae* Oshima et Maki, *P. minutus* Haviland).

Table 1. Species composition of termites in Hanoi area

		Feeding group	Mount. area	Hills	Plain
	KALOTERMITIDAE				
	Kalotermitinae Grasse' (3g; 7sp)				
1.	<i>Cryptotermes domesticus</i> Haviland	W	+	+	+
2.	<i>Glyptotermes almorensis</i> (Gardner)	W	+		
3.	<i>G. montanus</i> Kemner	W	+		
4.	<i>G. satsumensis</i> (Masumura)	W	+		
5.	<i>G. ceylonicus</i> (Holmgren)	W	+		
6.	<i>G. longnanensis</i> Gao et Zhu	W	+		
7.	<i>Neotermes tuberogular</i> Xu et Han	W	+		
	RHINOTERMITIDAE				
	Coptotermitinae Holmgren (2g; 14sp)				
8.	<i>Coptotermes formosanus</i> Shiraki	W	+	+	+
9.	<i>C. ceylonicus</i> Holmgren	W	+	+	+
10.	<i>C. travians</i> (Haviland)	W	+		+
11.	<i>C. curvignathus</i> Holmgren	W		+	
12.	<i>C. emersoni</i> Ahmad	W			+
13.	<i>C. dimorphus</i> Xia et He	W			+
14.	<i>C. minutus</i> Li et Huang	W			+
15.	<i>C. gestroi</i> Wasmann	W		+	+
16.	<i>Reticulitermes affinis</i> Hsia et Fan	W	+		
17.	<i>R. flaviceps</i> (Oshima)	W	+		
18.	<i>R. chinensis</i> Snyder	W	+		
19.	<i>R. setosus</i> Li et Xiao	W	+		
20.	<i>R. fukiensis</i> Light	W	+		
21.	<i>R. solidimandibulas</i> Li et Xiao	W	+		
	TERMITIDAE WESTWOOD				
	Macrotermitinae Kemner (4g; 19sp)				
22.	<i>Macrotermes annandalei</i> (Silvestri)	W&L (F)	+	+	
23.	<i>M. barneyi</i> Light	W&L (F)	+	+	
24.	<i>M. guangxiensis</i> Han	W&L (F)	+	+	
25.	<i>M. orthognathus</i> Ping et Xu	W&L (F)	+		
26.	<i>M. chebalingensis</i> Ping et Xu	W&L (F)	+		
27.	<i>Odontotermes yunnanensis</i> Tsai et Chen	W&L (F)	+	+	
28.	<i>O. malabaricus</i> Holmgren	W&L (F)	+		
29.	<i>O. assmuthi</i> Holmgren	W&L (F)	+		
30.	<i>O. hainanensis</i> Light	W&L (F)	+	+	+
31.	<i>O. formosanus</i> Shiraki	W&L (F)	+	+	+
32.	<i>O. angustignathus</i> Tsai et Chen	W&L (F)	+	+	+
33.	<i>O. giriensis</i> Roonwal and Chhotani	W&L (F)			+
34.	<i>O. proformosanus</i> Ahmad	W&L (F)			+
35.	<i>O. latigula</i> Snyder	W&L (F)		+	
36.	<i>Hypotermes sumatrensis</i> Holmgren	W&L (F)	+	+	
37.	<i>H. makhamensis</i> Ahmad	W&L (F)	+	+	
38.	<i>H. obscuriceps</i> Wassman	W&L (F)		+	
39.	<i>H. wnifredae</i> Ahmad	W&L (F)		+	
40.	<i>Microtermes pakistanicus</i> Ahmad	W&L (F)	+	+	
	Termitinae Sjostedt (3g; 6sp)				

41.	<i>Dicuspitermes garthwaitei</i> (Gardner)	S/H	+		
42.	<i>Pericapritermes nitobei</i> Shiraki	S/H		+	
43.	<i>P. tetraphilus</i> Silvestri	S/H		+	
44.	<i>Procapritermes sowerbyi</i> Light	S/H		+	
45.	<i>P. mushae</i> Oshima et Maki	S/H		+	
46.	<i>P. minutus</i> Haviland	S/H		+	
	Amitermitinae Kemner (2g; 2sp)				
47.	<i>Euhamitermes hamatus</i> Holmgren	S/H		+	
48.	<i>Microcerotermes bugnioni</i> Holmgren	W		+	
	Nasutitermitinae Hare (5g; 9sp)				
49.	<i>Nasutitermes curtinasus</i> He	W	+		
50.	<i>N. medoensis</i> Tsai et Huang	W	+		
51.	<i>N. sinensis</i> Gao et Tian	W	+		
52.	<i>N. tiangtongensis</i> Zhou et Xu	S/H	+		
53.	<i>Peribulbitermes dinghuensis</i> Li	W	+		
54.	<i>Havilanditermes atripennis</i> (Haviland)	W	+		
55.	<i>Ahmaditermes perisinuosus</i> Li et Xiao	S/H	+		
56.	<i>Aciculioiditermes holmgreni</i> Ahmad	S/H	+		
57.	<i>A. sarawakensis</i> Ahmad	S/H	+		
	∑	57	40	25	13
	%	100	70.1	43.8	22.8

W: wood feeder; W&L (F): wood and leave feeder (fungus growing termites); S/H: soil and humus feeder

Termite distribution in Hanoi

Distribution in different landscapes

Three types of landscape, mountainous, hilly and plain ones, were chosen to analyze characteristics of termite distribution in Hanoi area. As presented in Table 1, it is notable that the termites were collected primarily in the mountainous area. There were 40 species (accounting for 70.1% of the total species) found in this landscape, whereas the species number of termites found in the hills were lower (24 species, 42.1%), the lowest in the plain (13 species, 22.8%). The observed species number of termites tends to increase if one goes from the plain to the mountainous area over the hills. Moreover, our data also indicated that 7 species (*Cryptotermes domesticus*, *Coptotermes formosanus*, *C. ceylonicus*, *C. travians*, *Odontotermes hainanensis*, *O. formosanus*, and *O. angstignathus*) encountered in all three types of landscapes, 9 species (*Coptotermes curvignathus*, *C. travians*, *C. gestroi*, *Macrotermes annandalei*, *M. Barneyi*, *Odontotermes yunnanensis*, *Hypotermes sumatrensis*, *H. makhamensis*, and *Microtermes pakistanicus*) in two and 47 species in only one landscape. Eight genera (*Glyptotermes*, *Neotermes*, *Reticulitermes*, *Nasutitermes*, *Peribulbitermes*, *Havilanditermes*, *Ahmaditermes*, and *Aciculioiditermes*) were collected only in the mountainous area, four genera (*Pericapritermes*, *Procapritermes*, *Euhamitermes*, and *Microcerotermes*) only in the hills. It was noticeable that each type of landscape in Hanoi area held specific species and termites groups. This finding was an important practical contribution for protecting the works from the attack of termites in different areas of Hanoi.

Regarding the composition structure of the subfamilies, it could be realized that 3 subfamilies (Kalotermitinae, Coptotermitinae and Macrotermitinae) distributed in all three types of

landscape, 1 subfamily (Termitinae) in two types of landscape, the remaining three (Heterotermitinae, Amitermitinae and Nasutitermitinae) in only one landscape. Besides, Nasutitermitinae and Heterotermitinae presented only in mountainous area, while Amitermitinae only in hilly area. However, it is notable that the percentages of species of each subfamily in landscapes were relatively different. For instance, in the mountainous area, Macrotermitinae made up the highest percentage (35%), followed by Nasutitermitinae (22.5%) and Kalotermitinae (17.5%), while in the hills, subsequent to Macrotermitinae (50.4%) were Termitinae (21%) and Coptotermitinae (16.8%). In the plain, the subfamily with the highest percentage of species was Coptotermitinae (62.5%), followed by Macrotermitinae (33.3%) and Kalotermitinae (6.3%) (Figure 1).

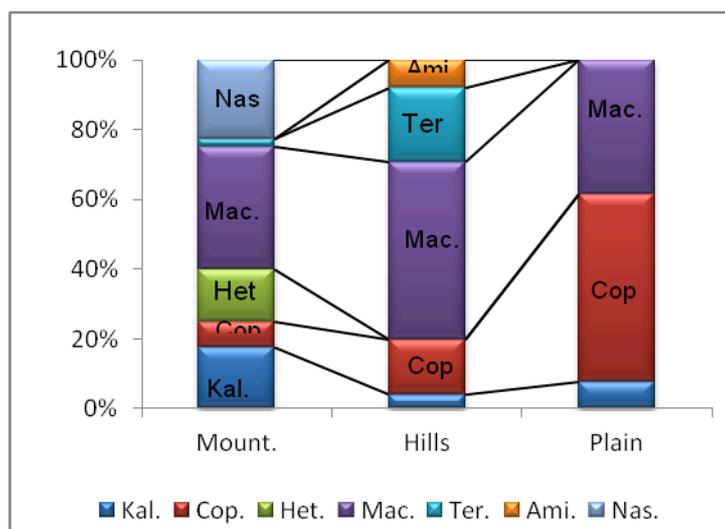


Figure 1. Taxonomic group composition of termites in the different landscapes in Hanoi area

Functional group composition of termites showed in Table 1 and Figure 2 were classified into 3 groups: wood and leave or fungus feeder (W&L (F)); soil and humus feeder (S/H); wood feeder (W). The structure of functional groups in each sampling location was different to the others. In the mountainous and the plain area, the wood feeders dominated (52 % and 61% respectively) whereas the dominant group in the hilly area was the fungus feeders (52%). The soil and humus feeders were absent in the plain.

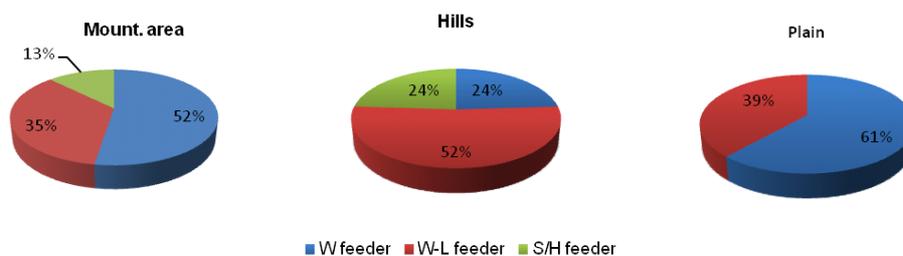


Figure 2. Functional group composition of termites in the different landscapes of Hanoi area.

Distribution of termites in different habitats in the plain

Although the termites are beneficial group in ecosystem, but on the economic aspects, some termite species are considered to be harmful, particularly in the plain area, where there are artificial habitats such as buildings, plants (timbers) and dikes. In development process, inhabitants have to protect their economic achievements, but on the other hand have to conserve the biodiversity including termites. Therefore, understanding the distribution characteristics of termites in the habitats is essential for the management of termites in the protected works.

Table 2. Distribution of termites species in different habitats of the plain.

	Scientific Name	Buildings	Plants *	Dikes
	HQ KALOTERMITIDAE			
1.	<i>Cryptotermes domesticus</i> Haviland	+		
	RHINOTERMITIDAE			
2.	<i>Coptotermes formosanus</i> Shiraki	+	+	
3.	<i>C. ceylonicus</i> Holmgren	+		
4.	<i>C. travians</i> (Haviland)	+	+	
5.	<i>C. emersoni</i> Ahmad	+	+	
6.	<i>C. dimorphus</i> Xia et He	+		
7.	<i>C. minutus</i> Li et Huang	+		
8.	<i>C. gestroi</i> Wasmann	+	+	+
	TERMITIDAE			
9.	<i>Odontotermes hainanensis</i> Light	+	+	+
10.	<i>O. formosanus</i> Shiraki		+	+
11.	<i>O. angustignathus</i> Tsai et Chen			+
12.	<i>O. giriensis</i> Roonwal and Chhotani			+
13.	<i>O. proformosanus</i> Ahmad		+	+
	Σ	9	7	6

The survey data on the species composition of termites in the plain area (Table 2) showed that there were 13 species of 3 genera found in this habitat, including 7 species of *Coptotermes*, 5 species of *Odontotermes* and 1 species of *Cryptotermes*. Genus *Coptotermes* dominated in buildings (7/9 species) while *Odontotermes* dominated in dikes (5/6 species). Both of these genera could be found feeding on plants. It was also noteworthy that some species of *Coptotermes* such as *C. formosanus*, *C. travians*, *C. emersoni*, *C. gestroi* were able to build their nest both in housings and in trees, and this finding should be taken in consideration when using solutions to prevent these termite species from damaging works.

Conclusion

A total of 57 species of 3 families, 7 subfamilies and 19 genera was found. Among them, the genera *Coptotermes* and *Odontotermes* had the largest number of species (8 species and 9 species respectively), 9 genera (*Cryptotermes*, *Neotermes*, *Microtermes*, *Dicuspiditermes*, *Euhamitermes*, *Microcerotermes*, *Peribulbitermes*, *Havilanditermes*, and *Ahmaditermes*) had the lowest number of species, each represented by only one species. To date, the present study has provided the most sufficient checklist of termites of Hanoi area.

There were 40 termite species (equal to 68.9% of the total species) found in the mountainous area, the lower number of species (24 species, 41.3%) found in the hills, and the lowest one found in the plain (15 species, 25,8%). Going from the plain over the hills to the mountainous area, the number of species, genus and subfamily tended to increase. Each type of the landscape consisted certain specific species or groups of species.

In the plain, genus *Coptotermes* dominated in the building while genus *Odontotermes* was common in dikes. Both of genera could be found in plants.

Acknowledgement

This study was supported by the Vietnam National University, Hanoi research grant. project code QGTĐ.10.05 .

References

- Ahmad, M. 1958 Key to the Indo-Malayan termites. *Biologia* **4**, 33-198.
- Ahmad, M. 1965 Termites (Isoptera) of Thailand. *Bull. Amer. Mus. Nat. His.* **133**, article 1.
- Bignell, D.E. and P. Eggleton 2000 Termites in ecosystems. In: Abe T, Higashi M, Bignell DE (eds) Termites: evolution, sociality, symbioses, ecology. Kluwer. Dordrecht.
- Bui Cong Hien, Nguyen Van Quang, Ngo Truong Son, Le Van Trien and Trinh Van Hanh 2000 Species composition of termites damaging the dikes in Hanoi and some biological characteristics of *Odontotermes hainanensis* (Isoptera: Termitidae). Proceedings of national conference on Biology, Hanoi 8-9 Aug. 2000: 367-371 (in Vietnamese).
- Huang Fusheng, Ping Zhengming, Li Guixing, Shu Shimo, He Xiusong and Gao Daorong 2000 "Isoptera", *Fauna Sinica*, Vol. 17, Science Press, Beijing, (In Chinese with English summary and keys).
- Jones, C.G., J.H. Lawton and M. Shachak 1994 Organisms as ecosystem engineers. *Oikos* **69**, 373-386.
- Lee, K.E. and T.G. Wood 1971 Termites and soils, Academic Press, New York.
- Nguyen Duc Kham, Trinh Duc Hanh, Nguyen Tan Vuong, Nguyen Van Quang, Nguyen Thuy Hien, Le Van Nghien, Vu Van Trien, Vo Thu Hien, Ngo Truong Son 2007 Fauna of Termites (Isoptera) in Vietnam. Scientific and Technical Publishing House (in Vietnamese).
- Sand, W. A. 1998 The Identification of Worker Castes of Termite Genera from Soils of Afrika and the Middle East, Cab International.
- Swift, M.J., Heal, O.W. and Anderson, J.M. 1979 *Decomposition in Terrestrial Ecosystem*. Blackwell Scientific, Oxford.
- Thapa, R. S. 1981 Termites of Sabah. *Sabah forest Record*, No.12 .

Tho, Y.P. 1992 Termites of Peninsular Malaysia. For. Res. ins. Malaysia, Kepong, Kuala Lumpur, Malayan forest records, No. 36.

Trinh V. H., Tran T.H and Nguyen, T. H. 2010 Biodiversity of termite species in Vietnam. Proceedings at the Eighth Conference of Pacific Rim Termite Research Group, Singapore.

Wilson, E.O. 1990 Success and dominance in ecosystems: the case of the social insects, Ecology Institute. Oldendorf Luhe, Germany.

Wood, T.G. and Sand, W.A. 1978 in: Brain, M.V. (ed.) Production Ecology of Ants and Termites. Cambridge University Press, Cambridge.

The characteristic of the isomorphs (Sibling species) and the heteromorphy of isoptera. The discussion on the isopteran classification

Nguyen Duc Kham

Head of Center for Bioproducts research and production, Hanoi, Vietnam.

Based on the fossil of Blatidae and termite, and the complexion as well as the anatomy, and according to many scientific document of many author, we can make a conclusion that the ancestor of termite is the blatidae.

It is supposed that the most antique termite is Mastotermitidae, it has the unique species named *M. darwinensis* living in Australia. This species has very little individual in the nest. Their nest is made very simple, that is the holes in the dry tree in the forest (it is same with the antique blatidae).

In one nest, the termites are very different from each other: the shape, the color, teeth, wings, this is called the heteromorphy in one species. The higher evolutionary termite will have more stable shape.

The termite in different nests can be copulate and reproduce because they have the same gene system and information system called phoremon, so that they can live with each other. On the other words, the phenotype is in one stable genotype.

The dispersion process to different areas base on the blow of wind, the moving of human, the good transportation of human, so that the termite will be divided into different polytype and different geographical characteristics.

In the new areas, they begin to get used to with the environment, after some generation, they will have more stable shape which is suitable with the environment and geographical condition. In the contrast, the different community which is dispersed in the same polytype and geographical characteristics, they will have same shape, in fact, there are some small different in that shape but it is very difficult to recognize. Because they belong to different community so that they cannot copulate and reproduce, this situation is called sibling species by E.Mayr.

When we classify the termite, it is important to record and keep the specimen at the dispersion areas or around dispersion areas. Some species which is widely dispersed in the world (cosmopoliste), they mostly live in human living environment.

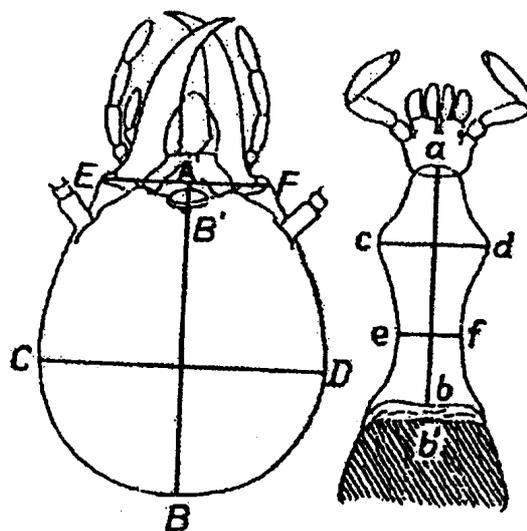
Some species belonging to coptotermitinae have nearly the same shape so that the classification is quite difficult. Due to the different biological characteristics but the same shape, it caused many difficulties for the prevention action. For example, Pro. Thai Pang Hoa said that China has only 02 species: *Coptotermes ceyloicus* and *Coptotermes formosanus* (1964).

These two species are different mostly in the vein of wings, the wings vein of *Coptotermes ceyloicus* is dark brown and the wings vein of *Coptotermes formosanus* is light yellow. But

recently document of China says that China has only one species called *Coptotermes formosanus*. But I think that Pro. Thai Bang Hoa is more reasonable because the *Coptotermes ceylolicus* species is living in the Southeast Asian. In order to classify the same species with *Coptotermes*, Light – David had created a classification table (1929) based on the size of termite's head.

Artaname	$\frac{CD}{AE}$	$\frac{EF}{CD}$	$\frac{B'B}{AB}$	$\frac{1}{2}(cd+6)$	$\frac{2ab}{cd+ej}$	$\frac{ej}{cd}$	$\frac{ab}{cd}$	$\frac{ab}{ej}$	$\frac{AB}{ed}$	$\frac{AB}{ej}$	$\frac{2AB}{e+ej}$	$\frac{AB}{eb}$
miclmeni SILYESTRI.....	0,740	0,631	0,936	0,2910	2,22	0,851	1,83	2,81	3,75	3,32	4,19	1,89
rajirayi WASMANN.....	0,756	0,720	0,924	0,3825	2,75	0,547	2,14	3,91	3,62	6,62	4,71	1,69
grandiceps SNYDER.....	0,794	0,703	0,639	0,3610	2,46	0,629	2,01	3,19	3,568	3,666	4,38	1,776
ceylonicus HOLMGREN.....	0,798	0,643	0,864	0,3506	2,25	0,018	1,82	2,95	3,25	5,27	4,02	1,78
acinaciformis (FBOGGATT)....	0,800	0,604	0,933	0,3828	2,42	0,564	1,88	3,33	3,28	5,81	4,26	1,74
jormosa pas SHIRARI	0,804	0,711	0,911	0,3272	2,65	0,604	2,13	3,52	3,73	6,17	4,65	1,75
travians HAVILAND	0,812	0,622	0,890		2,575	0,535	1,976	3,695	3,454	6,458	4,501	1,747
bomensis OSHIMA	0,818	0,684	1 +		2,576	0,512	1,947	3,803	3,332	6,508	4,407	1,711
crassas SNYDER	0,825	0,574	0,921	0,3236	2,71	0,617	2,19	3,55	3,88	6,29	4,80	1,77
lacteus (FROCCATT)	0,837	0,620	0,885	0,3655	2,09	0,720	1,80	2,50	3,32	4,61	3,86	1,84
dobonions OSHIMA	0,850	0,686	0,882	0,3230	3,03	0,520	2,30	4,42	3,84	7,38	5,05	1,67
niger SNYDER	0,860	0,616	0,900	0,3200	2,42	0,626	1,97	3,14	3,45	5,52	4,25	1,76
heimi WASMANN.....	0,866	0,560	0,928	0,3066	2,50	0,636	2,09	3,29	3,53	5,55	4,31	1,69
oshimas LIGHT & DAVIS.....	0,895	0,595	0,613	0,327	2,305	0,633	1,880	2,969	3,252	5,134	3,988	1,729
flavicephalus OSHIMA.....	0,896	0,025	0,864	0,3204	3,05	0,505	2,32	4,60	4,04	8,00	5,31	1,74
truncatus WASMANN.....	0,912	0,581	0,961	0,3421	2,11	0,610	1,60	2,97	3,27	5,36	4,06	1,92

Table. The differences of *Coptotermes*' head sizes (by Light – David, 1929)



Picture. Stipulation about points those used to measure *Coptotermes*' head size

The difference in size of termite species is very small so it is quite difficult to use this table to classify the species in *Coptotermes*.

Some document guide that we can classify the termite species based on the prickle on the termite *Coptotermes* legs. In 1981, M. Ampion and A.Queneday based on the epidemics exocrine

(where make the special smell - pheromone) to make a link between species in the evolutionary process of termite species (Phylogenetic).

Some Indian scientists base on the hair on the wings or on the lips of termite to classify species.

Comments: In 3000 species reported over the world, how many of them are really in the being? What the applicant researchers can be got from this? How many report of classification of the termite?

Thus, it is important to have a survey and research to classify the isomorphs (Sibling species) and the heteromorphy of termite, so that we can define which method is the best for the termite prevention.

Conclusion

The isomorphs (sibling species) and the heteromorphy of termite: the different species living in the same environment will have the same shape (but the gene system is different), The species which is same as *Coptotermes* also have these characteristics. However, the same land termite species living in the different biological conditions will have the different shape (different color, size, the number of internode of antenna...), that's why some species are defined with the same name 32 times, for example the species of *Macrotermes* and *ternsternes*, if we have the deep research on this, the species of *Temifidae* also have the some characteristics. This causes many difficulties for scientists to classify if they just base on the shape of termite. And in 3000 termite species announced in the world? How many of them are erroneous.

References

Nguyen Duc Kham 1976 *Termites in North Vietnam*. Science and Technology Publishing House in Hanoi, 83 -121 pp.

Nguyen Duc Kham, Trinh Duc Hanh, Nguyen Tan Vuong, Nguyen Van Quang, Nguyen Thuy Hien, Le Van Nghien, Vu Van Trien, Vo Thu Hien, Ngo Truong Son 2007 *Fauna of Vietnam 15* (Printed by Agriculture publishing house), 303pp .

Nguyen Duc Kham 1985 *Termites and termites control* (Printed by Agriculture publishing house).

Phylogeography of fungus-growing termite, *Macrotermes gilvus* (Blattodea: Termitidae) in South East Asia as inferred by mitochondrial DNA.

G. Veera Singham, Ahmad Sofiman Othman and Chow-Yang Lee

Urban Entomology Laboratory, Vector Control Research Unit, School of Biological Sciences,
Universiti Sains Malaysia, 11800 Penang, Malaysia

Abstract

The phylogeography of fungus-growing termites has not been studied, despite the potential for understanding the effects of vicariance events on population genetics and elucidating the evolutionary history of regional fauna. Here we used cytochrome c oxidase subunit II mtDNA marker to investigate the genetic differentiation of *Macrotermes gilvus* (Hagen), an endemic fungus-growing termite of Southeast Asia (SEA). Distinct patterns of geographical divisions were observed based on phylogenetic tree topologies. Dispersal events were rare and we suggest that the current distribution and genetic differentiation amongst *M. gilvus* populations were resulted from allopatric range fragmentation. Habitat stability had allowed the persistence of this termite species for a long time despite of its extremely low dispersal activity. The modern ranges of *M. gilvus* are indicative of speciation pattern of regional terrestrial fauna in accordance to the complex geological history of SEA.

Key words: phylogeography, *Macrotermes gilvus*, termite, Southeast Asia

Introduction

Southeast Asia is one of the most intriguing place on earth and of great interest to biologist and geologist. Complex history of tectonic changes, rise of mountains and sea level changes in the past had strongly influenced the distribution of flora and fauna in this region and thus warrants a detail study in understanding the evolution and patterns of speciation of terrestrial flora and fauna in this region. *Macrotermes gilvus* (Hagen), an endemic fungus-growing termite of Southeast Asia offers great potential to understand the effects of vicariance events on the genetic structure, population biology and biogeography of terrestrial fauna in this region due to its limited dispersal ability and large spatial distribution encompassing entire Southeast Asia from IndoChina, Malayan Peninsula, Singapore, Borneo island, the Philippines, and Indonesian archipelago (Snyder 1949, Roonwal 1970, Thapa 1981, Tho 1992). Mitochondrial DNA (mtDNA) is an excellent molecular marker for phylogenetics, population genetics, evolutionary and phylogeography studies (Brown et al. 1979, Marshall et al. 2009, Menke et al. 2010) as it is typically inherited maternally (Birky 2001) and hence no recombination occurs (Gyllensten et al. 1985, Marshall et al. 2009). Among the 13 protein coding genes, we chose the moderately conserved cytochrome c oxidase subunit II gene (COII) to illustrate the phylogenetic relationship of *M. gilvus* populations in South East Asia. We implemented neighbor-joining method, maximum parsimony and maximum likelihood analysis to interpret the data generated from sequence analyses. Genetic differentiation and distribution patterns of *M. gilvus* populations were inferred based on the tree topologies produced.

Materials and Methods

Sample collection. Worker termites of *M. gilvus* were sampled from 75 colonies in Peninsular Malaysia, Borneo, Singapore, West Java, Central Java, East Java, and the Philippines from August 2009 to October 2011. Samples were preserved in absolute ethanol for DNA analysis. Voucher specimens were deposited in the Vector Control Research Unit, Universiti Sains Malaysia.

Molecular techniques. Total genomic DNA was extracted from 75 whole individual worker termites each representing a single colony from various localities in Southeast Asia following CTB Tissue Extraction Kit protocol (Intron, Seongnam-Si, Gyeonggi-do, Korea) after being pulverized in liquid nitrogen. COII mtDNA gene was amplified using primers Atleu (5'-ATGGCAGATTAGTGCAATGG-3') and Btlys (5'-GTTTAAGAGACCAGTACTTG-3') (Liu and Beckenbach 1992, Simon et al. 1994), which targeted a ~ 693 bp fragment. Polymerase chain reaction (PCR) were performed in 25 µl volumes containing approximate 1 ng of template DNA, 10X PCR buffer, 1.7 mM MgCl₂, 5 mM dNTPs, 5 µM of forward and reverse primers, 0.5 U of *Taq* DNA polymerase (Promega), and sdH₂O added up to final volume. PCR amplifications were carried out on a thermal cycler (Model PTC200, MJ Research, Inc., Waltham, MA) with initial denaturing at 94 °C for 3 min, followed by 35 cycles of denaturation step at 94 °C for 30 s, annealing at 54.2 °C for 30 s, and extension at 65 °C for 3 min, followed by a final extension of 65 °C for 10 min. All the samples produced positive PCR amplifications under these conditions. PCR products were then purified using Wizard SV gel and PCR clean-up system (Promega) and sent for bidirectional sequencing to 1st BASE Laboratories Inc. (Singapore), which was conducted under BigDye terminator (Applied Biosystems, Foster City, CA). The reacted products were then purified under ethanol precipitation and ran for analysis using a DNA analyzer (Automatic Sequencer 3730xl, Applied Biosystems).

Phylogenetic analysis. The COII mtDNA sequences were edited using Molecular Evolutionary Genetic Analysis (MEGA v.4.0) software package (Tamura et. al 2007) and aligned by Clustal W incorporated in the MEGA v.4.0 under default settings. Phylogenetic relationships were initially determined using MEGA v.4.0 based on UPGMA and Neighbor-joining (NJ) method using Kimura-2 parameter genetic distance. Subsequently, Modeltest v.3.7 (Posada and Crandall 1998) was used to determine the optimal model of DNA substitution at COII mtDNA gene. Maximum likelihood (ML) tree was then reconstructed under a heuristic search in PAUP* v.4.0b10 (Swofford 2002) based on the best-fit model, which was selected by the Akaike information criterion (AIC) as implemented in Modeltest v.3.7. We chose AIC criterion for the phylogenetic tree reconstruction as it is more advantageous than the hierarchical likelihood ratio test (Posada and Buckley 2004). The reliability of the nodes in ML analysis was assessed by 100 bootstrap iterations. Additionally, Maximum parsimony (MP) analysis was performed with tree-bisection-reconnection (TBR) branch-swapping algorithm and 10 random taxon addition replicates under a heuristic search in PAUP* v.4.0b10, saving no >100 equally parsimonious trees per replicate and nonparametric bootstrap values for branch support were assessed with 1,000 bootstrap pseudo-replicates (Felsenstein 1985). The phylogenetic trees (ML and MP) were rooted with outgroups *M. malaccensis* and *M. annandalei*.

Results and Discussion

Mitochondrial DNA sequences for the COII gene were obtained from a total of 75 termites: Peninsular Malaysia (n = 15), Borneo (n = 20), Singapore (n = 9), the Phillipines (n = 6), West Java (n = 2), Central Java (n = 10) and East Java (n = 13). The mtDNA sequences specified 119 variable sites (of 693 total characters) defining 25 haplotypes. Sixty characters were found to be parsimony informative and 51 of the 60 changes were transitions. Phylogenetic analysis of the mtDNA haplotypes using NJ, MP and ML approaches produced congruent topologies that defined major geographic partitions. The consistency index (CI), homoplasy index (HI) and the retention index (RI) are 0.7574, 0.2426 and 0.8340 respectively for the MP analysis. The best-fit model of sequence evolution selected for ML analysis was HKY+I+G with the following parameter settings: Nst = 2, Base = (0.4204, 0.2368, 0.1183, and 0.2245), Tratio = 9.1960, rates = gamma, shape = 1.7872 and Pinvar = 0.7256, and a single tree was recovered for the COII gene (-ln L 1780.42475). The trees indicate that *M. gilvus* represents a monophyletic clade, with *M. malaccensis* and *M. annandalei* rooted as outgroups (Fig. 1). The basal branches were collapsed and formed polytomies in bootstrap 50 % majority-rule consensus tree topology. Five major clades (I – V) were observed in the tree topology that partitioned the taxa into distinctive geographic groups. Four haplotypes generated from 21 *M. gilvus* samples from Peninsular Malaysia and Singapore formed a monophyletic group (65 % MP/ 62 % ML bootstrap support, clade IV). Philippine population formed a monophyletic cluster (clade V) with two haplotypes generated from four individuals (80 % MP bootstrap support). A deep split was observed in Borneo with two clusters forming paraphyletic relationship (green lines in Fig. 1; 68 % MP/ 67 % ML, clade II, 96 % MP/ 92 % ML, clade III); partitioning Borneo into two distinctive phylogeographic regions (see Fig. 2). The Javanese populations were segregated into two main clusters (clade I and clade II). East Java and West Java populations formed monophyletic groups in clade I (blue lines) and II (purple lines) respectively. Central Java samples, however, showed a paraphyletic relationship with haplotypes distributing between clade I and II (red lines) suggesting a population admixture of *M. gilvus* colonies along the Java island due to either natural dispersal by alates or human mediated movement.

Despite the close proximity of main land SEA and its surrounding islands (Borneo, Java, and the Phillipines), the genetic analysis revealed a distinctive pattern of phylogeographic division in this region. Generally, *M. gilvus* populations are genetically segregated into clusters of geographic origin. Haplotype sharing across populations are rare indicating a low gene flow amongst populations. This phylogeographic patterns (genetic differentiation) suggest that dispersal events are rather rare and unlikely to have occurred recently. Thus, we hypothesized that the most recent dispersal event would probably have taken place during the Last Glacial Maximum in the presence of Sunda shelf; an exposed land mass that connects mainland Asia and the rest of Sunda islands, allowing discontinuous movements of terrestrial fauna across the regions. Interestingly, the deep split observed in the Borneo Island suggests that prehistoric geological processes may have caused the restricted gene flow between the two population clusters found on the island. Indeed, it is clear that dispersal via alates is fairly limited to short geographic distances (admixture in Java) and human mediated movement seems not to significantly influence the genetic differentiation of *M. gilvus* populations in SEA but is rather affected by past vicariance events in the region.

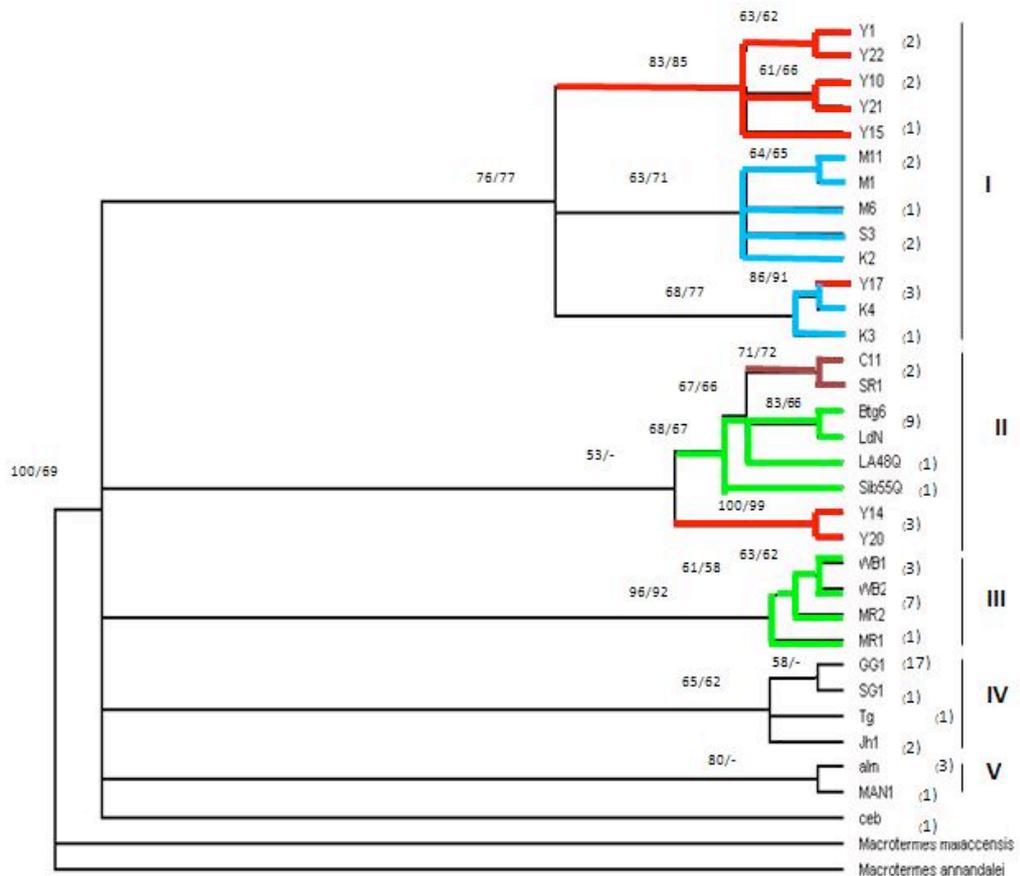


Fig. 1. Phylogenetic relationship based on rooted MP tree topology among *M. gilvus* mtDNA haplotypes. Trees derived from MP and ML have identical topologies. Numbers above branches represent bootstrap support from MP followed by ML analyses (only those above 50 % are indicated). Numbers in parantheses represents numbers of individuals sharing the same haplotypes. The alphabets I – V represent the clades.



Fig. 2. Map of Borneo showing the distinct phylogeographic division among the *M. gilvus* populations from Sarawak and Sabah. (http://www.aguide2malaysia.com/Map_Malaysia)

Conclusion

This study has demonstrated the importance of phylogeography in understanding the patterns of speciation and dispersal events of terrestrial fauna in SEA. The mtDNA sequence analyses have revealed a general perspective on the genetic differentiation of *M. gilvus* populations in SEA in accordance to their geographic distributional pattern. Further study using other mtDNA markers as well as nuclear gene markers is essential in illustrating the real extent of vicariance events on speciation of terrestrial flora and fauna of this region.

Acknowledgements

We thank pest management professionals, Ikhsan Guswenrivo, Foo Foong Kuan, Wan Nurainie, Nadiah, Aiman Hanis and Norazalily for their technical assistance in collection of the termite samples. G. Veera Singham is supported under a PhD. Scholarship provided by the USM Fellowship Scheme. The work reported here was funded by the USM Postgraduate Research Scheme USM-RU-PRGS, and Bayer Environmental Science (Singapore).

References

- Birky, C.W.Jr. 2001 The inheritance of genes in mitochondria and chloroplasts: Laws, mechanisms, and models. *Annual Review of Genetics* **35**, 125-148.
- Brown, W.M., M.J. George and A.C. Wilson 1979 Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences of the United States of America* **76**(4), 1967-1971.
- Felsenstein, J. 1985 Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783-791.
- Gyllenstein, U., D. Wharton and A.C. Wilson 1985 Maternal inheritance of mitochondrial DNA during backcrossing of two species of mice. *Journal of Heredity* **76**, 321-324.
- Liu, H. and A.T. Beckenbach. 1992. Evolution of the mitochondrial cytochrome oxidase II gene among 10 orders of insects. *Mol. Phylogenet. Evol.* **41**, 41–52.
- Marshall, H.D., M.W. Coulson, and S.M. Carr 2009 Near neutrality, rate heterogeneity, and linkage govern mitochondrial genome evolution in Atlantic cod (*Gadus morhua*) and other gadine fish. *Molecular Biology and Evolution* **26**, 579-589.
- Menke, S.B., W. Booth, R.R. Dunn, C. Schal, E.L. Vargo and J. Silverman 2010 Is it easy to be urban? Convergent success in urban habitats among lineages of a widespread native ant. *PLoS One* **5**, e9194. doi:10.1371/journal.pone.0009194
- Posada, D., and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**, 817- 818.
- Roonwal, M.L. 1970. Termites of the Oriental Region, pp. 315-384. *In*: Krishna, K., and F.M. Weesner (eds.), *Biology of termites*, vol.2. Academic, New York.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, H. Liu, and P. Flook. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann. Entomol. Soc. Am.* **87**, 651–701.

Snyder, T. E. 1949. Catalog of the termites (Isoptera) of the world. *Smithsonian Miscellaneous Collection* **112**, 1-490.

Swofford, D. L. 2002. PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sinauer, Sunderland, MA.

Tamura K, J. Dudley, M. Nei, and S. Kumar. 2007. MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) Software Version 4.0. *Mol Biol Evol* **24**, 1596–1599.

Thapa, R. S. 1981. Termites of Sabah. *Sabah Forest Record* **12**, 374p.

Tho, Y. P. 1992. Termites of Peninsular Malaysia. *Malaysia Forest Records* **36**, 224p.

Posada, D., and T. R. Buckley. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio test. *Syst. Biol.* **53**, 793- 808.

Development and isolation of 17 microsatellite loci in *Coptotermes formosanus* Shiraki

Bing-rong Liu, Jun-hong Zhong, Zhi-qiang Li, Qiu-jian Li and Wen-hui Zeng

Guangdong Entomological Institute, Guangzhou, 510260 China

Abstract

A set of primers to amplify 17 microsatellite DNA loci was developed for *Coptotermes formosanus*. A total of 32 *Coptotermes formosanus* individuals were genotyped. An average of 4.6 alleles per locus (3-8 alleles) was detected. Observed and expected heterozygosities ranged from 0.2500 to 1.0000 and from 0.5591 to 0.8562, respectively. Six loci were found to have deviated from Hardy-Weinberg equilibrium in the sampled population after Bonferroni correction. No significant linkage disequilibrium was detected. These markers will be useful in population genetics, conservation and phylogenies of the *Coptotermes formosanus*.

Keywords: *Coptotermes formosanus* , microsatellite loci, polymorphism

Introduction

The Formosan subterranean termite (FST) *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) is a major economic pest worldwide , It is thought to have originated in China, but has spread to many areas around the world where it is a highly destructive pest of wood structures (Su & Tamashiro 1987) .

These few years, many molecular genetic approaches have been used to study the genetic diversity of FST, including DNA fingerprinting (Husseneder *et al.* 2001, 2002), cuticular hydrocarbons (Haverty *et al.*1990), allozymes (Strong & Grace 1993; Wang & Grace 2000), microsatellite DNA (Vargo *et al.* 2003), and mitochondrial DNA sequencing (Jenkins *et al.* 2002; Austin *et al.*2006; Fang *et al.*2008). However, besides the problem of samples (size and numbers), many studies used molecular markers (COI, COII, 16SrDNA) detected low genetic diversity. To overcome drawbacks of some of these molecular markers, using non-specific primers, Simple Sequence Repeats (SSR) is considered as the good markers of choice, given to their abundance, high polymorphism, reproducibility, and codominant in heritance (Varshney *et al.*, 2005; Wunsch and Hormaza, 2002; Morgante *et al.*, 1998, 2002).

In this study, a set of primers to amplify 17 microsatellite DNA loci was developed for *Coptotermes formosanus*. And these microsatellite DNA loci would use to investigate the genetic diversity of FST in 209 colonies of 7 populations in China.

Materials and methods

A microsatellite enrichment protocol modified from Yu *et al.* (2010) was used to isolate loci from *C. formosanus*. The mixed genomic DNA of twenty individuals was digested with restriction enzyme *Sau* 3AI (Promega), and the size-selected DNA (400 - 900 bp) was isolated from an agarose gel using DNA purification kit (TaKaRa). Fragments were then ligated to a

blunt-end adapter (SAULA: GCGGTACCCGGGAAGCTTGG, SAULB: GATCCCAAGCTTCCCGGGTACCGC) with T4 DNA ligase (Takara) at 16°C for 14h. The ligation products were amplified by polymerase chain reaction using the adapter SAULA as primers. After denaturation at 95°C for 10 min, microsatellite-bearing amplified fragments were selected with the biotin-labeled (AC)₁₂ and (AGG)₈ (Sangon) oligonucleotide probes in sodium phosphate buffer (0.5 M sodium phosphate, 0.5% SDS, pH 7.4) at 50°C for 16h. The target fragments were amplified by PCR and the size-selected DNA (400 - 900 bp) were excised from agarose gel and recovered. These recovered DNA product were ligated with pMD19-T vector (TaKaRa) and transformed to *E. coli* DH5a competent cells (TaKaRa).

Eighty-two positive clones were identified from 196 recombinant colonies via PCR with the adapter SAULA as primers, and were sequenced with M13 primers in one direction. Forty sequences contained microsatellites, of which thirty-five possess sufficient flanking sequence appropriate for primer design. Thirty-five pairs of primers were designed by Premier 5.0 program (PREMIER Biosoft International, Silicon Valley, USA). A range of annealing temperatures (50 °C - 68 °C) were tested and the temperature producing the cleanest and strongest PCR product when observed on an 1.5% agarose gel stained with Goldview was selected for PCR. After these optimization procedures, 17 of the 35 primer sets designed amplified successfully and were used to assess polymorphism based on 32 unrelated *Coptotermes formosanus* individuals.

Genomic DNA of termites was extracted using a modified phenol-chloroform procedure as described by Zhang and Hewitt (1998). PCR was performed in a volume of 20 ul containing 50 - 100 ng of total DNA, 0.25 - 0.5 units of Taq polymerase (Tiangen, China), 19 PCR buffer, 1.0 - 2.0 mM MgCl₂, 0.2 mM dNTPs, and 0.2 - 1 uM of each primer. The PCR profile were as follows: initial denaturation at 95 °C for 3 min, followed by 30 cycles of 94 °C for 30 s, a primer-specific annealing temperature for 30 s and 72 °C for 40 s, with a final extension at 72 °C for 10 min. The PCR products were checked by electrophoresis on 8% non-denaturing polyacrylamide gel, and visualized with silver staining. Allele size was determined with software Gel-Pro Analyzer 4.5. Number of alleles, heterozygosity, test of Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were analyzed using GENEPOP4.0.6 (Raymond and Rousset 1995) and POPGENE 1.32 (Yeh *et al.*, 1999).

Results and Discussion

Conditions and characteristics of the 17 loci were showed by Table 1. The number of observed alleles per locus ranged from 3 to 8. The expected and observed heterozygosity values ranged from 0.2500 to 1.0000 and 0.5591 to 0.8562, respectively. Six loci (Copf02, Copf04, Copf09, Copf10, Copf13 and Copf17) significantly deviated from Hardy-Weinberg equilibrium after Bonferroni correction (P<0.0029). No significant linkage disequilibrium was detected.

So all the markers obtained will be useful in population genetics, conservation and phylogenies of the *Coptotermes formosanus*.

Acknowledgments We acknowledge the financial support of the National Natural Science Foundation of China (31172163) and the Guangdong Academy of Sciences Foundation for Excellent Young Scientists and Technicians (200901) to Zhiqiang Li. We are grateful to Dr. Yuchun Wu to revise the draft of the manuscript.

References

- Austin J.W., A.L. Szalanski, R.H. Scheffrahn, M.T. Messenger, J.A. Mckern and R.E. Gold 2006 Genetic evidence for two introductions of the Formosan subterranean termite, *Coptotermes formosanus* (Isoptera:Rhinotermitidae), to the United States. *Florida Entomologist* **89**, 183-193.
- Raymond M., F. Rousset 1995 Genepop (version 1.2): population genetics software for exact tests and ecumenicism. *J Hered* **86**, 248-249.
- Su N.Y. and M. Tamashiro 1987 An overview of the Formosan subterranean termite (Isoptera:Rhinotermitidae) in the world. In: Biology and Control of the Formosan Subterranean Termite. *College of Agriculture and Human Resources*, 3-15.
- Vargo E.L. and G. Henderson 2000 Identification of polymorphic microsatellite loci in the Formosan subterranean termite *Coptotermes fomosanus* Shiraki. *Mol. Ecol.* **9**,1935–1938.
- Varshney, R.K, A. Graner and M.E. Sorrells 2005 Genetic microsatellite markers in plants: features and applications. *Trends Biotechnol.* **23**, 48–55.
- Yu, D.M., B. Ma, Y.H. Sun and J.J. Peng 2010 Isolation and characterization of 16 microsatellite loci in an endangered fish Ussuri cisco, *Coregonus ussruensis*. *Conserv Genet* **11**, 1107-1109.
- Zhang D.X. and G.M. Hewitt 1998 Isolation of animal cellular total DNA. *Molecular Tools for Screening Biodiversity* (eds. A. Karp, P.G. Isaac & D.S. Ingram), Chapman & Hall, London, 5-9 pp.

Table 1 Characterization of 17 polymorphic *Coptotermes formosanus* microsatellite loci. Motif, repeat sequence of the isolated clone; *Ta*, annealing temperature; *A*, the number of alleles; *Ho*, the observed heterozygosity; *He*, expected heterozygosity and *P*, associated probability value of conformation with Hardy–Weinberg equilibrium (HWE).

Locus	Accession no.	Primer sequence (5'–3')	Motif	<i>Ta</i> (°C)	Allele size (bp)	<i>A</i>	<i>Ho</i>	<i>He</i>	<i>P</i>
Copf01	JQ313794	ATTCCTTCACTTACGCACTT GTACCCGACATCATACGC	(CT)10TT(CT)8	65	297	7	0.4074	0.7932	0.0113
Copf02	JQ313795	GGAAGAAGGACCAATCTG TAACCAAGGAGCGTAATG	(GT)8	62	190	4	0.9688	0.6652	0.0000
Copf03	JQ313796	ACCGACTCCTCTGATTGA CACATTATGTTTCCACGAC	(GT)22 (GA) 9	64	184	5	0.5806	0.5711	0.0069
Copf04	JQ313797	TACCGACTCTAACAGACA TCAGAGGATTCTTACCGA	(AC)7TC(AC)5	62	360	3	0.3125	0.7287	0.0000
Copf05	JQ313798	GCAATGAAGTGCTCTGA AACCTGGACTCGACCTTT	(AC)19	65	261	8	0.8438	0.6935	0.0827
Copf06	JQ313799	CAGTGGCAGCGACGTATA ATCCTGGAGTCCTAAGAAGC	(AC)8GC(AC)14	65	186	6	0.2500	0.6696	0.0196
Copf07	JQ313800	CTCTTTGCTGCCATACGT CTCAGTTCCATGCGGACA	(GT)18	65	242	5	1.0000	0.5591	0.0512
Copf08	JQ313801	TCAATGGCGTGCCTTCAC AGCTCAACCACTGCGTTT	(CACT)13 (CATT)16	62	263	6	0.9688	0.7277	0.1835
Copf09	JQ313802	GTGCTGGCGTTCGGTATT TTTGCTTGCCTAAAGTCG	(AC)8N(AC)6 N(AC)6	62	257	4	0.8750	0.8562	0.0001
Copf10	JQ313803	AGGTGTTGAATGGGCTGTT CCAAGCCTGCCAGAAAGT	(AC)17	65	333	3	1.0000	0.7142	0.0000
Copf11	JQ313804	CGAAGTTATGCCTCTGTT TTTGGATGCCTGGATTAG	(AC)8	62	286	4	0.8710	0.6785	0.0345
Copf12	JQ313805	GTGCTGGAGTTTGGATTT GAGGCGGTAGTAACAATAAG	(GT)6N(GT)6	62	334	3	0.8333	0.6870	1.0000
Copf13	JQ313806	TATTGTTGTTGCGGAAGC GTCGGCAGCACTGAAGTA	(GT)13	62	196	5	0.3929	0.6714	0.0000
Copf14	JQ313807	CTACAAGGCTACCATCAGG GGAACAGCGAGACGAGAT	(CT)13	64	222	4	1.0000	0.7821	0.5283
Copf15	JQ313808	TCTCCGTTATCACAGCC CAGGGAAAGCAACCACATC	(GT)16	65	323	4	0.7037	0.7659	0.1070
Copf16	JQ313809	CGTCACGTTATGGAGCAT AGCGGACTTGAGGTTAGA	(GT)16	64	267	4	0.9259	0.6981	0.0945
Copf17	JQ313810	TGTTTCACAGCCATCAGA TGCTTGGTAAATGGGTAG	(AC)12	62	184	4	1.0000	0.5714	0.0000

Session 5

Ecology and behavior

Evaluation of trail pheromone and attractants against drywood termite *Incisitermes minor* (Hagen)(Blattodea: Kalotermitidae)

Emiria Chrysanti and Tsuyoshi Yoshimura

RISH-Kyoto University, Uji, Kyoto 611-0011, Japan

Abstract

Information on the response of drywood termites towards chemicals such as trail pheromones and attractants could be valuable as lure or bait for their control. In this study, we investigated responses on workers of the drywood termite, *Incisitermes minor* (Hagen), against a candidate trail pheromone, (*Z*)-3-dodecenol, and three attractants, 2-phenoxyethanol, *d*-camphor, and maple lactone. All compounds have been reported as a trail pheromone or attractants by the circular open-field trail-following test and Y maze odor test. Using the same testing methods, we found that (*Z*)-3-dodecenol showed positive responses in the trail-following bioassay when used at amounts of 10^{-4} and 10^{-5} ng/cm. On the other hand, the odor bioassay did not show any response. As for the attractants, tests against 2-phenoxyethanol showed significant results for a number of individual test termites in both bioassays. Significant results were also found in the distance travelled by the test termites in both *d*-camphor and maple lactone circular open-field bioassays but not in the odor bioassay.

Keyword: trail pheromone, attractant, drywood termite

Introduction

Chemical communications have been suggested to play an important part in termite social interactions, including recruitment, individual orientation, and foraging activity (Abe et al. 2000, Sillam-Dussès *et al*, 2009). Since the 1960s, many reports on termite trail pheromones especially on subterranean termites have been published (Matsumura et al. 1968, Tokoro *et al*, 1991; Reinhard *et al*, 2002). In 1989, Klochkov and Pushin described the first trail pheromone of the drywood termite *Kaloterмес flavicollis* (nonan-1-ol, decan-1-ol, undecan-1-ol and dodecan-1-ol). The most recent report on drywood termite trail pheromones was given by Sillam-Dussès *et al* (2009), identifying (*Z*)-3-dodecenol as the main component from seven species of Kalotermitidae, including *Kaloterмес flavicollis*.

Besides following trail pheromones, some termite species have also been reported to follow trails using trail-pheromone mimics or attractants (Cornelius *et al*. 2009, Costa-Leonardo *et al*, 2009). However, this information is still limited simply to subterranean termites. The lack of information on chemical behavior of drywood termites is partially due to their feeding behavior being a single piece feeder (Cabrera and Scheffrahn 2005). In this study, we submitted drywood termite *Incisitermes*

minor (Hagen) workers to circular open field trail-following and Y maze odor bioassays to quantify their response to a trail pheromone and several other possible attractants.

Materials and Methods

Termites

Workers of *I. minor* were obtained from naturally infested lumbers collected from a warehouse in Wakayama Prefecture, Japan in 2009. The infested lumbers were stored and maintained in a termite rearing facility at the Research Institute for Sustainable Humansphere (RISH), Kyoto University. After being visually inspected regarding kick-out holes and pellets, the candidate lumbers were selected, cut into pieces and dissected with hand chisels and hammers, and then tapped for collecting termites. Termites were kept in the termite rearing facility at 25-26 °C and 64-65% relative humidity.

Trail Pheromone and Attractants

A synthetic trail pheromone (*Z*)-3-dodecenol (Tama Kagaku Kogyo Co, Ltd, Japan) was used for trail following experiments. Chemical attractants used in the study were 2-phenoxyethanol (Nacalai Tesque, Kyoto, Japan), known as one of trail pheromone mimics and attractants (Fei *et al.* 2005), *d*-camphor (Nacalai Tesque, Kyoto, Japan), an attractant for termites (Becker, 1971), and maple lactone (Sigma Aldrich, German), an attractant for cockroaches (Wolfe *et al.*, 1997). For untreated controls; distilled water was used for 2-phenoxyethanol and maple lactone assays, methanol for *d*-camphor assays, and hexane for (*Z*)-3-dodecenol assays.

Circular Open-field Trail-following Bioassay

An individual termite was forced to walk on a circular path drawn on a piece of filter paper applied with the chemicals. This was an adaptation of the method from Tokoro *et al.* (1991). A 7-cm diameter (22 cm circumference) circle was drawn with a pencil on a 12.5 cm diameter filter paper (Whatman no. 2, UK). Using a 25 µl micro syringe, each chemical was applied clockwise onto the line starting at the 9 o'clock position, which is also set as the launch point. Each individual termite was positioned at the launch point by using a plastic tube (1-cm in diameter with two openings) directed to the test arena. The entire assemblage was then covered with a 12-cm diameter Petri dish layered with red transparent plastic wrap (Rengo, Osaka, Japan) to shield the termite from ambient light levels. The test period was three minutes. A positive response was determined when the termite walked along the drawn line applied with the chemicals for at least 3 cm (Fig. 1). New filter papers, chemicals, and termites were used for each test. Seven different concentrations were used for (*Z*)-3-dodecenol assays (10^{-6} -1 ng/cm) and three different concentrations (0.01-1 ng/cm) were used for the other assays.

Odor Bioassay

A Y plastic maze (5-mm in both width and height, and 5-cm in both stem and branch length) was placed on a 15-cm diameter filter paper (Whatman no.2, UK). A 1cm diameter plastic cylinder with an opening was placed at the stem end of the Y plastic maize as a starter area. Twenty five μ l of each chemical vs control (solvent) were applied to separate 8-mm diameter paper discs (Tokyo Seisakusho Co., LTD, Tokyo, Japan). Each paper disc was placed inside a plastic holder at the end of the branches (Figure 1). A positive response was noted if the test termite walked for at least 6-cm from the start line (1-cm a part from starter area) within four minutes (Fig.2).

Statistical Analysis

In the circular open-field bioassay, the number of positive counts, distance travelled, and walking speed of termite was compared using non-parametric Kruskal-Wallis analysis of variance. For the odor assay, Chi-Square test was used under null hypothesis 1:1 for termite response to control vs. chemicals. All statistic analysis was done with Brightstat (www.brightstat.com).

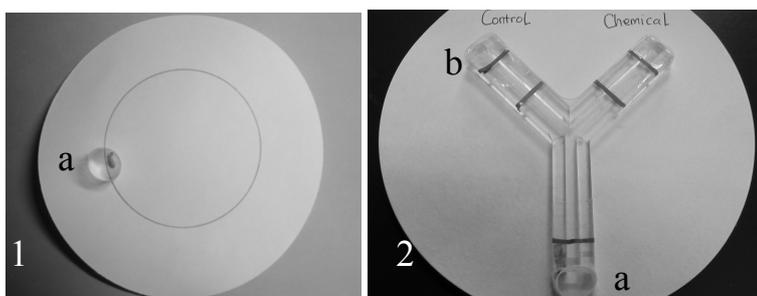


Fig. 1 Circular open-field trail-following bioassay, termite position in launch position (a).

Fig. 2 Y maze odor bioassay. (a) start point, (b) paper disc with chemical.

Results and Discussion

Circular Open-field Bioassay

Statistical analysis showed that (*Z*)-3-dodecenol gave positive responses for all concentrations tested. The lowest number of positive individuals was at 10^{-6} ng/cm ($n=16$). For distance travelled (*Z*)-3-dodecenol did not show any significant results for all concentrations ($P>0.05$) (Table 1). The control (solvents) did not show any positive response, with only 2-cm in the furthest distance travelled. Walking speed was significantly higher ($P<0.05$) at 10^{-4} ng/cm with an average of 7.4 mm/s. For bioassays with the attractants, 2-phenoxyethanol-0.01 ng/cm and *d*-camphor-0.1 ng/cm showed significant results in numbers of positive individuals ($P<0.05$). Interestingly, for 0.01 ng/cm concentration of *d*-camphor, only one positive individual was observed. In the case of distance travelled, in bioassays with *d*-camphor-0.1 ng/cm and maple lactone-0.01 ng/cm the test termites travelled for significantly longer distances (Table 2).

Table 1. *Incisitermes minor* response against (Z)-3-dodecenol in circular open-field bioassay (n=20) (means ± SE)

	Concentration						
	10 ⁻⁶	10 ⁻⁵	10 ⁻⁴	10 ⁻³	10 ⁻²	10 ⁻¹	1
Number of positive individual	16a	20a	20a	19a	18a	19a	19a
Distance travelled (cm)	17.5±1.7a	19.2±1.4a	20.5±0.9a	16.0±1.7a	16.8±1.7a	17.6±1.4a	14.3±1.7a
Walking speed (mm/s)	6.1±0.4ab	6.0±0.5ab	7.4±0.3b	6.0±0.8ab	5.2±0.6a	6.1±0.5ab	5.1±0.4a

I. minor tested for each concentration are twenty (n=20). Same small letter indicate there is no significant difference with Conover mean comparison test (P<0.05).

Table 2. *Incisitermes minor* response against attractants series in circular open-field bioassay (n=20) (means ± SE)

	2-phenoxyethanol			<i>d</i> -camphor			Maple lactone		
	10 ²	10 ¹	1	10 ²	10 ¹	1	10 ²	10 ¹	1
Number of positive individual	15c	14bc	8ab	1*	17c	3a	11bc	13bc	14bc
Distance travelled (cm)	9.2±1.5ab	6.0±1.3a	6.8±1.4ab	4.0*	13.6±2.0b	3.6±0.6a	12.6±1.9b	11.6±2.0b	11.7±2.0b
Walking speed (mm/s)	3.8±0.4a	3.7±0.6a	5.4±0.8a	2.4*	4.4±0.4a	4.8±1.3a	2.9±0.4a	3.1±0.3a	4.6±1.9a

I. minor tested for each concentration are twenty (n=20). Same small letter indicate there is no significant difference with Conover mean comparison test (P<0.05). *, the result did not consider in the statistic analysis since only showed one positive respond.

Odor Bioassay

With the Chi-Square test, a significant higher number of positive individual showed albeit to 1 ng/ul of 2-phenoxyethanol (P<0.05). Result of walking speed gave significant response (P<0.05), with the fastest speed in (Z)-3-dodecenol-10⁻⁴ ng/ul bioassays and followed by maple lactone-0.1 ng/ul bioassays (Table 3).

Table 3. *Incisitermes minor* response against trail pheromone and attractants in Y-maze odor bioassay (n=20) (means ± SE)

Chemicals	Concentration (ng/ul)	Treated/control	No response	Chi-square	Walking speed (mm/s)
(Z)-3-dodecenol	10 ⁻⁵	10/5	5	NS	3.7±0.9bc
	10 ⁻⁴	11/9	0	NS	4.9±0.6c
	10 ⁻³	13/5	2	NS	2.8±0.4b
2-phenoxyethanol	10 ⁻²	10/7	3	NS	3.8±0.7bc
	10 ⁻¹	13/5	2	NS	3.2±0.4bc
	1	15/2	3	P<0.05	4.2±0.2bc

<i>d</i> -camphor	10 ⁻²	11/8	1	NS	1.6±0.1a
	10 ⁻¹	11/9	0	NS	1.9±0.3a
	1	13/7	0	NS	2.1±0.2b
Maple lactone	10 ⁻²	10/9	1	NS	3.7±0.9bc
	10 ⁻¹	9/8	3	NS	4.3±0.9c
	1	10/9	1	NS	3.7±0.6bc

No respond, meaning termite did not choose any branch or walk less than 6-cm from the start line within four minutes.

Same small letter indicate there is no significant difference with Conover mean comparison test (P<0.05).

Our findings in the circular open-field bioassay, reinforce the results on the significance of (Z)-3-dodecenol as a trail pheromone for Kalotermitidae (Sillam-Dussès *et al* 2009, Costa-Leonardo *et al.* 2009). *I. minor* lives in their food sources, so the impact of trail following pheromone on foraging behavior is hard to discriminate from other activities that include building gallery systems, removing pellets, and taking care of the royal chamber with queen, king, eggs, and newly hatched instars. Our results also confirm the findings and observations from other researchers that drywood termites have the ability to move from one piece of wood to another (Grace *et al.* 2009), suggesting the use of trail pheromones. Comparing results of the number of positive individual response in the circular open-field bioassay, to that in the odor test for (Z)-3-dodecenol did not show any significantly positive effect. Since in the trail-following test termite had a direct contact with the chemical, lower concentration gives higher significant results, while in the odor bioassay higher concentration gives higher positive respond, especially in the 2-phenoxyethanol data. Distance between termite and chemical source is suggested to be a factor in termite respond.

2-phenoxyethanol had a trail-following and attractant effect for workers of *I. minor*. This chemical was reported as an attractant to subterranean termites (Fei *et al.* 2005). Our results on *d*-camphor in the trail-following bioassay may also support the proposal of this chemical being an attractant for termite (Becker, 1971). In Guangzhou, China, termite attacks are becoming a problem, especially in conserved ancient camphor trees, *Cinnamomum camphora* (Li *et al.* 2007). Maple lactone, a known cockroach attractant may also be useful to attract drywood termites, since our results showed the higher significant differences in distance travelled in the trail-following bioassay. Recent phylogenetic investigations using molecular genetics methods have confirmed the close association of termites to cockroaches, and have even made the recommendation that all termites are eusocial cockroaches within the order Blattodea (Inward *et al.* 2007). With the knowledge of termite attracting chemicals we could expectantly use them as a lure or supplement in bait system for drywood termite control (Yuliati, 2008), which is already reported mostly in controlling subterranean termites (Cornelius and Lax, 2005; Cornelius *et al.* 2009).

References

Abe, T., D. E. Gignell, and M. Higashi. (eds.) 2000 Termites: evolution, sociality, symbioses,

ecology., Kluwer Academic, Dordrecht.

Becker, G. 1971 Physiological influences on wood-destroying insects of wood compounds and substances produced by microorganisms. *Wood science and technology* 5,236-246.

Cabrera, B. J. and R. H. Scheffrahn 2005 Western drywood termite, *Incisitermes minor* (Hagen) (Insecta: Isoptera: Kalotermitidae). [Http://creatures.ifas.ufl.edu](http://creatures.ifas.ufl.edu). *EENY* 248.

Cornelius, M. L. and A. R. Lax 2005 Effect of summon preferred food source on feeding, tunneling and bait station discovery by the Formosan subterranean termite (Isoptera: *Rhinotermitidae*). *J. Econ. Entomol.* 98, 502-508.

Cornelius M.L., M. Lyn, K.S. Williams , M.P. Lovisa, A.J. de lucca and A.R. Lax 2009 Efficacy of bait supplements for improving the rate of discovery of bait stations in the field by formosan subterranean termites (Isoptera: *Rhinotermitidae*). *J. Econ. Entomol.* 102(3), 1175-1181.

Costa-Leonardo, A. M., F. E. Casarin and J. T. Lima 2009 Chemical communication in Isoptera. *Neotropical Entomology* 38(1), 1-6.

Fei, H., G. Henderson and R. A. Laine 2005 Trail-following behavior of *Coptotermes formosanus* (Isoptera: *Rhinotermitidae*) on concentration gradients of 2-phenoxyethanol. *Sociobiology* 45, 483-494.

Grace, J. K., R. J. Woodrow and R. J. Oshiro 2009 Expansive gallery systems of one-piece termites (Isoptera: *Kalotermitidae*). *Sociobiology* 54 (1), 37-44.

Klochkov, S.G., A.N. Pushin 1989 Trail pheromone of *Kalotermes flavicollis*. *Chem. Nat. Comp.* 25,115-118.

Li, D., Z. Suo and B. Man-zhu 2007 Effect of plant growth regulators on direct somatic embryogenesis in camphor tree (*Cinnamomum camphora* L.) from immature zygotic embryos and embryogenic calli induction. *For. Stud. China* 9(4), 267–271

Matsumura, F., H. C. Coppel and A. Tai 1968 Isolation and identification of termite trail-following pheromone. *Nature* 219, 963-964.

Reinhard, J., M. J. Lacey, F. Ibarra, F. C. Schroeder, M. Kaib and M. Lenz 2002 Hydroquinone: a

general phagostimulating pheromone in termites. *J. Chem. Ecol.* 28, 1-14.

Sillam-Dussès, D., E. Sémon, A. Robert and C. Bordereau 2009 (Z)-dodec-3-en-1-ol, a common major component of the trail-pheromone in the termites Kalotermitidae. *Chemoecology* DOI 10.1007/s00049-009-0017-7.

Tokoro, M., M. Takahashi, K. Tsunoda, R. Yamaoka, and K. Hayashiya 1991 Isolation and identification of the trail pheromone of the subterranean termite *Reticulitermes speratus* (Kolbe) (Isoptera: Rhinotermitidae). *Wood Research* 78, 1-14.

Wolfe J., D. Leseiwicz, W. Mehra, J. Mares 1997 Cockroach bait stimuli. US PATENT 5676961. www.brightstat.com.

Yuliati I., Y. Tsuyoshi and I. Yuji 2008 A novel control strategy for dry-wood termite *Incisitermes* minor infestation using a bait system. *Journal of Wood Science* 3, 220-224.

Parasitism by parasitoid *Misotermes mindeni* (Diptera: Phoridae) increases frequencies of trophallaxis and allogrooming in *Macrotermes gilvus* (Blattodea: Termitidae)

Foong-Kuan Foo, Ahmad Sofiman Othman and Chow Yang-Lee

Urban Entomology Laboratory, Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

Abstract

This study compared the frequencies of trophallaxis and allogrooming between workers of parasitized major soldiers (PAMS) and workers of unparasitized major soldiers (UPMS) and examined if the frequencies of trophallaxis and allogrooming between workers and UPMS and PAMS were influenced by the existence of PAMS or UPMS in the colony, respectively. Results indicated that the frequencies of trophallaxis and allogrooming of workers/PAMS were significantly greater ($P < 0.05$) than that of workers/UPMS. There were no significant differences ($P > 0.05$) in the frequencies of trophallaxis and allogrooming between workers and PAMS in the group of termites with or without UPMS. Similarly, no significant difference ($P > 0.05$) in the frequency of trophallaxis between workers and UPMS was observed in the groups with or without PAMS. However, workers of UPMS displayed significantly greater ($P < 0.05$) allogrooming behavior when PAMS were present. The implications of the behavioral changes of *Macrotermes gilvus* (Hagen) (Blattodea: Termitidae) carrying larval parasitoid of *Misotermes mindeni* Disney and Neoh (Diptera: Phoridae) were discussed.

Key words: *Macrotermes gilvus*, trophallaxis, grooming, endoparasitoid, host-parasitoid interaction

Introduction

The development of the parasitoid is generally relied on resources provided by a single host (Barber et al. 1988). The dependence of parasitoids' development on their host may lead to numerous behavioral modifications of the latter including behavioral fever, foraging, feeding, reproduction, and various social interactions such as trophallaxis and grooming (Loehle 1995, Roy et al. 2006, Libersat et al. 2009, Neoh and Lee 2010). Oftentimes, these behavioral changes are deliberated to be adaptive for either host or parasite (Poulin 1995).

Trophallaxis is the transfer of regurgitated food among members in colonies through stomodeal (mouth to mouth) and proctodeal (anus to mouth) contact. Only stomodeal feeding occurs in *Macrotermes gilvus* (Hagen) (Blattodea: Termitinae) (McMahan 1969). However, soldiers of *M.*

gilvus were unable to feed themselves and they were thus completely dependent on workers for nutrition (Huang et al. 2008). This was largely due to the modification of their mandibles for purposes of defense (Huang et al. 2008).

Allgrooming, a unique behavior of termite, is an activity of cleaning the body surface and appendices of another individual (Maistrello and Sbrenna 1996). This behavior serves for both hygienic and social functions (Loehle 1995).

The aims of the present study were to compare the frequencies of trophallaxis and allgrooming between workers/parasitized major soldiers (PAMS) and workers/unparasitized major soldiers (UPMS) and to examine if the frequencies of trophallaxis and allgrooming between workers and UPMS and PAMS were influenced by the existence of PAMS or UPMS in the colony, respectively.

Materials and Methods

Sample collection. PAMS and UPMS were randomly collected from previously surveyed colonies at Minden Campus of Universiti Sains Malaysia (Foo et al. 2011). To break up the mound, we dug a trench around the base of the nest and subsequently applied sideways pressure to remove the mound casing. PAMS were identified based on a rounded head capsules with remarkably short mandibles (Neoh and Lee 2010).

Frequencies of trophallaxis and allgrooming. To determine if the frequencies of trophallaxis and allgrooming between workers and UPMS and PAMS were influenced by the existence of PAMS or UPMS in the colony, respectively, the following termite groups were formed. Six PAMS and six UPMS were maintained together with 42 major and 58 minor workers in a plastic container (9.5 cm diameter x 6.5 cm height) in the first experiment, while workers were combined with either 12 PAMS or 12 UPMS in the second experiment. The proportion of workers and soldiers in each group was determined based on Lee and Lee (2011). Twenty gram of moist sand and 3 g of fungus comb were added in each container. The containers were subsequently arranged in an opaque plastic box (35.0 cm x 23.5 cm x 19.5 cm) with a glass cover and were kept in total darkness at 30 ± 1 °C and 90% relative humidity. After three days of the experimental setup, trophallaxis and allgrooming behaviors of termites were video-recorded using a digital camera (Canon Digital Ixus 80 IS, Japan) at 0700, 1200, and 1700 hours daily over three days. Each video was recorded for 30 min under red light, starting 10 min later to allow the termites to acclimate to the observation conditions. Trophallaxis was considered to occur if the mouthparts of the workers and major soldiers were held in close contact for at least 30 s (Sieber and Leuthold 1981), while allgrooming was regarded as taking place if the workers moved their mouthparts to clean the body surface and appendices of the major soldiers (Maistrello and Sbrenna 1996). Concurrently, the frequencies of trophallaxis and

allogrooming in every sample period (30 min) were also recorded. A total of four colonies were observed.

Statistical analysis. We compared the frequencies of trophallaxis and allogrooming behaviors between workers/PAMS and workers/UPMS using Student *T*-test (SPSS version 12.0, Inc., Chicago, IL). A similar test was also used to examine if the frequencies of trophallaxis and allogrooming between workers and UPMS and PAMS were influenced by the existence of PAMS or UPMS in the colony, respectively.

Results and Discussion

The frequencies of trophallaxis and allogrooming between workers/PAMS were significantly higher ($P < 0.05$) than that between workers/UPMS (Table 1). The increased in food demands of PAMS was likely to ensure that they have sufficient food for their growth and development in the host, while extensive allogrooming was essential to remove the foreign particles from the host's outer body surface body to ensure long-term survival of the host.

Table 1. Frequencies of trophallaxis and allogrooming of workers/PAMS and workers/UPMS in the tested group

Experimental unit	Behavior	Major soldier	n	Mean \pm SE	Range	t	df	P
Workers together with PAMS and UPMS	Trophallaxis	PA	4	0.14 \pm 0.05	0.07–0.18	4.911	3	$P < 0.05$
		UP	4	0.01 \pm 0.01	0.00–0.02			
	Allogrooming	PA	4	0.63 \pm 0.16	0.42–0.81	5.317	6	$P < 0.01$
		UP	4	0.19 \pm 0.04	0.15–0.23			
Workers with either PAMS or UPMS	Trophallaxis	PA	4	0.06 \pm 0.02	0.05–0.09	6.037	3	$P < 0.01$
		UP	4	0.01 \pm 0.00	0.00–0.01			
	Allogrooming	PA	4	0.38 \pm 0.06	0.31–0.46	10.162	6	$P < 0.01$
		UP	4	0.06 \pm 0.02	0.05–0.09			

No significant differences ($P > 0.05$) in the frequencies of trophallaxis and allogrooming between workers and PAMS were observed in the group of termites with or without UPMS (Table 2). Similarly, no significant difference in the frequency of trophallaxis between workers and UPMS was observed in the groups with or without PAMS (Table 3). However, workers/UPMS displayed significantly greater ($P < 0.05$) allogrooming behavior when PAMS were present (Table 3).

Table 2. Frequencies of trophallaxis and allogrooming of workers/PAMS with or without UPMS in the tested group

Behavior	UPMS	n	Mean \pm SE	Range	t	df	P
Trophallaxis	Present	4	1.35 \pm 0.05	0.07–0.18	-2.741	4	$P > 0.05$
	Absent	4	0.06 \pm 0.03	0.05–0.09			
Allogrooming	Present	4	0.63 \pm 0.16	0.42–0.81	-2.865	4	$P > 0.05$
	Absent	4	0.38 \pm 0.06	0.31–0.46			

Table 3. Frequencies of trophallaxis and allogrooming of workers/UPMS with or without PAMS in the tested group

Behavior	UPMS	n	Mean \pm SE	Range	t	df	P
Trophallaxis	Present	4	0.01 \pm 0.01	0.00–0.02	-1.916	4	P > 0.05
	Absent	4	0.01 \pm 0.00	0.00–0.01			
Allogrooming	Present	4	0.19 \pm 0.04	0.15–0.23	-5.388	4	P < 0.01
	Absent	4	0.06 \pm 0.02	0.05–0.09			

Conclusions

In conclusion, this study reveals that larval parasitoid changes trophallaxis and allogrooming of workers/PAMS. An increase in feeding activity of PAMS ensures the development of the larval parasitoid. Furthermore, the extensive allogrooming behavior of workers/PAMS could enhance the larval parasitoid's fitness by reducing the pathogen infections of the hosts.

Acknowledgements

We thank Universiti Sains Malaysia (USM Postgraduate Research Scheme USM-RU-PRGS) and DuPont Professional Products (Wilmington, DE) for financial support. F.K. Foo was supported under a Ph.D. scholarship provided by the USM Fellowship Scheme.

References

- Foo, F.K., G. V. Singham, A.S. Othman and C.Y. Lee 2011 Prevalence of a koinobiont endoparasitoid *Misotermes mindeni* (Diptera: Phoridae) in colonies of the fungus-growing termite *Macrotermes gilvus* (Blattodea: Termitidae) in Malaysia. *Journal of Economic Entomology* **104**, 1675–1679.
- Lee, C.C. and C.Y. Lee 2011 Population size and caste composition of a fungus-growing termite, *Macrotermes gilvus* (Blattodea: Termitidae). 78–82 pp. In K. Tsunoda [eds.], *Proceedings of the 8th Conference of the Pacific Rim Termite Research Group*, Bangkok, Thailand.
- Libersat, F., A. Delago and R. Gal 2009 Manipulation of host behavior by parasitic insects and insect parasites. *Annual Review of Entomology* **54**, 189–207.
- Loehle, C. 1995 Social barriers to pathogen transmission in wild animal populations. *Ecology* **76**, 326–335.
- Maistrello, L. and G. Sbrenna 1996 Frequency of some behavioural patterns in colonies of *Kaloterme flavicollis* (Isoptera: Kalotermitidae): the importance of social interactions and vibratory movements as mechanisms for social integration. *Ethology Ecology and Evolution* **8**, 365–375.

Neoh, K.B. and C.Y. Lee 2010 Morphological and behavioural changes in soldiers of *Macrotermes gilvus* (Hagen) (Termitidae: Macrotermitinae) parasitized by the fly *Misotermes mindeni* Disney & Neoh (Diptera: Phoridae). *Environmental Entomology* **39**, 835–840.

Poulin, R. 1995 “Adaptive” changes in the behaviour of parasitized animals: a critical review. *International Journal for Parasitology* **25**, 1371–1383.

Roy, H.E., D.C. Steinkraus, J. Eilenberg, A.E. Hajek and J.K. Pell 2006 Bizarre interactions and endgames: entomopathogenic fungi and their arthropod hosts. *Annual Review of Entomology* **51**, 331–357.

Sieber, R. and R.H. Leuthold 1981 Behavioural elements and their meaning in incipient laboratory colonies of the fungus-growing termite *Macrotermes michaelseni* (Isoptera: Macrotermitinae). *Insectes Sociaux* **28**, 371–382.

Foraging behavior and territory of *Coptotermes formosanus* Shiraki (1909) in Hanoi city.

Trinh Van Hanh, Nguyen Quoc Huy, Nguyen Thi My, Nguyen Thuy Hien, Tran Thu Huyen

Institute for termite control and works protection, Hanoi, Vietnam

Abstract

Coptotermes formosanus Shiraki (1909) is the harmful termite to the architectures in Vietnam. The article points out some data concerning the territory as well as normally operating positions in the works of this termite. The findings help research orientation and termite treatment in the field.

Keywords: *Coptotermes formosanus*, foraging, territory, a triple mark – release protocol,

Introduction

Coptotermes formosanus is regarded as one of the harmful termites to the architectures and crops in many countries in the world. According to the annual statistics, the damage caused by termites in the USA is more than 3 billion USD, including at least 80% of the damages caused by this species of termite (Ruan Carr, 2000) [6]. *C. formosanus* is the species with the fastest growth rate of distribution area in the world and having fast growth rate, great quantity of individuals, fast destruction rate, thereby causing big loss in a short time (Culliney T.W. and Grace J.K., 2000) [2]. For this very reason, the researches on prevention as well the researches on ecological biology related to preventing this species of termite have been paid special attention.

Cabrera (2001) argued that this species of termite may create nest in the structure that is not too hot or too cold but has high humidity [1]. Su et al. (1989) discovered that this species of termite causes harm on the 14th storey (at the height of about 42m) of the high-rise building [5]. Nan-Yao Su and Rudolf H. Scheffrahn (1988) determined the path and scope of food searching of 7 herds of *C.formosanus* in the urban area in USA and argued that a herd of termites may cause harm to several high-rise buildings within their scope of food searching [4]. The findings also pointed out that the characteristics, distance of food searching as well their scope of operation depend on many factors such as kind of food, source of food, quantity of individuals in the herd and climatic conditions.

Unlike most countries with the distribution of this species of termite, Hanoi is located in the tropical monsoon climatic zone, where construction density is as high as 85%. The research on the habit of food searching of a herd of *C. formosanus* will be a basis for selecting the measure of appropriate prevention as well as helping to identify and judge the encroachment possibility into the works.

Materials and methods

Survey the foraging positions of termite

Investigate buildings around Hanoi to find works that have been attacked by *C. formosanus*. Notice all the foraging sites of termite in the building. Statistics and analysis.

Research of foraging territory

Using the fat – soluble dye Nile Blue A (0.5%) to mark termites. The triple - mark – release – recapture was used to estimate the size of the foraging population and territory range of three nests at the address from 56 to 60, Tran Phu street, Hanoi city. Termites are captured, marked, released and recaptured over a period of five consecutive weeks (table 1).

Table 1. Sequence of the triple marked – released - recapture

week 1, 1 st capture	week 2, the 2 nd capture	week 3, the 3 rd capture	week 4, the 4 th capture	week 5, the 5 th capture
Mark	Mark	Mark		
	The 1 st release	The 2 nd release	The 3 rd release	
		The 1 st recapture	The 2 nd recapture	The 3 rd recapture

Estimated number of termite by Lincoln index (L.I.). Lincoln index were computed using the 1st marked – released - recapture cycle where marked termites were recovered from that colony (Lincoln 1930).

Time study was conducted from January to October 2010

Result and discussion

Common positions of food searching

We surveyed and sampled at 136 architectural works in inner Hanoi city encroached upon by termite and determined 74 architectural works encroached upon by *C. formosanus*. The encroachment rate of this species of termite is not even in various kinds of works (table 2).

Table 2. The rate (%) of works damaging by *C. formosanus* in Hanoi

Type of works	works surveying		works damaging	
	number	percent (%)	number	percent (%)
high-rise building	18	13.24	12	66.67
semi-detached building	68	50.00	33	48.53
garden villa	29	21.32	14	48.28
tile-roofed houses	21	15.44	15	71.43
Total	136	100.00	74	54.41

The result show that all kinds of architectural works are encroached upon by *C. formosanus*, at least achieving 48.3% in garden villas and at most achieving 71.4% in tile-roofed houses.

When determining and evaluating the termite encroachment by the height of the buildings, we gained the results shown in table 3.

Table 3. The rate (%) of encounter *C. formosanus* follows the floor of the building

Order of floor	The rate (%) of encounter <i>C. formosanus</i> follows the floor of the building				
	high-rise building	semi-detached building	garden villa	tile-roofed houses (pagoda, ancient house...)	Total
Floor 1	33.3	100.0	78.6	100.0	85.1
Floor 2	16.6	75.7	100.0		55.4
Floor 3	8.3	45.5	71.4		35.1
Floor 4	25.0	1.5			5.4
Floor 5	8.3	3.0			4.1
Floor 10	8.3				1.4

The results in table 3 show that the termites destruct most in low stories and the possibility of seeing them in low stories is the biggest.

Table 4. The rate (%) building components damaging by *C. formosanus*

Foraging site	Type of works				
	High-rise building	Semi-detached building	Garden villa	Tile-roofed houses (pagoda, ancient house...)	Total
kitchen counter	91.7	78.8	28.6	26.7	60.8
Stairwell	-	21.2	85.7	-	25.7
Frame doors	8.3	72.7	85.7	20.0	54.1
Wooden floor, wooden wall	8.3	45.5	7.1	-	23.0
Wooded pillar	-	-	-	80.0	16.2
Others	-	3.0	-	93.3	20.3

In terms of position by the interior subject in the works (table 4), the result show that the kitchen counter is most attacked by *C. formosanus* (occupying 60.8% of total works harmed by such species of termite), in which the rate of high-rise building apartments and semi-detached buildings attacked by such species of termite is 91.67% and 78.79% respectively. The two remaining types of building with kitchen counter attacked by termite occupy the much lower rate. The second subject most attacked by such species of termite is door and window frame and strip (making up 54.1% of total works harmed by such species of termite). The types of building harmed by termite are semi-detached buildings and garden villas with 72.7% and 85.7% respectively; the third most subject

harmed by termite is the space under the staircases (occupying 25.7% of total works harmed by such species of termite) - this subject harmed mainly in the semi-detached building (21.2%) and garden villa (85.7%); next, wainscot panel and wood flooring are harmed by termite mainly in semi-detached buildings (45.5%), garden villa (7.1%) and apartment building (8.3%); wood column base is harmed by termite mainly in the tile-roofed houses (making up 93.3% in tile-roofed houses, communal house in the village, pagoda and ancient house).

As such, it can be seen that all construction types in Hanoi are at the risk of being attacked by *C. formosanus*. The rate of works attacked by this species in Hanoi amounts to 54.41% of total works attacked by the termites in the species of *Coptotermes*. These subjects attacked by this species of termite are often wood, fabric and paper materials placed in humid and low positions in the space under the staircases, in the kitchen counter or column base, frame, window and door strip, bathroom door close to the ground or near water source or static position.

Estimate number of individuals and territory in the colony of Coptotermes formosanus

We researched and estimated the number of individuals and determined their territory of colony at 56-60 Tran Phu. This area includes 5 households numbered N1, N2, N3, N4 and N5 with total area of 14,579.5m². In which, four house areas N1, N2, N4 and N5 are seriously attacked by *C. formosanus*; only house N3 is not attacked by such species of termite. Through the termite samples collected to determine species composition, it can be seen that they belong to *C. formosanus*. Through four times of marking - releasing - arresting termite, it can be seen that each time termite is released at different positions.

The research result indicates that the marked termite that is released in house N1 is collected in house N1 only. Similarly, the termite released in house N4 is not collected in houses N1, N2 and N5. However, the termite released in house N2 is collected in house N2 and house N5 and vice versa. As such, the result determines that there are 3 independent termite nests in the research area. In which, the termites in house N2 and house N5 belong to the same nest with scope of operation of 630m², house N1 and house N4 are two separate nests and have scope of operation of 2,846m² and 350m² respectively. This research result is suitable for the research on the territory of food searching of 7 population of *C. formosanus* in the urban area of the USA by Nan-Yao Su et al. (1988).

The result estimated the quantity of individuals in the population according to Lincoln index at each time of releasing and arresting of each nest shown in table 5.

Table 5. Population estimates using Lincoln index

Nests	Territory	Population estimates			
		Cycle 1	Cycle 2	Cycle 3	L.I. ± SE (range)
N1	2,846m ²	214,483	229,005	63,736	214,483 ± 52,835
N2,N5	630m ²	1,411,850	1,855,800	430,800	1,411,850 ± 420,989
N4	350m ²	149,211	209,699	74,325	149,211 ± 39,153

The result indicated that the quantity of individuals of *C. formosanus* of 3 populations ranged from 149,211 to 1,411,850 individuals. The quantity of individuals in the population in this research is smaller than the estimates Su & Scheffrahn (1988) in the countryside in the south of Broward, Florida (ranging from 1,440,000 to 6,860,000 individuals) and it is impossible to see the clear correlation between the territory and the quantity of individuals in the population. According to Waller & La Fage (1987), the change in the quantity of individuals in the population of termites depends on the species, age, environmental conditions, the competition or the applicable method for estimation.

Conclusions

Most of the works types in Hanoi are attacked by *C. formosanus*. This species of termite operate mainly in low stories of the works, in humid, low and closed places such as the space under the staircases, in the kitchen counter, wall corner, column base, window and door frame and strip, bathroom door, especially in the wood parts close to the ground or water source.

The research result on 3 nests indicates that the territory of *C. formosanus* ranges from 350m² to 2,846m² and the number of individuals in the population in Hanoi ranges from 144,412 to 1,232,817 individuals.

References

- Cabrera, B. J., N.Y. Su, R.H. Scheffrahn, F.M. Oi and P.G. Koehler 2001 “Formosan subterranean termites” University of Florida: Extension (Institute of Food and Agricultural Sciences). *ENY* **216**, 157-164.
- Culliney T.W. and J.K. Grace 2000 Prospects for the biological control of subterranean termites (Isoptera: Rhinotermitidae), with special reference to *Coptotermes formosanus*, Review article of University of Hawaii, *Bulletin of Entomological Research* **90**, 9 – 219.

Forchler, B.T. and M.L. Townsend 1996 Mark-releaserecapture estimates of *Reticulitermes* spp. (Isoptera: Rhinotermitidae) colony foraging populations from Georgia, U.S.A. *Environ. Entomol.* **25**, 952–962.

Lai, P.-Y., M. Tamashiro, J.K. Fujii, J.R. Yates and N.Y. Su 1983 Sudan Red 7B, a dye marker for *Coptotermes formosanus*, *Proc. Hawaiian Entomol. Soc.* **24**, 277 - 282.

Su, N-Y and R. H. Scheffrahn 1988 Foraging population and territory of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in an urban environment, *Sociobiology* **14**(2), 353-359.

Su, N-Y., R. H. Scheffrahn and P. M. Ban 1989 Method to monitor initiation of aerial infestations by alates of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in high-rise buildings. *J. Econ. Entomol.* **82**, 1643-1645.

Carr, R. 2000 Review of the behavioral ecology of subterranean termite (Isoptera: Rhinotermitidae: *Coptotermes* sp. and *Reticulitermes* sp.) with discussion on applications to alternative control methods, http://www.treknature.com/gallery/South_America/Brazil/Southeast/Rio_de_Janeiro/RJ/photo153420.htm.

Waller, D. A. and J. P. La Fage. 1987 Nutritional ecology of termites. In: F. Slansky, Jr. and J. G. Rodriguez (eds.) *The Nutritional Ecology of Insects, Mites, and Spiders*. John Wiley and Sons, New York, 487-532 pp.

Laboratory maintenance of a fungus-growing termite, *Macrotermes gilvus* (Blattodea: Termitidae)

Ching-Chen Lee, Foong-Kuan Foo, and Chow-Yang Lee

Urban Entomology Laboratory, Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

Abstract

We determined the optimum maintenance condition for a fungus-growing higher termite, *Macrotermes gilvus* (Hagen) in the laboratory. Various group sizes of termites and container sizes were tested in this study. Termite survivorship was measured under different conditions. Results indicated that the survival rate in smaller group was better than that of the larger group. Irrespective of container volume, survival rate decreased with termite number. Container volume did not significantly ($P > 0.05$) affect termite survivorship. However, there was significant interaction effect ($P < 0.05$) between group size and container volume on the survival rate of termites.

Key words: *Macrotermes*, termite survivorship, group size, container volume

Introduction

The fungus-growing higher termites from subfamily Macrotermitinae are widely distributed in South and Southeast Asia (Neoh et al. 2010). *Macrotermes gilvus* (Hagen), a mound-building species is commonly found in gardens and plantations. However, they can become a secondary pest in homes that were previously baited for lower termites such as *Coptotermes* spp. (Lee et al. 2007). Acda (2004) reported that *M. gilvus* caused major problem to wooden structures and was known as the most destructive termite species in the Philippines after *C. gestroi* (formerly *C. vastator*). Because of its economic importance, field tests of insecticide efficacy have been carried out (e.g., Peters et al. 2008). However, due to the influences of various environmental conditions, field efficacy studies often resulted in inconsistent results. In addition, field tests are expensive and rigorous in nature. There is a need to test insecticides under laboratory condition for this species. However, past experiences have revealed that it is difficult to maintain the survivorship of *M. gilvus* in the laboratory for adequate time during the test. Thus, there is a serious need to find a way to optimally maintain the survivorship of this species under laboratory condition. Unlike the lower termites, Macrotermitinins exhibit irreversible fork developmental pathway and cultivate symbiotic fungus, *Termitomyces* spp. as food source (Rouland-Lefevre 2000). Thus, maintaining fungus-growing higher termites is more challenging than the other termite species.

In laboratory test, survival and food consumption are measured in the assessment of the effectiveness of insecticides. Survival rate in untreated control groups should reach acceptable levels to ensure the reliability of experimental results. Numerous factors such as colony origin, container size, group size, and type of matrix were known to influence termite survivorship (Lenz 2009). Lenz et al. (1984) found the relationship between group size and container volume in order to achieve constant population density under different combinations, and indicated that termite performance was optimum at specific group size. This is crucial to ensure that the termites are able to maintain their environment clean from contamination. Termite survival also varies with container volumes. When *Nasutitermes nigriceps* (Haldeman) was provided with increasing amount of living space, the survival rate tend to decrease (Lenz and Williams 1980). Termites are easily stressed when they have to maintain over large space. Group size and container volume exert variable effects on different termite species (Lenz et al. 1987). Clearly, there is a need to define the optimum maintenance condition for *M. gilvus*. Therefore, relationship between group size and container volume were studied to investigate their effects on termite survival.

Materials and methods

Termite sampling. *M. gilvus* individuals were collected from mounds located in a residential area (5°35'N, 100°29'E) near Universiti Sains Malaysia Minden campus in Penang island, Malaysia. The mounds were carefully broken into manageable pieces and brought back to the laboratory. The insects were separated from the fungus combs and other nest materials by gently tapping the mound materials onto a tray. Termites were sorted into different castes (i.e. major workers, minor workers, major soldiers, and minor soldiers). Only healthy termites were selected for testing.

Experimental set up. Four (4) groups of termites ranging from 0.3 g (\approx 55 termites) to 3.0 g (\approx 550 termites) were maintained in different container volumes (i.e., 120 ml, 480 ml, and 2300 ml) (Table 1). Airtight rounded base containers were used in this study. Caste composition in each group size was based on Lee and Lee (2011) with 10% proportion of soldier castes and minor: major worker ratio of 1.4: 1. Seven replicates were set up for each combination. Each container was filled with 1 cm depth of moistened sand matrix with 20% water w/w. Three (3) g of fungus comb was added as food source. Fungus comb was provided *ad libitum* during the experiment. The termite groups were maintained under conditions of $27 \pm 1^\circ\text{C}$ and 24-hour scotophase. The experiment was terminated after 28 days. The number of surviving termites was recorded. The percentage of survival was calculated.

Table 1. Caste composition, group size, and container volume used in the experiment.

Group size(g)	Caste composition ^a	Container volume (ml)	Amount of sand (g)
0.3 (≈55 termites)	21Mw, 29mw, 1Ms, 4ms	120	40
		480	120
		2300	342
0.6 (≈110 termites)	42Mw, 58mw, 2Ms, 8ms	120	40
		480	120
		2300	342
1.8 (≈330 termites)	126Mw, 174mw, 6Ms, 24ms	120	40
		480	120
		2300	342
3.0 (≈550 termites)	210Mw, 290mw, 10Ms, 40ms	120	40
		480	120
		2300	342

a: Mw = major worker; mw = minor worker; Ms = major soldier; ms = minor soldier

Data analysis. Termite survival (%) was transformed into arcsine values prior to statistical analysis. Two-way ANOVA were performed using SPSS version 12.0. Means were separated using Tukey HSD at $\alpha = 0.05$.

Results and discussion

The survival rate of *M. gilvus* differed significantly among four group sizes ($F = 10.524$; $df = 3$; $P < 0.01$). Small group size such as 0.3 g of termites, registering survivors of $64.3 \pm 5.0\%$ had significantly higher survival rate than those of the larger groups (1.8 g and 3.0 g) ($P < 0.05$) (Figure 1). Termites were also found to construct tunnels throughout the sand matrix and the fungus comb appeared to be highly consumed. It was found that survival rate declined with increasing group size

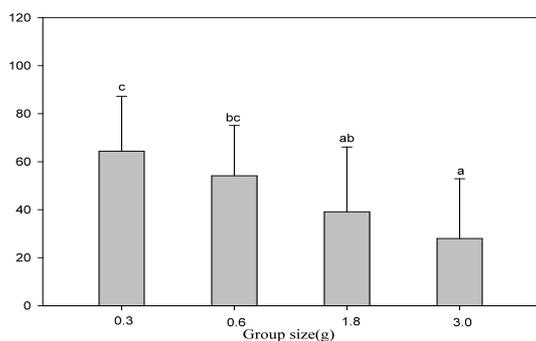


Figure 1. Mean survival (%) of *M. gilvus* under different group sizes. Means followed by same letter are not significantly different (Tukey HSD, $p < 0.05$)

for each container volume (Figure 2). Termites reared in larger group, to certain extent, might be overcrowded. Howick (1978) found that mortality of *Nasutitermes exitosus* significantly increased when maintained in overcrowding condition.

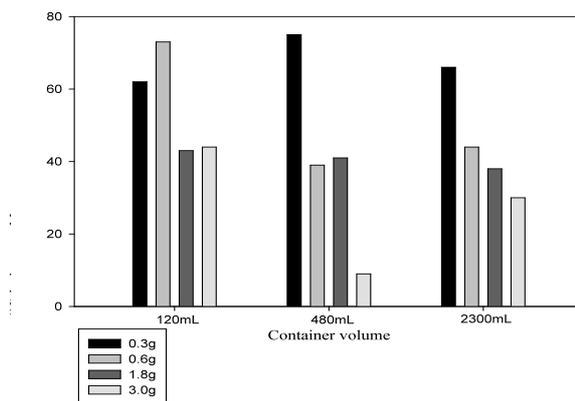


Figure 2: Mean survival (%) of four group sizes of *M. gilvus* maintained under different container volumes.

The termite survival rate was not significantly influenced by the volume of container ($P = 0.075$). In contrast, Lenz and Williams (1980) found that survival was highest in the smallest container for *Coptotermes acinaciformis* and *Nasutitermes exitiosus*. It was found earlier that the effect of population density to a laboratory-maintained termite differed among different termite species (Lenz et al. 1984, Lenz et al. 1987). However, there was a significant interaction effect between group size and container volume on survivorship of *M. gilvus* ($F = 2.443$; $df = 6$; $P < 0.05$). For example in 0.6 g of termites, significant higher survivorship was recorded in 120ml container (mean survival percentage \pm SE, $73.0 \pm 5.4\%$) compared to those in 480 ml container (mean survival percentage \pm SE, $38.7 \pm 8.4\%$) ($P < 0.05$).

Conclusion

In conclusions, termite survivorship varied between different combinations of group size with container volume. In general, *M. gilvus* survive better at smaller group size of approximately 55 termites compare to larger group, regardless the container size ranged from 120ml to 2300ml tested in the present study.

Acknowledgement

We thank Universiti Sains Malaysia (USM Postgraduate Research Scheme USM-RU-PRGS) and DuPont Professional Products (USA) for funding this project. CCL and FKF were supported under USM Fellowship Scheme.

References

Acda, M. N. 2004 Economically important termites (Isoptera) of the Philippines and their control. *Sociobiology* **43**, 159–168

Howick, C. D. 1978 Influence of crowding during transportation on survival and laboratory feeding of termites (Isoptera). *Journal of the Institute of Wood Science* **8**, 28–31

Lee, C. C. and C. Y. Lee 2011 Population size and caste composition of a fungus-growing termite, *Macrotermes gilvus* (Blattodea: Termitidae), pp. 78–82. In: Proceedings of the Eight Conference of the Pacific- Rim Termite Research Group, 28 February-1 March, Bangkok, Thailand.

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

Lenz, M. 2009 Laboratory bioassays with subterranean termites (Isoptera) – The importance of termite biology. *Sociobiology* **53**, 573–595

Lenz, M. and E. R. Williams 1980 Influence of container, matrix volume and group size on survival and feeding activity in species of *Coptotermes* and *Nasutitermes* (Isoptera: Rhinotermitidae, Termitidae). *Material and Organismen* **15**, 25–46

Lenz, M., R. A. Barrett and E. R. Williams 1984 Implications for comparability of laboratory experiments revealed in studies on the effects of population density on vigour in *Coptotermes lacteus* (Froggatt) and *Nasutitermesexitosus* (Hill) (Isoptera: Rhinotermitidae & Termitidae). *Bulletin of Entomological Research* **74**, 477–485

Lenz, M., T. L. Amburgey, D. Z. Rong, H. Kühne, J. K. Mauldin, A. F. Preston and M. Westcott 1987 Interlaboratory studies on termite–wood decay fungi associations: I. Determination of maintenance conditions for several species of termites (Isoptera: Mastotermitidae, Termopsidae, Rhinotermitidae, Termitidae). *Sociobiology* **13**, 1–56

Neoh, K. B., M. Lenz and C. Y. Lee 2010 Impact of orphaning on field colonies of Southeast Asian *Macrotermes gilvus* (Hagen) and *Macrotermes carbonarius* (Hagen) (Termitidae, Macrotermitinae). *Insect Sociaux* **57**, 431–439

Peters, B. C., S. Broadbent and P. Dhang 2008 Evaluating a baiting system for management of termites in landscape and orchard trees in Australia, Hong Kong, Malaysia, and the Philippines, pp. 379–383. In: Proceedings of the Sixth International Conference on Urban Pests. (W. H. Robinson and D. Bajomi, eds.).

Roonwal, M. L. 1970 Termites of the Oriental Region. In: K. Krishna and F. M. Weesner, eds. Volume 2: Biology of termites. pp. 315–384. Academic Press, New York.

Rouland-Lefevre, C. 2000 Symbiosis with fungi. In: T. Abe, D. E. Bignell and M. Higashi, eds. Termites: Evolution, Sociality, Symbioses, Ecology. pp. 289–306. Dordrecht: Kluwer Academic Publishers

The potential of subterranean termites *Macrotermes gilvus* Hagen (Blattodea: Termitidae) in modifying soil in rehabilitation critical land

Niken Subekti

Biology Department, FMIPA, Semarang State University, Indonesia

Abstract

Termites of the subfamily Macrotermitinae play an important role in decomposition processes in tropical forest ecosystem. In the process of mound building, termite modify the physical, chemical, and biochemical characteristics of the soil they use for construction, as well as the soil of nearby areas so that organic compounds. The result was physical soil two location in Ungaran Nature Reserved and main campus garden Semarang State University, Centre of Java, Indonesia more contain clay rich soil than sand and silt. Meanwhile, hydraulic conductivity, pH and temperature soil two location in Ungaran Natural Reserved (before removal) the same as main campus garden contain (after removal). *Macrotermes gilvus* Hagen can survival remove from native habitat to another habitat. We concluded that *Macrotermes gilvus* Hagen have potential rehabilitation critical land.

Keywords: *Macrotermes gilvus* Hagen, Potential, Rehabilitation, Critical Land

Introduction

Termite are essentially detritivores, feeding on a wide range of dead plant material at various stages of decomposition. Utilization of such a wide of food resources has been made possible by the close association with microbial symbionts, in both gut and nest. As a autotrophically fixed carbon, they can exert a significant effect on carbon mineralization and nutrient recycling, especially where termite biomass is high, as in some tropical areas (Noirot *et al.* 2000).

Mounds building from *Macrotermes gilvus* Hagen, for protection from the environment and to provide more suitable micro climatic conditions for the colony and its symbionts, together with subterranean gallery construction activity by termites, may have a strong influence on soil profile development. The cumulative effect of thousands of year of soil translocation by termites through mounds building activity is a notable example. Repacking and cementing together of soil particles during mound building usually results in mound soil having a higher bulk density and reduces porosity compared to non mounds soil. The construction of subterranean feeding galleries, however, results in increased soil porosity and improved soil water transmission properties (Bignell & Eggleton, 2000).

The chemical properties of soils are largely determined by the nature of their parent materials, but with additional influence from climate, vegetation cover and the activities of soil organism, especially for older soils. Termites use nutrient rich salivary secretations and faecal material as cementing agents in the course of construction, and in some regions where termite activity is high they are able to significantly modify soil chemical properties. Because of its higher clay content and usually higher exchangeable cation concentration, the translocation of soil from deeper in profile to the surface during termite gallery and mound construction may also enrich the surface soil with nutrients useful to plants (Lisa & Conacher, 2000).

Materials and methods

Sampling was conducted of their Natural Reserved in Ungaran, Centre of Java, Indonesia and main campus Garden Semarang State University, Centre of Java, Indonesia. Observation of the termite population size was carried out by the demolition of the mounds little by little and carefully both vertically and horizontally in each of the mounds (4 mounds). The demolition was carried out in until the depth of 1 m below land surface. However if the sample mounds is more than 1 m deep, the demolition it was continued as far as all the colony member was taken. After then, all of colony *Macrotermes gilvus* Hagen from nature reserved remove to main campus garden. Furthermore, soils were also sampled from outside the mounds from 0-20 cm deep (outer zone) for chemical and physical analyses. The physical analyses included soil texture, porosity, water content, temperature, soil humidity. Meanwhile, the chemical observation included pH. Soil samples were taken in each mounds with three replications (Fort, 1978).

Results and discussion

Table 1. The physical analyses texture of subterranean termite *M. gilvus* in Natural Reserved and Main Campus Garden, Centre of Java, Indonesia

LOCATION	TEXTURE		
	Sand (%)	Silk (%)	Clay (%)
Natural Reserved (Before Removal)			
A1	10.45±11.49	34.01±38.48	51.07±54.8
B1	8.7±10.22	36.31±46.33	44.97±54.91
C1	12.31±13.81	17.94±38.91	47.28±69.75
D1	11.94±13.99	36.42±38.55	47.46±51.64
Main Campus Garden (After Removal)			
A2	12.57±16.37	28.59±37.23	50.2±55.04
B2	10.38±14.92	20.49±33.67	52.96±69.13
C2	25.47±30.08	30.44±32.07	38.56±44.09
D2	22.43±27.91	30.06±36.02	36.8±44.54

Table 1 shows soil physical contain mound building of subterranean termite *M. gilvus* in Nature Reserved (before removal) and main campus garden (after removal), Centre of Java, Indonesia. The result was texture soil two location in Semarang, Centre of Java, Indonesia more contain clay rich

soil than sand and silt. Clay is important in the architecture of *Macrotermes gilvus* Hagen buildings, and it has been postulated that termites could modify the mineralogical properties of some clays (Jouquet *et al.* 2004).

This enrichment has occurred because *Macrotermes gilvus* Hagen select particle from clay rich horizons within the soil profile for the construction of their mounds (Holt & Lepage, 2000). Termite activity has also been suggested as major contributing factor in the formation of some lateric profiles and ferrisols although there is caused primarily by soil physic-chemical processes. The evidence for termite involvement in the formation of lateritic landscape has been recently reviewed (Miedem & Vuure, 2006).

Table 2. The physical analyses hydraulic conductivity soil contain mound building of subterranean termite *M. gilvus* in Ungaran Natural Reserved and main campus garden, Centre of Java, Indonesia

LOCATION	Hydraulic Conductivity (%)	pH	Temperature (°C)
Natural Reserved (Before Removal)			
A1	42,81	4,9	29,3
B1	41,02	5,0	29,2
C1	43,72	4,9	29,3
D1	39,11	5,1	29,0
Main Campus Garden (After Removal)			
A2	42,38	5,2	30,4
B2	39,40	5,2	30,2
C2	45,93	5,0	29,5
D2	44,16	5,1	29,1

Table 2 shows soil physical hydraulic conductivity soil contain mound building of subterranean termite *M. gilvus* in Nature Reserved (before removal) and main campus garden (after removal), Centre of Java, Indonesia. The result was hydraulic conductivity, pH and temperature soil two location in Ungaran Natural Reserved the same as main campus garden contain.

There has been considerable speculation regarding the influences of termite activity on the hydraulic properties of soils, but it is only in recent years that the influence of termite activity on infiltration rate and hydraulic conductivities have actually been measured (Holt & Lepage, 2000).

The termites have roles to answer the future of the environment, where they can regulate soil structure, nutrition cycle within the natural forest ecosystem. In the future, subterranean termites *M gilvus* can be used in the rehabilitation or reforestation of the critical land, poor land, bare land, and former mining areas.

Conclusions

The termites have also roles at the ecosystem management process in the ground which has a role at maintenance of diversity, stability, nutrition cycles, and biomass productions in the ecosystem. *Macrotermes gilvus* Hagen can survival remove from native habitat to another habitat. We concluded that *Macrotermes gilvus* Hagen have potential rehabilitation critical land.

References

- Bignell, D.E. and P. Eggleton 2000 Termites in ecosystem. In *Termites: Evolution, Sociality, Symbioses, Ecology*. Edited by: Takuya Abe, David Edward Bignell and Masahiko Higashi. Kluwer Academic Publisher London. 363-388pp.
- Fort, H.D. 1978 *Fundamentals of Soil Science*. 6th.Ed. John Wiley & Sons, New York.
- Holt, J.A. and M. Lepage 2000 Termite and Soil Properties. In *Termites: Evolution, Sociality, Symbioses, Ecology*. Edited by: Takuya Abe, David Edward Bignell and Masahiko Higashi. Kluwer Academic Publisher London. 389-408pp.
- Jouquet, P., D. Tessier and M. Lepage 2004 The soil structural stability of termite nest: role of clays in *Macrotermes bellicosus* (Isoptera, Macrotermitinae) mound soils. *European Journal of Soil Biology* **40**, 23-29.
- Lisa, A.L. and A.J. Conacher 2000 Soil modification by termites in the central wheatbelt of Western Australia. *Australia Journal Soil Res* **33**,179-193.
- Miedem, R. and W.V. Vuure 2006 The morphological, physical and chemical properties of two mounds of *Macrotermes bellicosus* (Smethman) compared with surrounding soils in Sierra Leone. *European Journal of Soil Science* **28**, 112-124.
- Noirot, C. and J.P.E.C. Darlington 2000 Termite mound : architecture, regulation and defence. In Abe T, Bignell DE and Higashi M. *Termites : Evolution, Sociality, Symbioses, Ecology*. London, Kluwer Academic Publisher. 121-140pp.

Do termites fear carcasses?

Kok-Boon Neoh^{1,2}, Beng-Keok Yeap^{1,3}, Kunio Tsunoda^{4,5}, Tsuyoshi Yoshimura⁴, and Chow-Yang Lee¹

¹Urban Entomology Laboratory, Vector Control Research Unit, School of Biological Sciences, Universiti Sains Malaysia, 11800 Penang, Malaysia

²Present address: Center for Southeast Asian Studies, Kyoto University, Yoshida Sakyo-ku, Kyoto 606-8501, Japan

³Present address: Department of Entomology, University of Georgia, Athens, GA 30602, USA

⁴Research Institute for Sustainable Humanosphere, Kyoto University, Uji, Kyoto 611-0011, Japan

⁵Deceased.

Abstract

Undertaking behavior is pivotal to social life in enclosed nests. Workers are known to remove dead colony members from the nest. Such behavior prevents the spread of pathogens that may be detrimental to a colony. Four species from different subterranean termite taxa: *Coptotermes formosanus* Shiraki, *Reticulitermes speratus* (Kolbe), *Microcerotermes crassus* Snyder, and *Globitermes sulphureus* Haviland were tested in the present study. Different types of carcasses (freshly killed, 1-, 3-, and 7-day-old carcasses) were also used to investigate whether the termites exhibited any behavioral responses that were specific to carcasses in certain conditions. In the present study, we found that for *C. formosanus* and *R. speratus* more behavioral acts and effort were spent when the worker termites encountered fresh carcasses; but the worker termites only had a cursory examination when encountered old carcasses. In contrast, *M. crassus* and *G. sulphureus* workers performed relatively few behavioral acts and usually ignored or fled away from the carcasses.

Key words: carcasses, necrophobia, grooming, cannibalism, antennation, undertaking behavior

Introduction

Undertaking behaviour is an essential adaptation in the evolution of eusocial insects. Insects use many sophisticated behaviours when they encounter disease, parasitic infection, injury, and death (Rosengaus et al. 1999; Wilson-Rich et al. 2007; Neoh & Lee 2010) to prevent the spread of pathogens that may be fatal to a colony. For example, many social insects, particularly ants (Julian & Cahan 1999), bees (Trumbo & Robinson 1997), and social wasps (Espelie & Hermann 1990), remove dead colony members from the nest (necrophoric behaviour). Wilson-Rich et al. (2007)

reported that the termite *Zootermopsis angusticollis* (Hagen) significantly increased the frequency of allogrooming and vibratory displays and exhibited two other specific behaviours (abdominal-tip raising and self-scratching) when exposed to the soil nematode *Steinernema carpocapsae* (Weiser). This termite species also released a pathogen alarm and exhibited many more allogrooming acts when in contact with the spores of or termites infected by the pathogenic fungus *Metarhizium anisopliae* (Metsch.) (Rosengaus et al. 1998; Rosengaus et al. 1999). To date, little is known about the ethological aspects of how termites deal with carcasses. To investigate this issue, we studied the reaction to carcasses of four species from different termite taxa: *Coptotermes formosanus* Shiraki, *Reticulitermes speratus* (Kolbe), *Microcerotermes crassus* Snyder and *Globitermes sulphureus* Haviland. We also used different types of carcasses to further compare termites's behaviour when exposed to dead nestmates.

Materials and methods

Termite species. Laboratory culture of *C. formosanus* in the laboratory of the Research Institute for Sustainable Humanosphere (RISH) of Kyoto University was used in this study. Samples of *R. speratus* were collected from a heavily infested tree branch at the Uji Campus of Kyoto University, Japan. Samples of two Southeast Asian mound-building termite species, *M. crassus* and *G. sulphureus*, were collected in Penang, Malaysia.

Experimental set up. A test unit consisted of two containers (inner diameter: 40 mm; height: 50 mm) connected by an acrylic tube (inner diameter: 5 mm; length: 100 mm) (Figure 1). The bottom of the containers was layered with plaster of Paris (GC Corporation, Tokyo, Japan), which was covered by a 25 mm thick layer of 20% water content sandy loam (for *C. formosanus* and *R. speratus*) or sand sieved through 40 mesh with 20% water content (for *G. sulphureus* and *M. crassus*) to provide humidity. Crosland et al. (1997) found that carcass-burying activity was mostly performed by old workers. Thus, in these experiments we used 100 workers that were at least at the third worker stage. We assumed that the termites would perform the task equally. Soldiers were added based on the worker: soldier ratio from the field populations: 100 workers: 2 soldiers for *R. speratus* and *M. crassus* and 100 workers: 10 soldiers for *C. formosanus* and *G. sulphureus*. The termites were introduced into the test container (a) (Figure 1) and acclimatized for 1 day prior to testing for the purpose of allowing termites to tunnel to container (b).

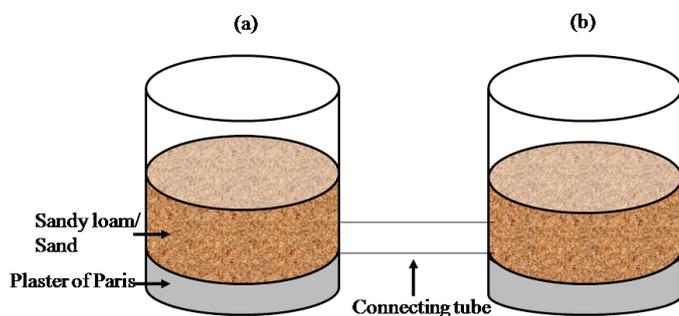


Figure 1. Experimental setup for behavioral assay

Test samples. Freshly killed, 1-, 3-, and 7-day-old carcasses (crushed) were tested. To age the carcasses, they were kept in covered Petri dishes under the laboratory conditions. Healthy termites were as a control. We marked the healthy termites with a dot of black paint on the head capsule to differentiate them from the acclimatized termites. Laboratory tests prior to the experiments showed that the paint caused no significant behavioural changes or mortality in termites.

Behavioural assay. Approximately 20 termites of each type were introduced into container (b) separately (Figure 1). Because the time that termites took to approach the test samples varied, we began video recording (Sony[®] DRC142 TRV340E, Tokyo, Japan) when worker termites first contacted the test samples. Video recording was conducted for 15 min. Next, direct observations (~1 min with the naked eye) were made at selected time intervals (i.e., 30 min, 1, 2, 3, 4, 8, and 24 hrs) to see if any construction, carcass-burying activity, etc. had occurred. Final observations were recorded at 48 hrs. The tests were replicated three times for each sample tested. All behaviours exhibited during the 15 min video recordings were identified and categorized as exploratory behaviour, grooming, alarming behaviour, and/or carcass burying behaviour.

Data analysis. Mean frequency (F) of each behaviour performed by healthy workers was calculated as $F = B/T$, where B = total number of times the particular behaviour was shown and T = total number of healthy worker termites that appeared in container B during the first 15 min. The mean frequency was then subjected to Log (n+0.5) transformation to produce normality of the distribution. The data were separated by carcass type and compared among these types for each species of termite

tested using analysis of variance (ANOVA) Tukey HSD (STATISTIX 9 User's Manual, Tallahassee, FL).

Results and discussion

Our result showed that *C. formosanus* and *R. speratus* exhibited (1) high frequency of grooming and carcass removal of freshly killed, and 1-day-old carcasses, but these behaviours decreased as the carcasses aged; (2) the termites repeatedly crawled under the aging carcass piles; and (3) only newly dead termites were consumed as a food source. In contrast, *G. sulphureus* and *M. crassus* workers performed relatively few behavioural acts.

Previously, several common types of carcass-burying behaviour and necrophagy were documented. In the present study, we documented the following novel behavioural responses that were specifically performed on certain type of carcasses or mutilated termites:

1. The act of crawling under aging carcass piles: To our knowledge, this is the first documentation of this behaviour in termites. One of the proximate observations was made by Saran & Rust (2007) whom previously reported that healthy *Reticulitermes hesperus* Banks showed no tendency to avoid 7-day-old termite carcasses.
2. As predicted, the carcasses often were cannibalized by healthy workers. However, in the present study, we found that freshly killed and 1-day-old (only in *R. speratus*) carcasses were fed upon.
3. For *C. formosanus* and *R. speratus*, the old carcasses (3- and 7-day-old carcasses) often were walled-off with sand at the spot where the carcasses were deployed. On the other hand, newly dead termites were intensively carried into the soil or connecting tubes to be disposed of or fed upon.
4. Avoidance behaviour (or necrophobia) was demonstrated by *G. sulphureus* through the limited contact of healthy termites with carcasses and by *M. crassus* as the blockage that was constructed in the access tubes. In contrast, *C. formosanus* and *R. speratus* performed relatively more behavioural acts towards carcasses. These contrasting behaviours of the latter termite species cast a new light on the earlier study conducted by Su et al. (1982), which led to the interpretation that termites are necrophobic (Su 2005; Yeoh & Lee 2007). The premise of necrophobia was further disapproved by Hu et al. (2006) and Campora & Grace (2007).

Conclusions

Based on the results of the present study, we propose that the behavioural response towards carcasses depends largely on the nature of the carcasses, the contamination source, and termite species, and the response is more complex than previously thought.

References

- Campora, C.E. and J.K. Grace 2007 Behavioral response of the Formosan subterranean termite to borate-treated wood. *Proceedings of the Hawaiian Entomological Society* **39**,127-137.
- Espelie, K.E. and H.R. Hermann 1990 Surface lipids of the social wasp *Polistes annularis* (L.) and its nest and nest pedicel. *Journal of Chemical Ecology* **16**, 1841-1852.
- Hu, X.P., D. Song and W. Presley 2006 Horizontal and vertical transfer of fipronil within functional colonies. In: National Conference on Urban Entomology (Ed. By Sutphin, T., B. Cartwright and R. Houseman), pp. 40-45. Raleigh-Durham, North Carolina.
- Julian, G.E. and S. Cahan 1999 Undertaking specialization in the desert leaf-cutter ant *Acromyrmex versicolor*. *Animal Behaviour* **58**, 437-442.
- Neoh, K.B. and C.Y. Lee 2010 Behavioral and morphological changes in soldiers of *Macrotermes gilvus* (Termitidae: Macrotermitinae) parasitized by *Misotermes mindeni* (Diptera: Phoridae). *Environmental Entomology* **39**, 835-840.
- Rosengaus, R.B., C. Jordan, M. L. Lefebvre and, J.F.A. Traniello 1999 Pathogen alarm behavior in a termite: A new form of communication in social insects. *Naturwissenschaften* **86**, 544-548.
- Rosengaus, R. B., A.B. Maxmen, L.E. Coates and J.F.A. Traniello 1998 Disease resistance: a benefit of sociality in the dampwood termite *Zootermopsis angusticollis* (Isoptera: Termopsidae). *Behavioral Ecology and Sociobiology* **44**, 125-134.
- Saran, R.K. and, M.K. Rust 2007 Toxicity, uptake, and transfer efficiency of fipronil in western subterranean termite (Isoptera: Rhinotermitidae). *Journal of Economic Entomology* **100**, 495-508.
- Su, N.Y. 2005 Response of the Formosan subterranean termites (Isoptera: Rhinotermitidae) to baits or nonrepellent termiticides in extended foraging arenas. *Journal of Economic Entomology* **98**, 2143-2152.
- Su, N.Y., M. Tamashiro, J.R. Yates and M.I. Haverty 1982 Effect of behavior on the evaluation of insecticides for prevention of or remedial control of the Formosan subterranean termite. *Journal of Economic Entomology* **75**, 188-193.

Wilson-Rich, N., R.J. Stuart and R.B. Rosengaus 2007 Susceptibility and behavioral responses of the dampwood termite *Zootermopsis angusticollis* to the entomopathogenic nematode *Steinernema carpocapsae*. *Journal of Invertebrate Pathology* **95**, 17-25.

Wong, N. and, C.Y. Lee 2010 Effects of disturbance and the presence of termite and other invertebrate carcasses at feeding sites on the behavior of the subterranean termite *Microcerotermes crassus* (Blattodea: Termitidae). *Sociobiology* **55**, 353-367.

Yeoh, B.H. and C.Y. Lee 2007 Tunneling responses of the Asian subterranean termite, *Coptotermes gestroi* in termiticide-treated sand (Isoptera: Rhinotermitidae). *Sociobiology* **50**, 457-468.