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A device with non-looping electromagnetic induction for remote monitoring of termites and its application

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Abstract

Remote monitoring plays a very important role in termite management. Currently, there are some devices developed. However, there is room to improve in accuracy, real time warning, and durable characters of the device. Here we introduced a novel system and a device for remote detecting termite activity developed by DEKAN. A non-looping electromagnetic induction technology was first introduced in this system. This system and device is comprised of termite monitoring information bar, signal receiver, DEKAN database and DEKEN management software. The monitoring information bar is a core part of this device and consists of wooden stakes, particles, and a magnetic bar. While the bait is consumed by termites, the magnetic bar drops and causes a signal. All these signals are sent to receivers and host computers which are programmed to access this data. Field trials proved its efficacy and accuracy in detecting termite activity. This device can be widely used for termite monitoring and management in households, historic buildings, reservoirs and sea banks as well as natural parks.

Introduction

Monitoring baiting stations have been widely used for termite management especially for remedial control of termites (Kakkar, 2018; Webb, 2017). Unlike traditional liquid treatment, baiting stations use significantly less termiticide. However, manually monitoring baiting stations relies on routine inspection by pest control operators to identify subterranean termite activity. The shortcomings of manual monitoring are obvious, such as labor intensive and therefore costly. Moreover, frequent inspection may disrupt the feeding behavior of some termite species in the stations. So, there is a need for an automated monitoring system instead of manual inspection of bait stations to reduce the labor and cost.

There are some automated monitoring devices with patents. For instance, Su et al. (2001, 2002) described a sensor consisting of a wooden stake painted with a conductive circuit of silver particle emulsion. The sensors were wired to a datalogger read by a host computer and form a computerized remote monitoring system. The mean monthly accuracy reporting the presence or absence of termites in the stations was 85%, but, at 6month after installation, the accuracy ranged from 41 to 79%.

However, there are some limitations of these automated termite monitoring devices in termite management such as accuracy, real-time warning, and durable characters associated with looping induction of system. Non-looping induction technology provides an alternative approach involving a novel system and device. Here we introduced a novel system and device for remote detection of termite activity developed by DEKAN. A non-looping electromagnetic induction technology was first introduced for monitoring termite activity with this system.

Materials and Methods

DEKAN non-looping Electromagnetic induction technology is a termite monitoring and warning technology that detects the presence of termites by changes in magnetic amount during termite consumption.

In this device, bait station is set up as an informational bar, where spherical particles have been installed along with wood stalks. Spherical particles are also built in with a permanent magnet and a magnetic switch on the circuit board. When termites eat a certain amount of the bait, spherical particulates and the permanent magnet within the information bar drop by gravity that result in a switch in magnetic force due to the disappearance of magnetic force. All signals are received and collected by wireless to a termite management database.

The patents for DEKAN non-looping of Electromagnetic induction technology and the devices have been registered.

Results and Discussion

Description of device

The monitoring system consists of an information bar, signal receiver and termite management computer and software (Figure1). An informational bar consists of wooden stakes, particles and magnetic bar (Figure 1A).

A1 A2

A. Information bar

B. Signal receiver

C. Termite management- Computer and Software

Figure 1. The components of a device with non-looping electromagnetic induction for remote monitoring of termites. The device consists of three parts, A. Information Bar. B. Signal Receiver. C. Termite Management Computer and Software. A1: The situation when termites consume the information bar. A2. The spherical particulate within the information bar and the permanent magnet. The magnet drops while the information bar is consumed by termites.

Features

This device with non-looping electromagnetic induction technology combines built-in technology, particle technology, and non-loop technology together to make it a unique device for automated monitoring of termites.

The features of this device include, 1) No wire. There is no any wire inside of this device. 2) No connection. All components exist independently without any connection. 3) Waterproof. The non-conductive particles, permanent magnets, circuit boards are not affected by water. 4) No metal.

These features of the device provide a base for the accuracy and real-time warning of detecting termite activity.

Application

Different types of devices have been invented. For instance, according to the source of energy, the device is divided into active energy technology (built-in) or passive technology. The area that the system is used can be divided into either underground or above ground stations. According to the information transmission method, the device can use one-way transmission, bidirectional transmission, or cellular communication technology. A unique type can be chosen for detecting termite activity for different situations. In general, this passive technology and one-way transmission technology has a low cost and can be used. Field trials in Zhejiang province, China and Guangdong province, China have proved its accuracy in monitoring termite activity. This device can be widely used for termite monitoring and management in household structures, historic buildings, reservoirs and sea banks as well as natural parks.

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Diversity of Termite Species Associated with Different Ages of Oil Palm Planted on Tropical Peat Soil

by
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Abstract

The palm oil industry is an important economic component of the agricultural sector in Malaysia and Indonesia. The diversity of termite species reflects the overall ecosystem production as well as the health of oil palm planted on peat soil. A study on the termite assemblages using a modified field transect was conducted on three different ages of oil palm: immature palms (IP), young mature palms (YP) and fully mature palms (MP). Based on the results, seven subfamilies were encountered, namely Coptotermitinae, Termitogetoninae, Rhinotermitinae, Heterotermitinae, Macrotermitinae, Termitinae and Nasutitermitinae. The wood feeders dominated all the studied sites with C. curvignathus Holmgren identified as a pest infesting standing palm. Other feeding groups recovered included wood-litter feeders; Macrotermes gilvus, soil-wood interface feeders Termes sp, and soil feeders Pericapritermes sp. The highest diversity was recorded at the MP site (H'=2.056) with high diversity richness (M=2.982), whereas, the IP site (H'=1.521) had the lowest species diversity (M=1.214) and richness (M=1.214). However, the YP (0.758) and MP (0.651) sites had a lower value of Evenness (J) compared to the IP site (0.915). Based on the results, termite assemblages increased notably as the age of oil palms increased.

Keyword: species composition, species abundance, diversity, oil palm, peat, pest

Introduction

Termites are very common in peat soil areas due to the abundance of pure, poorly decomposed organic material which directly results in the formation of a spongy, highly moist environment conducive for termites (Zulkifli et al., 1996; Lim and Silek, 2001). Malaysia is rich in

High termite biodiversity, approximately 42 genera and 175 species, has been recorded in Peninsular Malaysia (Tho, 1992) and Sabah with 103 species (Thapa, 1981). They are undeniably significant in the ecological system because, in nature, particularly in lowland dipterocarp forests, they are dead wood decomposers, continuously consuming the components of the humus, to form more fertile and stable soils. In Sarawak, conversion of peat forests to oil palm plantations is practiced to meet a huge industrial demand. Deforestation and land conversion, however, has resulted in significant impacts to termite species diversity and abundance (Vaessen et al., 2011). Hence, this study aimed to determine the composition and structure of termite communities as well as abundance and richness in different ages of oil palms planted on peat soil.

Materials and methods

Study sites and termite sampling method

Data were collected from two oil palm plantations located in Sg. Assan, Sibu (E2.200905, N111.872859) referred to as SG and in Durin, Sibu (E2.113758, N111.982639) referred to as DS (Table 1). Both plantations are located in Sarawak, Malaysia and consist of oil palms of different ages. Based on the palm age classification; immature palm site (IP), young palm site (YP) and full mature palm site (MP) (MPOB, 2011), a total of six transects were surveyed. Termite samples were collected within the modified transect protocol consisting of 20 non-contiguous sections (5x2 m2) that were sequentially sampled by two trained personnel for 30 min per section. Termites were sampled in the following microsites: Fronds heaps within the planting rows, the soil below the frond heaps, humus at the base of a tree and its root system, tree stumps, dead logs along stack rows, soils below rotten logs, subterranean nests, mounds, mud tubes on the palm and arboreal nests up to 2 m height. The presence of termites in each section of each transect was recorded as "encounters" (Jones et al., 2003). Encountered termites were hand-sorted according to caste for identification purposes and stored in a vial containing 70% ethanol. Information such as date, caste, grid, location and occurrence were recorded during the survey from December 2014 until July 2015.

Table 1. General characteristics of study sites

Sampling site	Transect location	Palm age	Peat depth*
IP	DS	25-month old	Shallow
(1-3 year old palms)	DS	36-month-old	Shallow
YP	DS	4-year-old	Shallow
(4-7 year old palms)	SG	7-year-old	Deep
MP	SG	12-year-old	Deep
(8 year old palms and above)	SG	12-year-old	Deep

*The peat depth was only assigned to deep (> 6' deep) and shallow (<3' deep) peat

Termite identification, feeding and nesting groups and data analysis

Termites were identified and categorized into two functional groups; the feeding group (FG) and the nesting group (NG) (Zulkefli et al., 2012; Fazly, 2008; Decaens et al., 2006; Donovan et al. 2001; Thapa, 1981), Tho, 1992; Krishna et al., 2013). Feeding groups consist of wood feeders (feeding on wood), wood-litter feeders (surface litter), soil feeders (humus and mineral soil), soil-wood interface feeders (highly decayed, soil-like wood) and epiphyte feeders (lichens, epiphytes and other free-living, non-vascular plants). The nesting groups were wood-nester (nesting on the main trunk or branches), arboreal (in trees), subterranean (in the ground), epigeal (above the ground), hypogeal (below the ground) and inquilines (nesting with other termites). Termite abundance was assessed based on the number of "encounters" in each site (Dawes, 2005). The taxonomic groupings of termites were to subfamily. The levels of sampling units (α-diversity) and sites (β-diversity) were compared to determine termite diversity and functional composition. Biodiversity data for termite species was generated using diversity indices which included the Shannon-Wiener (H'), Pielou's J Evenness (E), and Margalef index (M) across sampling units and sites. All data were analyzed using the biodiversity software EstimateS.

Result and discussion

A total of 14 species were recovered from the six transects, comprising two families (Rhinotermidae and Termitidae), seven subfamilies (Coptotermitinae, Termitogetoninae, Rhinotermitinae, Heterotermitinae, Macrotermitinae, Termitinae and Nasutermitinae) and nine genera (Coptotermes, Termitogeton, Parrhinotermes, Schedorhinotermes, Heterotermes, Macrotermes, Termes, Pericapritermes and Nasutitermes) (Table 2). As a whole, species diversity in this study was comparatively low compared to previous studies in oil palm agro-ecosystems in Indonesia and Sarawak (Vaessen et al., 2011; Bong et al., 2012; Luke et al., 2014).

The IP sites had the lowest diversity and species richness (H'=1.521, M=1.214) in comparison with the YP and MP sites (Table 3). The YP and MP sites produced close species diversity (H'=2.026 and 2.0565 respectively) and species richness (M=2.267 and 2.982 respectively) values, indicating similar species composition. The number of termite species collected at the YP and MP sites were 10 and 12 respectively, double the number of species from the IP sites.

Four feeding groups were identified in this study (Table 2). The Rhinotermitidae was represented by nine species of wood feeders compare to five species from several feeding groups from the Termitidae. Among these groups, wood feeders (78.57%) were most commonly found representing six genera; Coptotermes, Termitogeton, Parrhinotermes, Schedorhinotermes, Heterotermes and Nasutitermes. The wood-feeders dominated the IP site entirely (100%). The other groups were organic soil feeders, wood/litter-fungus feeders, and intermediate feeders. All these feeding group were represented by only one species from the YP and MP sites.

Among the subfamilies, the Coptotermitinae was considered the main pest group with the highest number encountered at the MP site (42.5%), followed by the YP (28.07%) and IP (22.5%) sites. This suggests oil palm encounters a higher chance of infestation as the palm grows older and resident termite colonies grow and multiply. The Coptotermitinae particularly C. curvignathus is an important pest in the oil palm industry as that species has the ability to kill oil palms planted on peat by feeding on the living tissues (Lim and Silek, 2001; Faszly, 2008; Chan et al., 2011; Zulkefli et al., 2012) and our data showed high densities in the MP sites.

The plantation soil surface may experience direct sunlight for 24-36 months after planting prior to canopy closure thus increasing soil surface temperatures and reducing the moisture level. Similar to this study, the dominance of Coptotermitinae and Rhinotermitinae was also reported in oil palms aged 28 months (Vaessen et al., 2011). The occurrence of these wood feeders increases over time and was the highest in oil palms planted on converted peat over 12 years of age (MP transect). In this study, a clear difference in terms of number of species found was observed in the IP transects (4 species) compared to the YP and MP where we collected a higher number of species. The Termitidae was found only in transect YP and MP. Faszly (2008) also stated that termite community structure and diversity increases over time after planting. The canopy of oil palm in the fourth year onwards is able to reduce direct sunlight on the soil surface due to canopy closure and height of the trees. The availability of termite food sources such as fronds in stacked rows and dead wood in the peat soil play an important role in providing microsites for termites in oil palm plantations (Keng and Homathevi, 2012). Over time, the soil surface layer will eventually be occupied by ferns such as Nephrolepis biserrta (Swatz) that help conserve soil temperature and moisture (Bong et al., 2012) allowing more species of termites to survive. The soil-feeding termite, Pericapritermes dolichocephalus (John) has been reported to be susceptible to landscape disturbance but was recovered from both YP and MP transects. The relationships between termite species compositions and their response to habitat disturbance may be regarded as one of the indicators in the process of evaluating disturbance effects within ecosystems (Davies, 2002; Aiman et al., 2014).

Table 2 List of termite species collected from oil palm plantations on peat in Sibu, Sarawak

Species	Feeding Group	Nesting Group	Sampling sites					
			IP		YP		MP	
			TPD 1	TNE 1	TPD 2	TNE 2	TNE 3	TNE 4
RHINOTERMITIDAE								
Coptotermitinae								
Coptotermes curvignathus (Holmgren)	*w*	w	0	3	4	0	2	7
Coptotermes sepangesis (Krishna)	*w*	w	0	0	2	1	0	1
Coptotermes kalshoveni (Kemner)	*w*	w	3	0	5	2	3	4
Termitogetoninae								
Termitegeton minor(Haviland)	*w*	w	0	0	0	0	1	0
Rhinotermitinae								
Parrhinotermes aequalis (Haviland)	*w*	w	0	0	0	0	1	0
Schedorhinotermes sarawakensis	*w*	w	1	7	1	14	5	4
(Holmgren)	*w*	w	5	3	3	4	4	2
Schedorhinotermes brevialatus (Haviland)	*w*	w	3	2	1	2	0	2
Schedorhinotermes tarakensis (Oshima)								
Heterotermitinae								
Heterotermes tenuior (Hagen)	*w*	w	0	0	0	0	1	0
TERMITIDAE								
Macrotermitinae								
Macrotermes gilvus (Hagen)	*l/f*	e	0	0	2	1	0	0
Termitinae								
Termes rostratus (Haviland)								
Pericapritermes dolichocephalus (John)	*i*	i/w	0	0	1	0	0	0
	o	h	0	0	1	0	1	0
Nasutermitinae								
Nasutitermes havilandi (Desneux)								
Nasutitermes matagensiformis (Haviland)	*w*	a	0	0	4	5	1	0
	w	a	0	0	0	0	1	0
Total no. of encounters			12	15	24	29	20	20
Total no. of species			4	4	10	7	10	6

Feeding groups were wood-feeders (w), wood/litter-feeders (l), fungus growers (*f*), soil-wood or intermediate-feeders (*i*) and organic soil feeders (*o*). Nesting groups are wood or tree-nesters (w), hypogeal-nesters (h), epigeal-nesters (e), arboreal-nesters (a), inquiline-nesters (i). Numbers refer to relative encounters (abundance) per species per transect (see Materials and Methods).

Table 3 Diversity indices for termite species between sampling sites

Diversity index	IP	YP	MP
Number of species (S)	5	10	12
Relative encounters	27	53	40

Species Diversity (H’)	1.521	2.026	2.056
Species Evenness (J)	0.915	0.758	0.651
Species Richness (M)	1.214	2.267	2.982

Conclusion

In conclusion, this study shows termite assemblages and diversity increase as the age of oil palm plantings increase. This correlation may not be directly due to palm age itself but improved ecosystem functioning as the trees grow older allowing more sensitive species to return to the area. Wood feeder termites were the most successful group able to establish themselves at all sites as the main organic matter decomposer as well as the main pest of oil palm plantations.

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Revision of the Subterranean Termite Genus, *Reticulitermes* (Isoptera: Rhinotermitidae), in Taiwan

by
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Abstract

Reticulitermes are an important economic pests most found in temperate zones. Many controversial issues regarding species identification occur because the morphology of the immature stages of *Reticulitermes* are similar. In the past few years, genetic analysis eased *Reticulitermes* identification of North American and European species. However, many taxonomic problems regarding *Reticulitermes* in the Far East Asia remain. In this study, we collected the *Reticulitermes* from 64 localities of Taiwan. Morphological and genetic data (COII and 16S rRNA), and the ecological traits indicate that three *Reticulitermes* species, *R. flaviceps* (Oshima), *R. kanmonensis* Takematsu, and *R. leptomandibularis* Hsia and Fan occur in Taiwan. The second one is a cryptic species and new record for Taiwan.

Keyword: *Reticulitermes*, cryptic species, COII, 16S, Taiwan.

Introduction

Rhinotermitidae, the subterranean termite, is responsible of 80% of worldwide termite damage, 40 billion dollars annually (Rust and Su, 2012). The genus *Reticulitermes* Holmgren, 1913, composites ca. 25% of the rhinotermitids, and is an important group widespread in temperate zones (Krishna et al., 2013). Identification these pest species with high economic impact is generally considered the first step for developing control strategies.

A total of 138 *Reticulitermes* species are recorded throughout the world including Nearctic Region, Neotropical Region, Oriental Region, and Palaearctic Region (Krishna et al., 2013). In Taiwan, based on the morphological characteristics of the solider caste, two species have been recorded. In 1911, Oshima described a new species, *Reticulitermes flaviceps*, from Taiwan as the first record of Taiwanese *Reticulitermes* termites, which was differentiated from Japanese *Reticulitermes speratus* (Kolbe, 1885) based the ratio of head width/ head length of soldier caste. There was a dispute, however, regarding identification of *R. flaviceps* and *R. speratus*, at the time, because the key character was not clear, (Hozawa, 1912). In 1968, Morimoto discovered an additional species, *Reticulitermes leptomandibularis*, from Taiwan, which was distinguished from *R. flaviceps* based on the shape of labrum tip.

Reticulitermes taxonomy in East Asia, e.g. Taiwan, China, Japan, and Korea, is still controversial due to insufficient morphological characteristics for identification. In recent years, molecular technology has been recognized as an efficient tool for the clarification of taxonomic debates, especially for *Reticulitermes* termites in North America and Europe (Ke et al., 2017; Szalanski et al. 2003; Ye et al. 2004). In the present study, both morphological and molecular characteristics were applied to re-examine the current taxonomic status of Taiwanese *Reticulitermes* termites.

Materials and methods

Sample collection site and collecting method

Winged imago, soldier, and worker samples of *Reticulitermes* termites were collected from 64 localities across Taiwan. Specimens of Japanese *Reticulitermes kanmonensis* were collected from the 6 sites in the Yamaguchi Prefecture in the vicinity of the type locale. All samples were preserved in 95% ethanol and deposited in the NCHU Termite Collection, Department of Entomology, National Chung Hsing University (NCHU), Taichung, Taiwan.

DNA extraction, amplification, and sequencing

Genomic DNA was extracted from the muscle tissue of both head and thorax using Puregene DNA Isolation kit (Gentra Systems, Minnesota, USA) and QuickExtract DNA extraction kit (Epicentre Biotechnologies, Madison, WI) according to the manufacturer's protocol. Two mitochondrial genes, i.e. COII and 16S rDNA, were amplified for the phylogenetic analyses.

Phylogenetic analyses

Sequences were edited and verified using Bioedit (Hall, 1999) and aligned using the Muscle Alignment option in SeaView4 (Gouy et al., 2010). A congeneric species, i.e. *Reticulitermes chinensis* (Accession no. KM216388), was rooted as an outgroup. Phylogenetic inference, on the basis of COII+16S rDNA, was conducted using Maximum likelihood (ML) and Bayesian inference (BI).

Measurement and photography

We selected 9, and 11 quantitative characters of winged imago, and soldiers (Takematsu, 1999), including the enteric valve of workers for measuring and photography. All sample measurements were record by using a Leica M205 C stereomicroscope and a Leica MC170 HD digital camera with LAS software (version 4.4.0, Leica Application Suite, Wetzlar, Germany). The measurements were taken according to Roonwal (1969). The color of samples was examined under the microscope and compared using the Munsell color system (Munsell Color Company 1975, Kelly and Judd 1976) to determine hue, value, and chroma of the described character.

Results and discussion

Phylogenetic inferences, on the basis of COII and 16S rDNA, showed three lineages in the Taiwan samples, which is incongruent with the accepted taxonomic status. Three groups could be referred to the known species via blast analysis in GenBank, i.e. Group A, B, and C represent *R. flaviceps*, *R. kanmonensis*, and *R. leptomandibularis*, respectively. Moreover, Group B contains all of the Japanese specimens and those from Taiwan. The average genetic divergence for describing species boundaries was for Groups A/B, A, B/C 4.5% and 6-8.7%, respectively. These results support that *R. flaviceps* and *R. kanmonensis* maybe sibling species.

We compared samples from the Taiwanese Group B with samples of *R. kanmonensis* from the type local in Japan to clarify the Group B connection to *R. kanmonensis*.

We found no external features that could distinguish (Figure 1A.) the Taiwanese Group A and Group B from *R. kanmonensis* from Japan, but show it can discriminated by the structure of the enteric valve (Figure 1B, 1C, and 1D). According to the phylogenetic analyses (Figure 2.), we show the samples could be dividing into three groups, Group A contained *R. flaviceps*; Group B was indistinguishable from the Japanese *R. kanmonensis* and the last group Group C, with *R. leptomandibularis*.

In addition to morphology and genetic data, we further compared the ecological habitat of *R. flaviceps* and *R kanmonensis* in Taiwan. The collection sites of *R. kanmonensis* (mean = 1080m) were significantly higher than that of *R. flaviceps* (mean = 437m), which also indicate that *R. flaviceps* and *R. kanmonensis* are different but cryptic species.

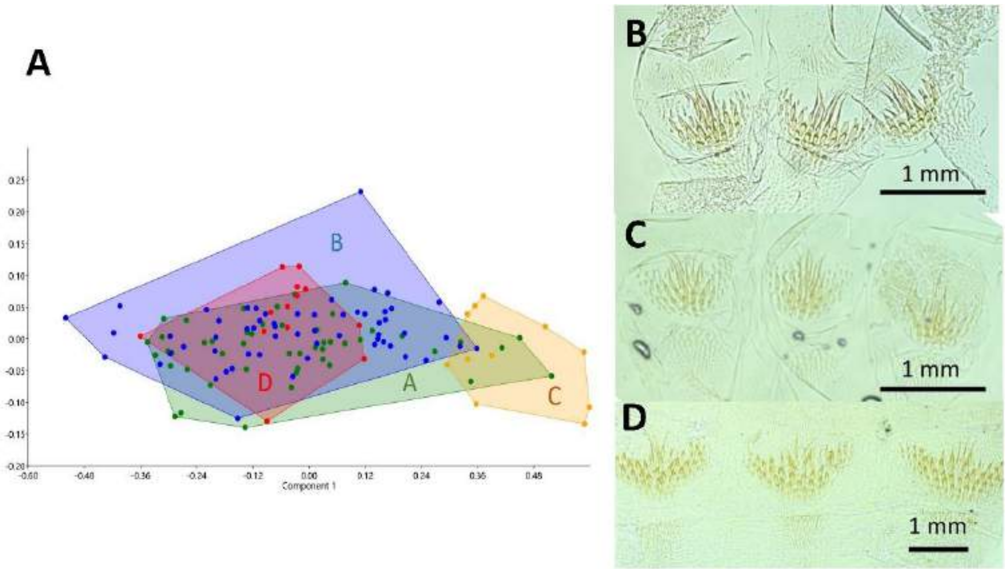

Figure 1. Morphological comparison of Taiwanese and Japanese samples. A, Comparison of three *Reticulitermes* group in Taiwan and *R. kanmonensis* from Japan using principal components analysis (PCA) (Head length, Head width, Head high, Left mandible length, Labrum length, Labrum width, Pronotum width, Pronotum length, Postmentum maximum width, Postmentum minimum width). (A) *R. flaviceps* (B) *R. kanmonensis* from Taiwan (C) *R. leptomandibularis* (D) *R. kanmonensis* from Japan. B, enteric valve of *R. flaviceps*. C, enteric valve of *R. kanmonensis*. D, enteric valve of *R. leptomandibularis*.

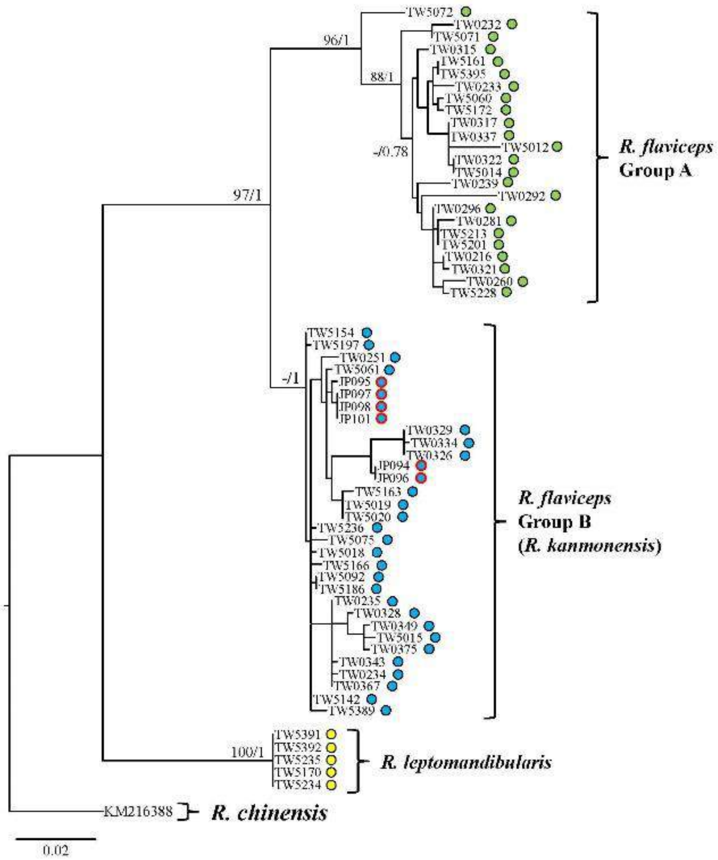

Figure 2. Phylogenetic analyses of COII and 16S rRNA. Blue dots with red frame are Japanese samples.

Conclusions

Three *Reticulitermes* species, *R. flaviceps*, *R. kanmonensis*, and *R. leptomandibularis* were found in Taiwan. *R. flaviceps* and *R. kanmonensis* are cryptic species, and *R. kanmonensis* is a new record to Taiwan.

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Species composition and distribution of termites according to altitude in the Central Highlands, Vietnam

by
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Abstract

A survey was conducted on the composition of termite species and distribution characteristics according to altitude in the whole Central Highlands, Vietnam over 3 years (2013 - 2015). We identified 95 species belonging to 26 genera, 8 subfamilies and 3 families (Kalotermitidae; Rhinotermitidae and Termitidae). There were 84 species we could determine from the literature while 11 species were determined to genus. The composition of termites was different according to altitude in which they were collected. The highest number of termite species was in the altitude range of 500m - 1,000m (82 species), that number decreased below 500 m (71 species) and the least number was found at the altitudes over 1,000m (32 species). There were 21 species distributed in all three altitude ranges. 15 species were distributed in the altitude range 500 m - 1000 m; 8 species were only found at an altitude below 500 m and 3 species were only distributed in altitudes above 1,000 m.

Keyword: termites, Isoptera, Central Highlands, Vietnam

Introduction

The Central Highlands is the largest plateau in Vietnam, with a relatively flat terrain, and an average altitude of 500 - 600m above sea level. The Central Highlands includes 5 provinces namely Kon Tum, Gia Lai, Dak Lak, Dak Nong and Lam Dong. This area is favorable for the development of long-term, large scale industrial timber production. The Central Highlands is also a region developing many reservoirs to service irrigation and hydropower. This region has favorable environmental conditions for termite species and it has been a long time since termites were a matter of special concern not only for managers and scientists, but also for farmers who have been following agricultural and forestry production directives. Research on termites in the Central Highlands includes Vu Van Tuyen (1991) on the issue of treating termites harming coffee trees in Lam Dong, the author recognized 6 species that caused low productivity and small beans, but provided no specific description of the indigenous termite fauna. To 2003, Le Van Trien et al. (2003) published a list of termite species and their distribution that harm trees in the Central Highlands, there were 34 species of 12 genera belonging to 2 families. Nguyen Tan Vuong et al. (2007) conducted a survey on the composition of termite species in the habitats of rubber, coffee and cocoa trees in the Central Highlands provinces and recorded 48 species belonging to 15 genera and 3 families. Among them, 32 species were harmful to coffee, 29 species to rubber and 25 species to cocoa. Nguyen Van Quang et al. (2007) also published data on the harmfulness of termites to coffee, rubber and cocoa trees. The authors showed that the level of injury was different according to the type and growth stage of the trees. Nguyen Minh Duc (2009) found 45 termite species belonging to 14 genera and 2 families in industrial tree plantations Dak Lak province and its vicinity.

The object of termites harming dams in the Central Highlands was investigated by Vu Van Tuyen (1982) in Lam Dong province who discovered 9 species. Le Van Trien et al. (1998) determined 19 species of termite damaging irrigation dams in Lam Dong, with the main species found in the genera *Macrotermes* and *Odontotermes*. Continuing, Le Van Trien et al. (2002) found 21 termite species when investigating termites harming the dam at a reservoir in the Central Highlands. Nguyen Quoc Huy et al. (2008) also investigated some reservoir dams in the Central Highlands and determined 25 species, of which 15 species were found in the dam itself.

It can be seen that the investigation on termites in the Central Highlands was carried out mainly in agricultural and industrial tree plantation areas or irrigation works with the aim of determining the species composition and main harmful species to limit the damage caused by termites. The overall survey on the composition of termite species in the Central Highlands and determination of their distribution according to habitats and altitude has not been previously accomplished. Our research contributes to supplementing the deficiencies stated above.

Figure 1. The map of the Central Highlands, Vietnam (survey locations)

Methods

The research was implemented over 3 years from 2012 - 2015, in 5 Central Highlands provinces with 58 locations for collecting samples. Specifically: Kon Tum had 8 locations, Gia Lai 12; Dak Lak 20; Dak Nong 9; and Lam Dong 9 (Figure 1). At each location, we conducted the sampling according to the method of Nguyen Duc Kham (1976), 1-3 km long transects. The transects were placed through the following habitats: primeval forest, secondary forest, plantation forest, brushwood, residential areas and reservoir dams. The sampling technique was carried out as follows: using digging tools such as trowel and screwdriver to collect termites from tree-trunks, rotten roots, dry branches, under the rotten coverage, in nests. We collected all castes, however, paid special attention to soldiers. Termite samples collected at each point were stored in 75% alcohol in a small plastic vial (1cm x 7cm). We used GPS to record the elevation in addition to the survey points along each transect line.

The treatment, cleaning, preservation and analysis of specimens was implemented at the laboratory of Institute of Ecology and Works Protection, Vietnam Academy for Water Resources.

The termite samples were described by observeration and measured according to Roonwal (1969) while species or genus determined by morphology according to Ahmad (1958, 1965); Akhta (1975); Thapa (1981); Huang Fu Sheng (2000); Nguyen Duc Kham et al. (2007).

Result and discussion

Composition of termite species in the Central Highlands

We analyzed 2,566 terminate specimen and recorded 95 species belonging to 26 genera from 8 subfamilies of 3 termite families Kalotermitidae; Rhinotermitidae and Termitidae. We determined 84 species, while 11 species were only identified to genus. Comparing the published results on termite species composition of Vietnam, Nguyen Duc Kham (2007) and Trinh Van Hanh (2010) (101 species and 133 species, respectively), our list from the Central Highlands added 15 species to Vietnam (Table 1). There were 6 genera and 30 species (excluding new species determining the genus) supplemented to the list from the Central Highlands area.

Considering the structure of species composition, the Central Highlands area has 8 subfamilies (Table 1), in which the family Macrotermitinae had the highest number of species (37 species, 38.9%). This subfamily has four genera, *Odontotermes* (20 species), *Macrotermes* (8 species), *Hypotermes* (5 species) and *Microtermes* (4 species). *Odontotermes* was collected the most, it always dominated the number of species and number of individuals in all habitats and heights. Abundance and diversity was also shown for the Nasutitermitinae with 15 species (15.8%) and Termitinae with 14 species (14.7%).

Table 1. The list of termites in the Central Highlands by Province

No.	Taxon	Province				
		Kon Tum	Gia Lai	Dak Lak	Dak Nông	Lam Dong
	KALOTERMITIDAE ENDERLEIN, 1909					
	KALOTERMITINAE FROGGATT, 1896					
1	*Cryptotermes* sp.			+		
	RHINOTERMITIDAE LIGHT, 1896					
	COPTOTERMITINAE HOLMGREN					
2	**Coptotermes curvignathus* Holmgren, 1913		+			
3	*Coptotermes ceylonicus* Holmgren, 1911			+	+	+
4	*Coptotermes emersoni* Ahmad, 1953		+	+		
5	*Coptotermes formosanus* Shiraki, 1909	+	+	+	+	+
6	*Coptotermes havilandi* Holmgren, 1911			+	+	
7	*Coptotermes travians* Holmgren, 1898			+		
8	**Coptotermes gestroi* (Wasmann, 1896)		+			
9	**Coptotermopsis dimorphus* Nguyen, 1971			+		
10	*Coptotermopsis* sp.		+			
	RHINOTERMITINAE FROGGATT, 1896					
11	***Schedorhinotermes brevialatus* (Haviland, 1898)				+	
12	*Schedorhinotermes javanicus* Kemner, 1934	+	+	+	+	+
13	**Schedorhinotermes magnus* Tsai et Chen, 1963					+
14	*Schedorhinotermes malaccensis* Holmgren, 1913			+	+	
15	*Schedorhinotermes medioobscurus* Holmgren, 1914			+	+	+
16	*Schedorhinotermes tarakensis* (Oshima)			+		
17	***Schedorhinotermes translucens* (Haviland, 1898)	+				+
18	*Schedorhinotermes sarawakensis* (Holmgren, 1913)	+	+	+		+
19	***Schedorhinotermes rectangularis* Ahmad, 1965		+	+		
20	***Prorhinotermes tibiaoensiformis* Ahmad, 1965			+		
	HETEROTERMITINAE FROGGATT, 1896					
21	**Reticulitermes wuganensis* Huang et Yin, 1983					+
	TERMITIDAE WESTWOOD, 1840					

	MACROTERMITINAE KEMNER, 1934					
22	*Macrotermes annandalei* (Silvestri, 1914)	+	+	+	+	+
23	*Macrotermes carbonarius* (Hagen, 1858)	+	+	+	+	+
24	*Macrotermes gilvus* (Hagen, 1858)	+	+	+	+	+
25	*Macrotermes latignathus* Thapa, 1981			+	+	+
26	*Macrotermes malaccensis* (Haviland, 1898)	+		+	+	+
27	*Macrotermes maesodensis* Ahmad, 1965			+		
28	*Macrotermes tuyeni* Vuong, 1996	+	+	+		+
29	*Macrotermes* sp.			+		
30	*Microtermes incertoides* Holmgren, 1913		+	+		
31	*Microtermes obesi* Holmgren, 1913	+	+	+		+
32	*Microtermes pakistanicus* Ahmad, 1955		+	+	+	+
33	*Microtermes* sp.			+		
34	*Odontotermes angustignathus* Tsai et Chen, 1963	+	+	+	+	+
35	*Odontotermes butteli* Holmgren			+		
36	*Odontotermes bruneus* (Hagen)			+		
37	*Odontotermes ceylonicus* Wasmann, 1902	+	+	+	+	+
38	***Odontotermes faeoides* Holmgren					+
39	*Odontotermes feae* (Wasmann, 1896)	+		+	+	
40	*Odontotermes formosanus* (Shiraki, 1909)		+	+	+	+
41	**Odontotermes graveli* Silvestri, 1914			+		
42	*Odontotermes hainanensis* (Light, 1924)	+	+	+	+	+
43	*Odontotermes longignathus* Holmgren, 1914			+	+	+
44	*Odontotermes malabaricus* Holmgren			+		
45	*Odontotermes maesodensis* Ahmad, 1965			+	+	+
46	*Odontotermes oblongatus* Holmgren		+	+	+	+
47	*Odontotermes pahamensis* Nguyen, 1971	+		+		+
48	*Odontotermes proformosanus* Ahmad, 1965	+	+	+	+	+
49	***Odontotermes pyriceps* Fan, 1985					+
50	***Odontotermes sarawakensis* Holmgren, 1913	+				+
51	**Odontotermes yunnanensis* Tsai et Chen, 1963		+			
52	*Odontotermes javanicus* (Holmgren, 1912)				+	
53	*Odontotermes*sp.			+	+	
54	*Hypotermes makhamensis* Ahmad, 1965			+	+	+
55	*Hypotermes obscuriceps* (Wasmann, 1902)		+	+	+	+
56	*Hypotermes sumatrensis* Holmgren, 1913	+	+	+	+	
57	***Hypotermes xenotermitis* (Wasmann, 1896)	+				
58	*Hypotermes* sp.			+		
	AMITERMITINAE KEMNER, 1934					
59	*Microcerotermes bugnioni* Holmgren, 1911			+	+	
60	*Microcerotermes burmanicus* Ahmad, 1947			+	+	
61	*Microcerotermes crassus* Snyder, 1934			+		
62	*Globitermes sulphureus* (Haviland, 1898)	+	+	+	+	+
63	*Globitermes* sp.		+			
64	*Speculitermes* sp.			+		
65	**Euhamitermes hamatus* (Holmgren, 1912)			+		
66	***Indotermes bangladeshiensis* Akhtar, 1975			+		
	TERMITINAE SJOSTEDT, 1926					
67	*Termes comis* Haviland, 1898			+	+	+
68	*Termes propinquus* (Holmgren, 1914)	+	+	+		
69	*Pericapritermes latignathus* (Holmgren, 1914)		+	+	+	+

70	*Pericapritermes nitobei* (Shiraki, 1909)			+	+	+
71	***Pericapritermes paraspeciosus* Thapa, 1981			+		+
72	*Pericapritermes sermarangi* Holmgren, 1913		+	+	+	+
73	*Pericapritermes tetraphilus* (Silvestri, 1922)		+	+		
74	*Pericapritermes* sp.1			+		
75	*Pericapritermes* sp.2			+		
76	***Procapritermes prosetiger* Ahmad, 1965			+		
77	***Pseudocapritermes albipennis* (Tsai et Chen)			+		
78	**Pseudocapritermes parasilvaticus* Ahmad, 1965			+		
79	***Pseudocapritermes sinensis* Ping et Xu, 1986			+		
80	**Dicuspiditermes garthwaitei* (Gardner, 1944)		+	+		
	NASUTITERMITINAE HARE, 1937					
81	***Nasutitermes fuscipennis* (Haviland, 1898)			+		
82	*Nasutitermes matangensis* (Haviland, 1898)			+	+	
83	*Nasutitermes ovatus* Fan, 1983			+		+
84	***Nasutitermes rectangularis* Thapa, 1981		+			
85	**Nasutitermes regularis* (Haviland, 1898)		+	+		
86	**Nasutitermes ninhthuanensis* Nguyen			+		
87	*Nasutitermes* sp.		+	+		
88	**Lacessititermes albipes* (Haviland, 1898)		+			
89	**Lacessititermes batavus* Kemner, 1993			+		
90	*Hospitalitermes medioflavus* (Holmgren, 1913)			+		
91	*Bulbitermes prabhae* Krishna, 1965	+	+	+		
92	*Bulbitermes laticephalus* Ahmad, 1965			+		
93	*Aciculioditermes holmgreni* Ahmad, 1968					+
94	**Aciculioditermes sarawakensis* Ahmad, 1968			+		
95	***Ahmaditermes guizhouensis* Li et Ping, 1982		+			
Sum.		**22**	**37**	**77**	**35**	**38**

Note: (**): *Termites species first identified in Vietnam*
(*): *Termites species first time identified in the Central Highlands*

The Central Highlands is divided into five provinces. The distribution of termite species in each province has practical significance. The results presented in Table 1 shows the number of termite species in Dak Lak province was the greatest with 77 species, accounting for 81.1% of the total number of species found in the Central Highlands. There were 29 species that showed a relatively narrow distribution, only obtained in Dak Lak. In Lam Dong province, there were 38 species (40%), of which 5 species were found only in Lam Dong. The number of species found in Gia Lai was approximately as that of Lam Dong with 37 species (37.9%) and 8 were only collected in Gia Lai province. Dak Nong province provided 35 species (36.8%) and 2 were found only in this province. Kon Tum province had the least number of species, 22 species, with one species only found in Kon Tum. The Di Linh plateau in the South Central Highlands sub-zone is adjacent to the plateaus of Dak Nong and has natural conditions relatively similar to Dak Nong and therefore the number of termite species discovered in Lam Dong was not much different.

Distribution of termite species according to altitude

The Central Highlands consists of many succeeding plateaus at different altitudes, of which the most are between 500 m to 1,000 m, but there are also areas below 500 m and mountainous areas above 1,000 m (such as Lam Vien plateau, 1,500 m). We therefore selected three altitude ranges for this termite survey in the Central Highlands because altitude is a major factor governing climate and directly affects the distribution of plants and animals.

The results of the termite samples collected in the three altitude ranges are presented in Table 2. The number of termite species at the altitude range 500m - 1,000m had the greatest number, 82 species (86.3%) from a total of 95 species and range of less than 500m, provided 71 species (74.7%) while the altitudes over 1,000m had 32 species (33.7%).

Table 2. Number of species according to altitude range in the Centre Highland

No	Subfamilies	Altitude (m)		
		< 500	500 - 1000	> 1000
1	Kalotermitinae	1	1	0
2	Coptotermitinae	9	6	0
3	Rhinotermitinae	8	8	4
4	Heterotermitinae	0	0	1
5	Macrotermitinae	29	35	17
6	Amitermitinae	6	8	1
7	Termitinae	10	13	6
8	Nasutitermitinae	8	11	3
	Number	71	82	32

It is notable that in the altitude range of less than 500m and from 500m to 1,000 m, provided no Heterotermitinae (Rhinotermitidae) (Table 2). At the altitude range of over 1,000 m, there were no Kalotermitinae or and Coptotermitinae. In general, the subfamilies Macrotermitinae, Amitermitinae, and Termitinae had the most species and these were found in the altitude range of 500 - 1,000 m, but the Coptotermitinae represented the largest number of species at the altitude range below 500 m (Table 2). Among the 95 termite species found in the Central Highlands, we found 21 species distributed at all three altitude ranges, 15 species were only found at the altitude range of 500 m - 1,000 m, 8 species only at altitudes less than 500m and 3 species were only found over 1,000 m. Thus, in the Central Highlands, the composition of termite species at altitudes below 500 m and 500 m to 1,000 m were not clearly different, but the number of termite species at the altitudes over 1,000m was reduced by more than half in comparison to those found at altitudes less than 1,000m. Our results were consistent with Nguyen Van Quang (2007) and Nguyen Tan Vuong (2007).

The results presented in Table 2 show that the adaptability of termite subfamilies at altitudes over 1,000 m was different because Kalotermitinae and Coptotermitinae were not found. The Amitermitinae and Nasutitermitinae subfamilies had few adaptable species, specifically, Amitermitinae which had one species of eight species in all altitude ranges, Nasutitermitinae had 3 of 14 species at all altitude ranges. Macrotermitinae dominated the number of species we collected in the Central Highlands with 29 species from a total of 71 species (40.8%) at altitudes less than 500 m, 35 species (42.7%) at altitudes between 500 m - 1,000 m, and 32 species (53.1%) at altitudes above 1,000 m. Our survey is not contrary to the previous judgments concerning the dominance of Macrotermitinae in the North and the Central of Vietnam. Nguyen Duc Kham (1976) reported that the Macrotermitinae was widely distributed at the altitude of 700 m. Nguyen Van Quang (2005) also reported that at higher altitude, the number of Macrotermitinae species decreased. For example, according to Nguyen Duc Kham (1976), *M. annandalei*, suffers at cold temperatures and was only found in the plains and edges of the forests, rarely found in the forest and at higher altitudes. Nguyen Van Quang (2003) reported that in North of Vietnam, *M. annandalei* was found only at the altitudes less than 1,000 m. But in the Central Highlands, we found *M. annandalei* distributed quite commonly at all altitude ranges, including over 1,000 m. Perhaps, the air temperature in the Central Highlands is higher than that in the mountainous areas in the North of Vietnam.

Conclusion

1. We identified 2,566 termite samples collected from 58 survey sites in 5 Central Highlands provinces over 3 years (2013 - 2015) and determined 95 species belonging to 26 genera representing 8 subfamilies in 3 termite families: Kalotermitidae; Rhinotermitidae and Termitidae. In the list of 95 species, 15 species were added to the termite fauna of Vietnam along with 6 genera and 30 species first recognized from the Central Highlands.
2. The highest diversity of termite species was in the altitude range of 500m - 1,000m (82 species), decreasing at the altitude range below 500 m (71 species) and the least number at the altitude range over 1,000m (32 species). There were 21 species distributed in all three altitude ranges. 15 species were distributed at the altitude range of 500 m - 1000 m; 8 species were only found at altitudes below 500 m and 3 species were only distributed at altitudes above 1,000 m. Macrotermitinae predominated in species and number at all 3 altitude ranges (in particular, below 500 m).

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Speciation in the Australian *Amitermes* Group: first insights from molecular genomics

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Abstract

The Australian *Amitermes* group (AAG) is the most speciose and diverse group of termites in Australia. The success of this group, diverging during a time of rapid climate change on the continent, can help us understand how termites have adapted to their environment and may continue to do so under future climate-change scenarios. We began by sampling from the species-rich area of Western Australia near the proposed origin of *Drepanotermes*, one of the major genera of the AAG, to reconstruct the diversification of this group after its colonization of Australia. The information from mitochondrial genome sequencing and selected nuclear genes recovered a monophyletic *Drepanotermes* split into a clade of species with extensive and another with restricted ranges. All *Amitermes* we have sequenced to date are basal to *Drepanotermes* and separate into a number of clades without clear geographical, morphological or nesting trait divisions. Our preliminary estimate of the divergence of *Drepanotermes* from *Amitermes* at 20 ± 5 Mya is probably not accurate; we expect future analysis will recover the split between the AAG and the rest of *Amitermes* to have occurred around this time, with the *Drepanotermes-Amitermes* division being more recent, in congruence with other taxa that arrived in Australia after its collision with the Southeast Asian plate. This initial dataset suggests intriguing stories of multiple introductions, waves of diversification, undescribed diversity, and incomplete lineage sorting and/or introgression during divergence, which will be revealed as we collect more complete data from additional AAG taxa.

Key words: molecular phylogenetics, *Amitermes*, *Drepanotermes*, cryptic species, diversity

Introduction

The Australian *Amitermes* group (AAG) currently consists of five genera and roughly 100 species. These termites represent one third of Australia's termite diversity and are hugely important to its ecology, cycling nutrients, enriching soil, and providing food and habitat for other species (Eggleton 2010). They diversified in a period of dramatic climate change, probably after the collision of the Australian and Southeast Asian plates 25 – 20 Mya through the end of the Pleistocene (1.6 Mya). The continent transitioned during this time from lush rainforests, to open forests and woodlands, then to the forests, savannas, and deserts of today. Rapid adaptation to these changing and sometimes challenging conditions, may have fostered the evolution of traits in the AAG species which are unusual in the termite world: foraging in the open, harvesting and storing vegetable matter in the nest, large numbers of reproductives in a single nest, loss of the soldier caste, and nest parasitism.

The genus *Amitermes* is thought to have originated in the Afrotropical or Indomalayan region (Bourguignon et al. 2015) during the Oligocene (Engel et al. 2009). The “other” AAG genera, *Drepanotermes, Ahamitermes, Incolitermes*, and *Invasitermes*, probably diverged from *Amitermes* following one or more invasions from southeast Asia, a pattern seen in *Coptotermes* (Lee et al 2017) and the Nasutitermitinae (Arab et al. 2017) as well as well as herpetofauna, birds, some plants, and other invertebrates (reviewed in Byrne et al. 2009). Earlier work found *Drepanotermes* to be nested within (i.e. derived from) *Amitermes*, with the Australian *Amitermes* + *Drepanotermes* forming a sister group to the Asian *Amitermes* (Inward et al. 2007). The distribution of *Drepanotermes* indicates a likely origin in the Northwest Cape zone (Watson and Perry 1981), so as a first step to resolving the relationships within the AAG and estimating their divergence times, we sampled *Amitermes* and *Drepanotermes* from a species-rich area south of the Northwest Cape of Western Australia.

Materials and methods

Samples were collected in February 2016 from a roughly 120,000 km^2 area of Western Australia, bordered by Mandurah, Kalgoorlie, Mt Magnet, and Geraldton and stored in RNAlater on ice (in the field) or at room temperature (lab). Morphological determination followed Watson and Perry (1981) and Gay (1968). DNA was extracted from whole specimens, minus the gut, using the DNeasy Blood & Tissue Kit (Qiagen; Hilden, Germany). The mitochondrial genome was amplified in two fragments using primers modified from Bourguignon et al (2015) or designed for this study. Multiplexed, paired-end reads were sequenced on the Illumina HiSeq2000 and assembled using SPAdes (Nurk et al., 2013) plugin in Geneious 11.0.5 (Kearse et al. 2012). Mitochondrial genomes were aligned with those from previous work (Bourguignon et al. 2015) using ClustalW (Larkin et al. 2007). Nuclear markers ITS1/2 were amplified in a single PCR reaction and Sanger sequenced (Seqlab, Göttingen, Germany). Sequences were aligned using ClustalW (Larkin et al. 2007). Evolutionary rate models were estimated using PartitionFinder2 (Lanfear et al. 2016) and separate phylogenetic trees for ITS and mitochondrial genes were constructed using MrBayes v3.2.6 (Ronquist et al. 2012; $5x10^6$ generations, burnin 2500, sampling frequency 2500). Divergence times were estimated with the Bayesian phylogenetic software BEAST v1.8.1 (Drummond et al, 2012) using a strict molecular-clock model, 10^6 generations sampled every 10,000 steps, and Yule speciation process.

Results and Discussion

No differences in mitochondrial genome structure (re-arrangements, inversions, GC-content, etc.) were found between *Drepanotermes* and *Amitermes* or between the AAG and non-Australian *Amitermes*. Genetic distances (COII sequence alignment) ranged from 5.8 – 10.4% between *Drepanotermes* and *Amitermes* and from 7.8 – 13.2% between AAG and non-Australian *Amitermes*. Both mitochondrial genomes and nuclear markers recovered *Drepanotermes* as a monophyletic group within *Amitermes*. The sister group to *Drepanotermes* differed between the trees, however *A. deplanatus* was consistently recovered near the *Drepanotermes* clade. While this is a small to medium-sized *Amitermes*, it does have distinctively long mandibles, a potential synapomorphy with *Drepanotermes*. Additional sampling, particularly in the Northwest Cape, Pilbara, and Kimberly will be required to truly identify sister species and traits that may have been under selection in the divergence of *Drepanotermes* from *Amitermes*.

The widespread *D. perniger* and *D. rubriceps* cluster together, as expected from their once synonymy, while three other *Drepanotermes* with more restricted ranges seem to form a sister group. Whether this indicates two separate origins of "*Drepanotermes*" or a second wave of divergence within the genus, perhaps coinciding with the Hill Gap and the end of the Pliocene mesic pulse, requires inclusion of the remaining *Drepanotermes* species. Included in our analysis are genetically supported morphospecies which we have been unable to associate with described species. This undescribed diversity is not surprising: Miller (1994) and Watson and Abby (1993) refer to "many" new *Amitermes* species, mainly soil-dwellers, which they suggest represent distinct genera as well as series of *Drepanotermes* which lack "biological data" to assist in their identification (Watson and Abby 1993).

Divergence between *Drepanotermes* and *Amitermes* is estimated at no earlier than 20 ± 5 Mya. While this is possible, we think that future analysis will estimate the split between global *Amitermes* and the AAG to have occurred in this time range, with even more recent divergences between lineages within the AAG, similar to patterns found in the Nasutitermitinae (Arab et al. 2017) and *Coptotermes* (Lee et al 2017). Our initial analysis also suggests multiple expansions of the AAG across Australia. The most basal taxa in our trees occur in southwestern Western Australia, while the limited sequences we have from northern and eastern Australia seem to fall into two separate clades also containing Western Australian taxa.

Conclusions

Our results suggest interesting hypotheses for further study although sampling covered only a portion of described AAG taxa. Differences between mitochondrial and nuclear trees are likely the result of incomplete lineage sorting or hybridization and introgression during divergence and will need to be examined in more detail. This is particularly relevant to widespread taxa, such as *D. perniger*, which already form distinct subclades despite our dataset covering a relatively small portion of their range. This and a number of other AAG species have previously been noted as having significant variability in morphological and/or feeding or nesting traits (Watson and Perry 1981, Gay 1968) and as currently defined likely represent species complexes. The undescribed diversity within the AAG may complicate future trait-based analysis. We are testing RADseq and expanding our taxon coverage to provide insight into these and other outstanding questions.

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Odontotermes formosanus regulate α-amino acids and fatty acids with their symbiont fungus

by
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Abstract

Odontotermes formosanus (Shiraki) is a common fungus-growing termite in Asia. *O. formosanus* culture the fungus *Termitomyces* spp. on plant tissue they collect. Fungal fermentation was hypothesized to transform the plant tissues to nutritious fermentation products. We tested this hypothesis by quantifying the fungal transformation and insect nutritional requirement for 18 α-amino acids and 56 fatty acids in *O. formosanus*. The results showed that fungal fermentation significantly decreased the proportional variation of each α-amino acid and increased that of fatty acids. The composition of α-amino acids and fatty acids in termite tissues were highly similar to that in post-fermentation materials, indicating that the fermentation products were a balanced and major nutrition source for fungus-growing termites. The results support that termite fungiculture homogenized α-amino acids, which is advantageous to broadening the termites feeding niche.

Keywords: *Odontotermes formosanus*, *Termitomyces* spp., acquiring enzyme hypothesis, ruminant hypothesis, solid-state fermentation

Introduction

Odontotermes formosanus is a common species of fungus-growing termite in Asia (Krishna et al. 2013). *O. formosanus* collect and ingest plant tissue, however, they digest that plant material through an external fungal mutualism. Termites mix plant tissue, lignocellulolytic enzymes, spores of *Termitomyces* and gut bacteria in their hindgut, and in less than 3.5 hours excrete the mixture on top of a growing substrate for the fungus, termed the fungus garden, (Li et al. 2017). The growing substrate is colonized by *Termitomyces* fungus and after around 45 days, fungal tissue and fermented products are accumulated in the bottom of fungus garden as a nutritious food for the termites (Li et al. 2017).

The fungus is capable of synthesizing all α-amino acids (α-AA) by two mechanisms, using non-protein nitrogen (Botha & Eicker 1992), or transform the α-amino acids by transamination reactions (Deacon 2006). In addition, fungus can generate fatty acids (FAs) by carboxylation reactions, from carbon-dioxide or methanol or ethanol (Deacon 2006).

Theoretically, reducing the differences in α-AA and FA composition in their diet and compared to body tissue reduces the cost of nutritional transformation. When an animals dietary α-AAs and FAs are adequate and highly assailable, the α-AA and FA compositions in their tissues are similar to that in the diet (Moir 1994).

In the *O. formosanus* fungiculture system, the fungus *Termitomyces* spp. may transform, synthesize, and accumulate α-AAs and FAs in the fungus garden (Botha & Eicker 1992). Fungal transformation may homogenize the nutrition value of the garden by decreasing variation of the α-AA and FA in the rough, collected diet of plant material. We hypothesized that the nutritionally demanding larval stage would provide a α-AA and FA profile in their tissues more similar to the fungus garden than the collected plant tissue.

We aimed to study two questions in this study. Firstly, how was the nutritional composition changed by fungal transformation? Secondly, does the transformation in the fungus garden increase its nutrition value for termites?

Materials and Methods

One-hundred grams of *O. formosanus* from each of the three localities (Houli: 24.3038°N, 120.7290°E; National Chung Hsing University: 24.1193°N, 120.6744°E; Tunghai University: 24.1785°N, 120.6031°E) were collected. Each 100 g termite sample contained 38,367±1,360 individuals, 95.2±2.9% were workers and 4.8±2.2% soldiers.

Three *O. formosanus* nests from different localities were located by the fruiting body of *Termitomyces eurhizus* on the soil surface (Huisun: 24.0916°N, 121.0331°E; National Chung Hsing University: 24.1193°N, 120.6744°E; Xiaping: 23.7729°N, 120.6731°E). Six to twenty-five fungus gardens were collected from each nest. Fungus gardens were visually subdivided into four quartiles. The top quartiles and bottom quartiles were categorized as fresh and aged substrates, respectively (Li et al. 2017).

α-AAs and FAs from whole termites, as well as, the top and bottom fungus gardens were analyzed. Analysis of α-AAs and FAs were performed by National Animal Industry Foundation, Pingtung, Taiwan. A 100g sample was homogenized and divided into two subsamples, 0.5g for analysis of α-AAs and 3.0g for FAs. To analyze the α-AA contents, samples were hydrolyzed by 4 N methanesulfonic acid with 0.2% 3-(2-aminoethyl) indole, under a vacuum condition with temperature of 115˚C for analysis of α-AAs (Simpson, Neuberger & Liu 1976). The contents of 18 α-AAs including Aspartic acid, Glutamic acid, Cystine, Serine, Histidine, Glycine, Threonine, Arginine, Alanine, Tyrosine, Valine, Methionine, Tryptophan, Phenylalanine, Isoleucine, Leucine, Lysine, and Proline were determined by high performance liquid chromatography. FA samples were extracted, saponificated and methyl esterificated according to the official methods for fatty acids analysis (Document No. 1021950978) of Food and Drug Administration, Taiwan. The FA content was determined by gas chromatography-flame ionization detector.

We compared the coefficient of variation (CV) of each α-AA and FA from the top and bottom of the fungus garden to test whether the fungal transformation homogenizes the nutritional value for termites using a paired Wilcoxon test.

The composition of α-AA and FA in the top and bottom substrates from the fungus garden were strongly correlated (α-AA: Pearson r = 0.89, $p < 0.0001$; FA: Pearson r = 0.91, $p < 0.0001$) because they shared a common source – the collected plant material. The non-correlated top and bottom substrates more likely represent the nutrition transformed by fungus. To test whether fungal transformation increases assimilation efficiency, α-AAs and FAs from the bottom substrate was compared with termite tissues by partial linear correlations, with the following formula:

$$r_{yx_1 \cdot x_2} = \frac{r_{yx_1} - r_{yx_2} r_{x_1 x_2}}{\sqrt{(1 - r_{yx_1}^2)(1 - r_{x_1 x_2}^2)}}$$

where x_1 and x_2 are the α-AA and FA compositions in bottom and top substrates, respectively, and y is the α-AA and FA compositions of the termite. The correlation between bottom and top substrates ($r_{x_1 x_2}$) were excluded in the calculation of correlation between termite and bottom substrates ($r_{yx_1.x_2}$). All statistical analyses were conducted by using the R software (v3.3.1) (R Development Core Team 2013), and partial linear correlations were computed with the R package *ppcor*.

Results and discussion

A total of 18 α-AAs and 42 FAs were detected in fungus garden or termites (Figs. 1, 2). Concentrations, by weight, of total α-AAs were 3.9±0.3% and 3.7±0.5% in the top and bottom fungus garden substrates, respectively. CV of concentrations of α-AAs in the top was significantly higher than that in the bottom (CV: top: 0.47±0.25; bottom: 0.18±0.19) (paired Wilcoxon test: W = 130, $p < 0.01$). CV of concentrations of FAs in the top fungus garden substrate was significantly lower than the bottom (CV: top: 0.93±0.69; bottom: 1.11±0.53) (paired Wilcoxon test: W = 176, $p < 0.01$). These results show that fungal transformation homogenized the α-AA composition but diversified the FA composition.

CV of α-AAs and FAs concentrations were low in termite tissues (Figs. 1C, 2C; α-AA CV: 0.41±0.52; FA CV: 0.69±0.63), supporting that the source and the nutritional requirement of termites were constant in different localities. The α-AA composition of termites was positively correlated with fungal transformed α-AA (Figure 1BC; partial linear $r = 0.91$, $p < 0.0001$). The FA composition of termites was positively correlated to that of fungal transformed FA (Figure 2BC; partial linear $r = 0.75$, $p < 0.0001$). These results support that termites depend on nutrition from old fungus garden, and the fungal transformation increased the diet assimilation and conversion efficiencies for termites.

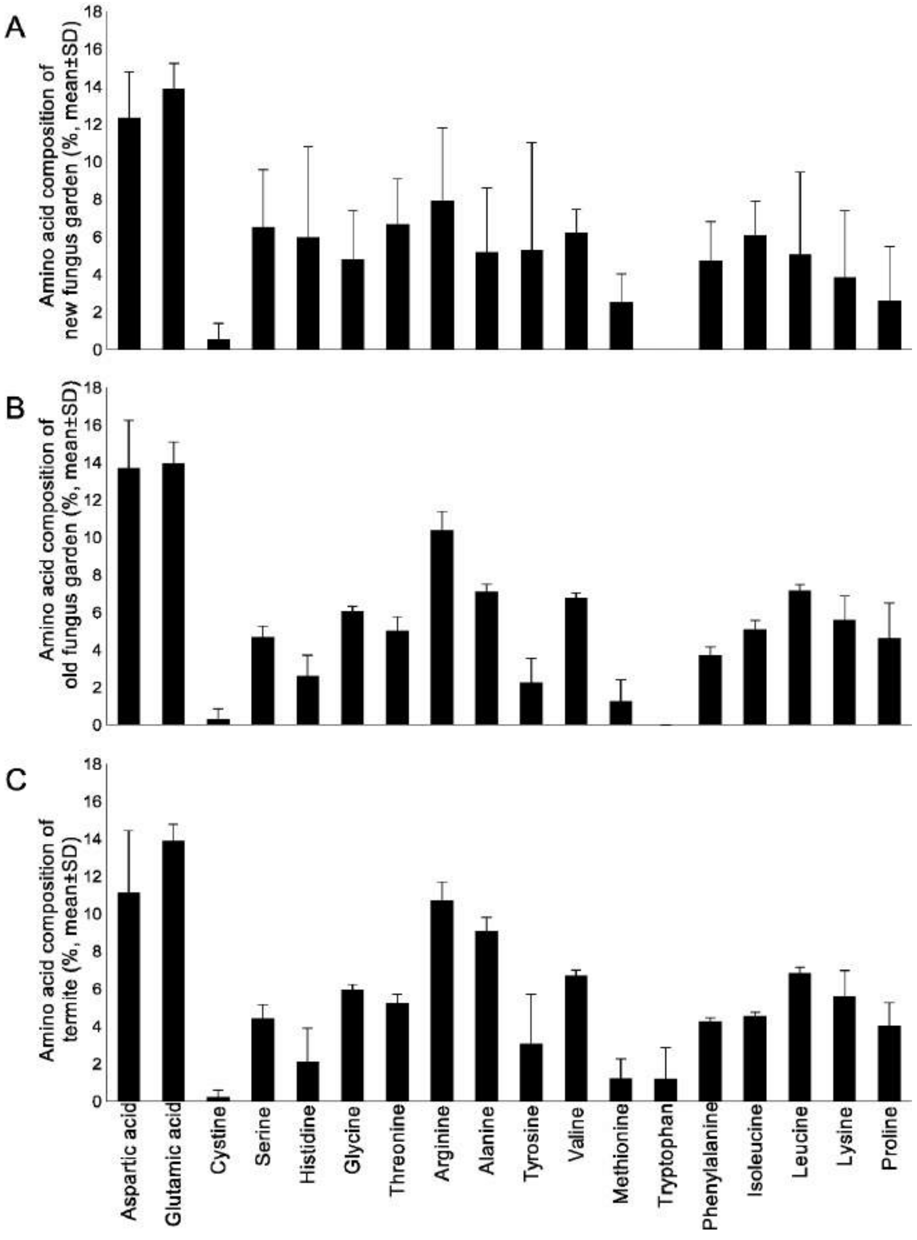

Figure 1. α-amino acid compositions of new (A) and old (B) fungus gardens, and termites (C).

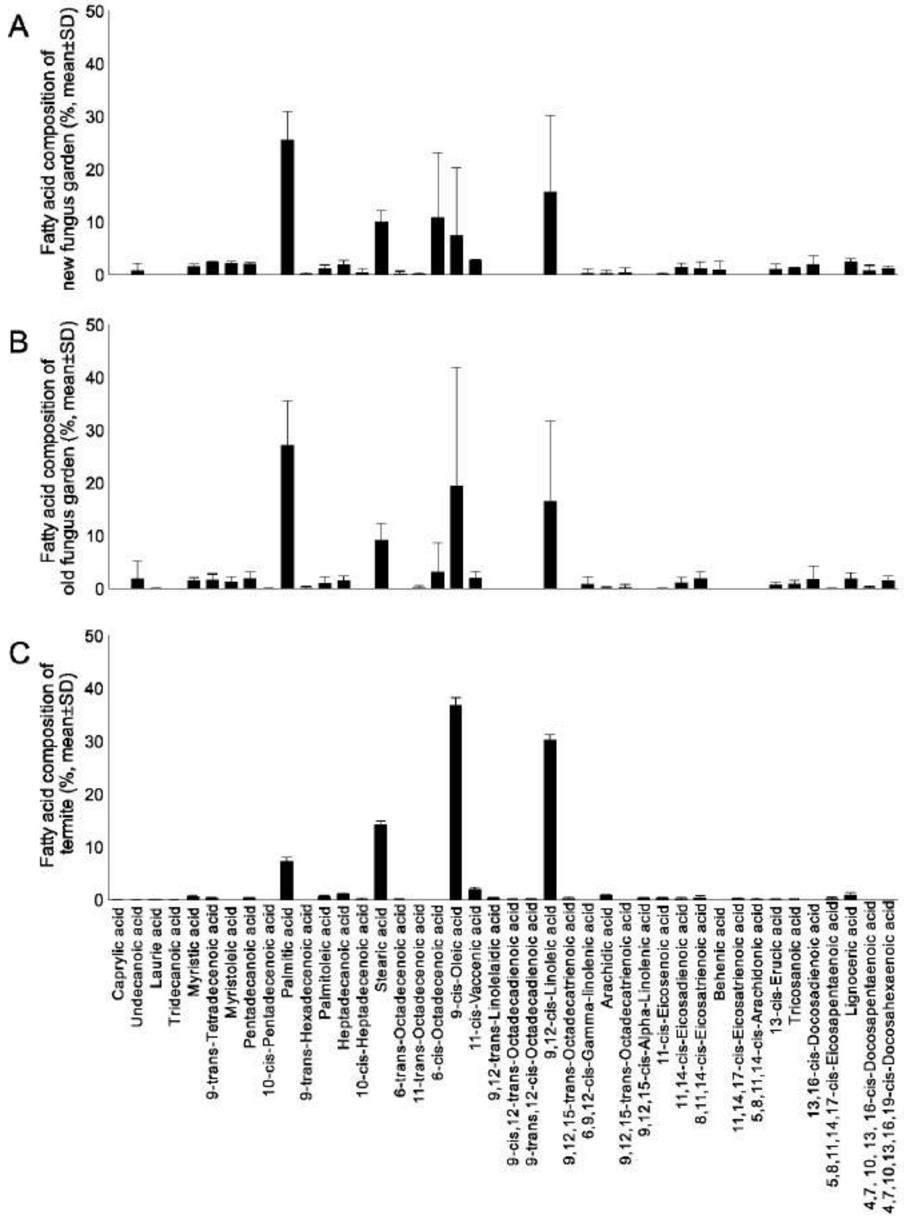

Figure 2. Fatty acid compositions of new (A) and old (B) fungus gardens, and termites (C).

Conclusions

Fungal fermentation significantly decreased the proportional variation of each α-amino acid and increased that of fatty acids, and provided a composition similar to that in termite tissues.

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Caste ratio and task division of termites *Macrotermes annandalei* (Silv.) (Insecta: Isoptera) in Vietnam

by
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Abstract

In this article, we present our research results on caste percentages and polyethism concerning food foraging and nest repair by *Macrotermes annandalei* (Silv.). Our experiments were conducted in the hills of Xuan Mai, Chuong My District, Hanoi Vietnam. The percentage of adult termites (soldiers and workers) was 34.7%, much lower than that of juvenile termites (65.3%). The average percentages of major workers, minor workers, major soldiers and minor soldiers were 11.1%, 19.4%, 0.4%, and 3.8% respectively. The percentage of minor workers was nearly twice of that of major workers, and that of the minor soldiers was approximately nine times of that of major soldiers when counting only adult termites.

The activities outside the nest was mainly carried out by adult termites. Food foraging was implemented mostly by major workers, making up 79.4%, about twelve times that of minor workers (6.1%). Major workers also dominated sites of building or repairing mounds with an average percentage of 53.3%, compared to minor workers (32.2%).

It was clear from our study that there is division of labor in food foraging and repairing mounds in *Macrotermes annandalei* (Silv.).

Key Words: caste percentage, polyethism, *Macrotermes annandalei*

Introduction

Macrotermes annandalei is a common termite species in Vietnam and neighbouring countries like Thailand, and China (Southern). In Vietnam, they can be found in various habitats and elevations, ranging from the north to the south of the country. This species causes damages not only to dams, dikes and electrical infrastructures (Nguyen Duc Kham & Vu Van Tuyen, 1985) but also to crops (Nguyen Duc Kham, 1976), especially to young trees such as *Eucalyptus*, *Acacia*. Although we do not have statistic data on the benefits of this termite species in natural ecosystems in Vietnam, *M. annandalei*, with a large number of individuals in each colony together with their associated fungus species has an important role in improving soil fertility by decomposing dead plant material.

Due to their importance in ecosystems and human life, the biology of *M. annandalei* has been studied in the aspects of nest structure, swarming and establishment of new colonies (Nguyen Duc Kham, 1976; Nguyen Tan Vuong, 1997; Nguyen Van Quang, 2003).

Furthermore, the mutual relationship between *M. annandalei* and the fungus (Termytomyces) regarding the existence and development of both termites and fungi have also been studied and documented by Nguyen Van Quang (2001, 2002). However, the important features of caste ratios and division of labour, which could be the basis for proposing effective management of this termite species, have still been unadequately documented.

Materials and Methods

Methods for the study of caste ratio

Field experiments were conducted on the hills of Xuan Mai area, Chuong My, about 30 km North West from Hanoi. Samples were examined in the lab of the Department of Invertebrate Zoology, Faculty of Biology, Hanoi University of Science, Vietnam.

The study of caste ratio followed Collin (1981) and Darlington (1984b). All fungus gardens and structures of the central cavities of termite mounds were collected placed in plastic bags and an insecticide sprayed into the bags before they were tied up. The small number of fungus comb without termites and insecticide was put back into the mounds in order to attract the remaining termites for subsequent sample collection.

The separation of termites from fungus garden and other structures of the nest were performed in 2 steps:

Step 1: Dry separation

Fungus combs and the other structures were cracked into small pieces, and termites swept out using a feather duster or shaking by hand to separate the majority of fungus garden and soils in a sample. The remaining fragments mixed with termites was used for the next step.

Step 2: Wet separation

All the fragments, fine grained soils and termites (see above) was submerged in a sink until all sank to the bottom of the sink. Termites were separated using different sieves. Then samples with termites were diluted in 25 litres of water and stirred to make sample homogeneous and separated into 10, 25 ml/pots. The water was decanted from each pot and replaced with the same volume of 75% EtOH to preserve the specimens. A 5ml of sample was from each 25ml pot to count the number of castes (major workers, minor workers, and minor soldiers), except major soldiers that were counted in each 25 ml pot.

Methods for the study on division of labour concerning food foraging and nest repair

We followed the methods described by Gerber *et al.* (1988) and Lys & Leuthold (1991):

Collecting samples at the sites of food acquisition

Enticements were made of small bundles of soft dry wood pieces, branches, or dry leaves set randomly around mounds of *M. annandalei*. They were laid 20-30m apart, within a radius of 2- 10m from a mound. After 3 to 7 days enticements were checked and all termites found at the enticements were collected and preserved in 70-75% EtOH. The number of preserved individuals of each caste was counted for calculation of percentages.

Collecting samples at the sites of mound repaired and rebuilt

The walls of mounds were destroyed with a pick or a shovel. After 1-2 hours the individuals involved in rebuilding and repairing the mound at the disturbed sites were collected. However, the time for collecting samples varied, depending on how serious the destruction of the mound was. The samples were also preserved in 70-75% EtOH and the number of individuals of each caste counted for the calculation of percentages.

Results

The caste ratio in M. annandalei colony

The termites collected in a colony of *M. annandalei*, aside from the young and reproductive termites, were divided into 4 groups: major workers (MW), minor (small) workers (SW), major soldiers (MS) and minor (small) soldiers (SS). These groups are easily recognized by morphology and size.

Caste percentages of the 10 *M. annandalei* colonies surveyed in Xuan Mai, Ha Tay are shown in Table 1.

The average percentage of mature individuals: workers, soldiers (W + S) and immature individuals (Im) were 34.7% and 65.3% respectively. The average percentage of soldiers (4.2%) was much lower than that of workers (30.5%). The castes were arranged in order of ascending percentage: major soldiers (0.4%); minor soldiers (3.8%), major workers (11.1%), minor workers (19.4%). It is clear that major soldiers were fewer than minor soldiers, the number of major workers was just over half of minor workers. The result of statistical analysis showed the difference between worker castes was significant (t-Test; t Stat = 4.24 ≥ 2.87 = t Critical).

Table 1. The caste percentages of 10 colonies *M. annandalei* in Xuan Mai area, Chuong My, Hanoi

No.	*MW*	*SW*	*MS*	*SS*	*Mature (W+S)*		*Immature (Im)*		*Total*
	(%)	*(%)*	*(%)*	*(%)*	*Indi.*	*%*	*Indi.*	*%*	
1	4.1	24.7	0.2	2.7	22298	31.8	47927	68.2	70225
2	7.0	17.9	0.9	4.6	20826	30.4	47710	69.6	68536
3	7.7	18.9	0.4	5.8	44175	32.7	90746	67.3	134921
4	18.6	20.7	0.9	4.9	29518	45.1	35964	54.9	65482
5	14.1	18.8	0.2	3.6	27298	36.6	47226	63.4	74524
6	18.4	27.9	0.3	4.2	31694	50.7	30765	49.3	62459
7	10.9	18.4	0.3	2.5	28639	32.0	60762	68.0	89400
8	11.6	17.0	0.5	3.9	38333	33.0	77901	67.0	116234
9	9.7	13.6	0.1	1.8	31906	25.3	94202	74.7	126108
10	9.3	16.4	0.2	3.6	22130	29.4	53088	70.6	75218
Mean (%)	**11.1±4.5**	**19.4±3.9**	**0.4±0.3**	**3.8±1.1**	**34.7±7.3**		**65.3±7.5**		

%: Counting based on the sum of individuals including Soldiers - S. Workers - W and Imatures (Im)

The same analysis for castes of workers and soldiers in mature colonies obtained the results presented in Table 2. In the soldier group, the average percentage of major soldiers was smallest (1.2% ± 0.8), and one-ninth of the minor soldiers (10.9% ± 3.2). In the worker group, the average percentage of major workers (31.4% ± 8.4) was much lower than minor workers (56.5% ± 8.0), about one-third of the total. These analytical data formed the basis for evaluating division of labour in *M. annandalei* colonies in their food-foraging and nest-repair activities.

Table 2. The percentage of workers and soldiers in mature populations of *M. annandalei*

Caste	Percentage (%)			Individuals
	Minimum	Maximum	Average	
Major workers	13.0	41.3	31.4 ±8.4	94586
Minor workers	45.8	77.8	56.5 ±8.0	165726
Major soldiers	0.4	3.1	1.2 ±0.8	3365
Minor soldiers	7.2	17.7	10.9 ±3.2	33139
Total		100		296816
Number of surveyed colonies		**10**		

%: Counting based on the sum of workers and soldiers

The caste ratio in the activities concerning to foraging food and building mounds of M. annandalei

The most important and regular activities needed to keep a termite colony alive are foraging for food and building/repairing the nest. In *M. annandalei*, individuals taking part in these activities are mainly adults, including major workers, minor workers, major soldiers, and minor soldiers. The analysed results on task division by *M. annandalei* are presented in Table 3.

Table 3. The percentages of workers and soldiers collected at sites used for food foraging and mound repair

Castes	Foraging food		Repairing mound		In mound	
	Indiv.	*%*	*Indiv.*	*%*	*Indiv.*	*%*
MW	7888	79.4 ± 3.7	5799	53.3 ± 10.5	94586	31.4 ± 8.4
SW	616	6.1 ± 2.4	3787	32.2 ± 8.0	165726	56.5 ± 8.0
MS	73	0.7 ± 0.4	28	0.2 ± 0.1	3365	1.2 ± 0.8
SS	1265	13.6 ± 3.0	1556	13.8 ± 3.5	33139	10.9 ± 3.2
W+S	9842		11170		296816	
Nr. of samples	15		17		10	

%: Counts based on the sum of adult termites including Workers and Soldiers

After analysing 9,842 individuals involved in food foraging and 11,170 involved in nest repair, we found that the majority of those foraging for food were major workers, accounted for 79.4% of the total collected, other castes were at lower percentages, i.e. minor soldiers (13.6%), minor workers (6.1%) and major soldiers (0.7%). Among those involved in rebuilding or repairing mounds, the percentage of major workers was also highest (53.3%), followed by that of minor workers (32.2%) and minor soldiers (13.8%), while major soldiers was almost not involved in (only accounted for 0.2% of individuals collected) (Table 3).

In general, major workers took part both in both activities, food acquisition and repairing mounds, while contributing the highest percentages, which were thirteen times and nearly twice those of minor workers in foraging and repair, respectively.

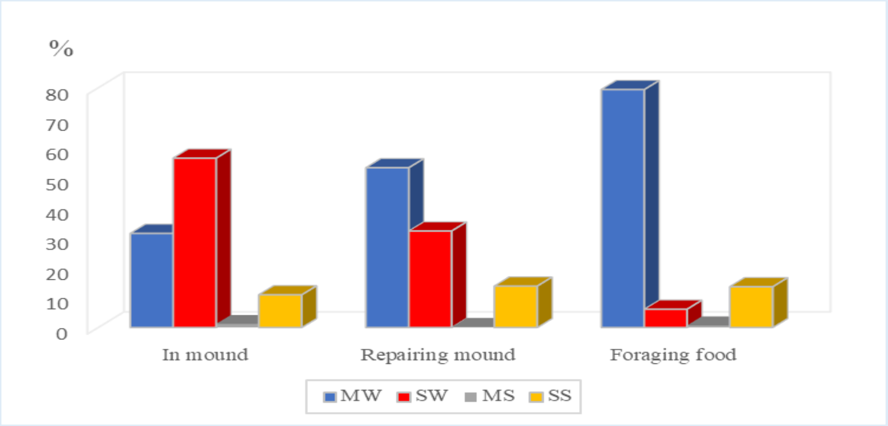

Figure 1. Percentage of different caste in foraging food and repairing nest of *M. annandalei*

Discussion

In the nest of *M. annandalei*, the average percentage of major workers (31.4%) was lower than minor workers (56.5%), while with foraging and rebuilding nests, major workers were dominant. Going from the inner part of the nest to outer areas to nest repair and foraging, the percentage of major workers increased (31.4%; 53.3% and 79.4% respectively), whereas the percentage of minor workers decreased (56.6%; 32.2% and 6.1% respectively) (Figure1).

The same results were found in the soldier caste. There was an increase in the percentage of minor soldiers and a decrease in major soldiers at sites of mound repair and food acquisition. The increase in quantity of minor soldiers outside the nest implies safety reasons. Dangers outside the nest are more conspicuous threats than inside the nest. Besides, the slight decline in percentage of major soldiers in activities outside the nest might be a result of specialisation in protective tasks, mainly being inside the nest. This result agrees with the previous comments by Nguyen Duc Kham (1976).

According to Badertscher (1983), in the nest of *M. subhyalinus*, the percentage of major workers, minor workers and minor soldiers was 88%, 9.2%, and 2% respectively. Oloo (1984) reported that 78% of major soldiers and 14% of minor soldiers of *M. michaelseni* were involved in food foraging. Gerber (1988) found there was larger percentage of major and minor workers taking part in food acquisition (70.4% and 26.0% respectively) in *M. bellicosus*. These findings show that major workers of *Macrotermes* species play the main role in food foraging.

However, among different species, the percentages within worker caste are not similar. Gerber (1988) stated that this feature related to different strategies of gathering food in each species. The minor workers of *M. bellicosus* and *M. michaelseni* take part in food foraging with a higher percentage than the other species (26% and 14% respectively), and that may be linked to a behaviour of covering food with soil before cutting into small pieces and carrying to the mound by major workers. Noirot (1969) and Lepage (1981) found that the percentage of minor workers of most Macrotermitinae involved in foraging fluctuated from 5% to 10%. In our study, the percentage of major workers (79%) and minor workers (6.1%) involved in this activity are similar to some results of other studies on *Macrotermes* in Africa.

Also according to Gerber (1988), minor workers of *M. bellicosus* hold the main task in building nest with proportion of 77.8%, major workers joined at 4.9%. On the contrary, in *M. subhyalinus* and *M. michaelseni* this task was carried out mostly by major workers similar to results by Grassé & Noirot (1961) on *Odontotermes magdalenae*. It is clear that task division concerning building nest is also different among different species.

Besides, the results in the study by Trainiello & Leuthold (2000) showed that task division in termites was flexible evidenced by task sharing in some activities. In our study, the data on polyethism in *M. annandalei* with 53.3% of major workers and 32.2% minor workers involved in nest repair agrees well with the remarks by those authors. Although labour tasks outside the nest usually involved major workers, nest rebuilding was the activity related to safety of colony therefore should be completed as fast as possible. For this reason, this task may be implemented by both worker castes.

In general, our results showed that the polyethism in *M. annandalei* concerning food acquisition and rebuilding or repairing nest was clearly distinct. Although major workers were a lower proportion in the nest, they were dominant in activities outside nest, reflected in higher percentage than minor workers. In addition, in other activities, the percentage of each worker caste was also different. These findings elucidate different roles for the castes in each task.

Our study contributes to understanding reasons for failure in managing fungus growing termite species, with distinct task division only a small proportion of adult individuals work outside the nest. So when using infectious agents for control it will not affect enough individuals to spread that substrate to kill all termites in a nest.

Conclusions

1. The average percentage of adult termites including workers and soldiers in the inner mound of *M. annandalei*, was 34.7%, which was much lower than that of juveniles (63.5%). The percentage of major workers, minor workers, major soldiers and minor soldiers were 11.1%, 19.4%, 0.4%, and 3.8% respectively.
2. The average percentage of minor workers was the highest (56.5%) among adult individuals whereas that of major workers was 31.4%. Major soldiers contributed the lowest proportion (1.2%), which was about one-ninth of that of minor soldiers (10.9%).
3. Task division showed clearly in *M. annandalei*. In the activities of food foraging and nest repair, the percentage of major workers were 79.4% and 53.3% while minor workers 6.1% and 32.2% respectively. Inside the nest, the percentage of minor workers was 56.6%, and that of major workers (31.4%).

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Host specificity and dependence of the termitophilous rove beetles associated with Formosan subterranean termite in Taiwan

by
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Abstract

The Formosan subterranean termite, *Coptotermes formosanus*, is a well-known invasive pest of wood structures. Its native range had been widely investigated for potential implications for pest control strategies. Two termitophilous staphylinid genera, *Japanophilus* Maruyama & Iwata and *Sinophilus* Kistner, found in the nest of *C. formosanus* had been used for inferring the native range of their host termite. However, the relationships between these beetles and *C. formosanus* have not been extensively studied and more information could clear up the uncertainty surrounding any inference of their association with the termites' native range. The present study aimed to address if the beetles were obligatory or voluntary inhabitants of *C. formosanus* nests. Our results show that in Taiwan both staphylinid genera are obligatorily associated with *C. formosanus* and highly depend on a living termite host. This result supports the presumption of the species-specific relationship between these termitophiles and this termite, and is appropriate for interpreting termite native range using termitophiles. We also found a non species-specific staphylinid genus, *Anacyptus* Horn in the nest of *C. formosanus* in Taiwan, and this genus cannot serve as the indicator of termite native range. The present study emphasized the use of termitophiles applied to termite biogeography and that considerations of endemic range should be based on a clear understanding of termitophile-host relationships.

Key words: Rhinotermitidae, social insect symbionts, Staphylinidae, Aleocharinae

Introduction

The Formosan subterranean termite, *Coptotermes formosanus*, is an economically important pest of the wood structures (Pimentel et al. 2005) and was described from Taiwan by Dr. Tokuichi Shiraki in1909. It has been proposed that *C. formosanus* might be endemic to several East Asia countries (Kistner 1985, Li et al. 2009) and spread to urban regions of the world via human activities (Evans et al. 2013). Determining the native distribution of an invasive pest is crucial for improving pest control from biological and ecological perspectives. Previous studies attempted to determine the native range and introduction routes of *C. formosanus* by genetic analysis of populations. However, multiple introduction routes are likely and global genetic variation among the populations is low (Wang and Grace 2000, Austin et al. 2006, Li et al. 2009, Husseneder et al. 2012). Defining the endemic range of *C. formosanus* is still vague among East Asia countries, such as Japan, China, and Taiwan.

Termitophiles, the organisms closely associated with termite societies, have diverse host relationships, such as obligatory predators, parasites, food thieves, and symphiles (Kistner 1969, Kistner 1979). Symphiles are often species-specific to a host and integrate into the termite society through mechanisms, like chemical mimicry or Wasmannian mimicry (Kistner 1979). As some of the termitophiles are species-specific and they are not likely transported by human activities or cross the natural barrier by themselves, so their presence within the termite nest is ideal for indicating the native range of their host termite (Kistner 1985, Maruyama et al. 2012). Three species of rove beetles belonging to two genera, *Japanophilus* Maruyama & Iwata and *Sinophilus* Kistner, had been found in the nest of *C. formosanus*, and used for interpreting the native range of the termite in China and Japan (Kistner 1985, Maruyama and Iwata 2002, Maruyama et al. 2012). However, none of the termitophile-host relationships of these beetles have been investigated, therefore there is uncertainty when inferring termite native range using these termitophiles. To address this problem, the present study investigated the relationship of the rove beetles associated with *C. formosanus* in Taiwan, through two crucial dimensions, host specificity and dependence.

Materials and methods

Host specificity assay

We investigated the occurrence of rove beetles on all termite species in Taiwan to assess the host specificity of the beetles associated with *Coptotermes formosanus*. We examined termite specimens deposited in the National Chung Hsing University (NCHU) Termite Collection, Taichung, Taiwan. The NCHU Termite Collection was established by the second author in 2005. At the time of this investigation, there were 4,990 termite colony specimens and termite-associated organisms deposited in NCHU Termite Collection, representing five termite families: Archotermopsidae, Kalotermitidae Rhinotermitidae, Termitidae, and Stylotermitidae. These specimens were collected through numerous termite biodiversity surveys in Taiwan and the citizen science project, Taiwan Termite Identification Service (http://termite.nchu.edu.tw/), founded by Urban Entomology Laboratory, Department of Entomology, NCHU.

Host dependence assay

We tested the survivorship of termitophilous beetles living with no termites, with dead termites, and with living termites, to assess the host dependence of the termitophilous rove beetles associated with *Coptotermes formosanus*. Two genera of termitophilous beetle, *Japanophilus* and *Sinophilus*, were collected from bucket traps of *C. formosanus* in the field. The tests were conducted in a petri dish (diameter: 50mm) with one moisture filter paper (diameter: 50mm; add 0.2 ml distal water) as the substrate for water and food (cellulose). One termitophilous beetle was put in a petri dish without additional resources, one dead *C. formosanus* worker, or one living *C. formosanus* worker. We tested three individuals for the first two treatments, as these termitophiles are seldom found in a termite nest and four and five beetles for the living termite treatment with *Japanophilus* and *Sinophilus*, respectively. All experimental devices were put in a dark environment at room temperature (around 25°C) and we recorded survivorship of the termitophilous beetles every day. In addition, some termitophilous rove beetles were kept in the laboratory for further recording their life history and observing their interaction with host termites.

Results and discussion

The termitophilous rove beetles associated with *C. formosanus* in Taiwan

Examination of the termite colony specimens deposited at NCHU Termite Collection, identified three termitophilous staphylinid genera associated with *Coptotermes formosanus* that belonged to two tribes of Aleocharinae, the Mesoporini (genus *Anacyptus*) and Termitohospitini (genus *Japanophilus* and *Sinophilus*). All specimens found in the present study exhibited no specific difference in morphology, so we presumed them to be three species. However, we found differences between the Taiwanese specimens and described species based on their original descriptions, we therefore refrained from assigning a species designation to our specimens and suggest a more comprehensive taxonomic revision be conducted.

Host specificity of rove beetles associated with *C. formosanus*

According to the specimens we examined, the genera *Japanophilus* and *Sinophilus* are only known from the nest of *C. formosanus* (Table. 1). That result indicates the obligatory host specificity of *Japanophilus* and *Sinophilus*, and both genera were also only recorded for *C. formosanus* in previous studies (Kistner 1985, Maruyama and Iwata 2002, Maruyama et al. 2012). However, the genus *Anacyptus* was not only be found from *C. formosanus* but also *C. gestroi*, as well as two species of *Neotermes*. The genus *Anacyptus* represented only one species, *A. testaceus*. This species has a wide distribution in North America associated with *Reticulitermes*, *Coptotermes*, and *Diversitermes* and also has been discovered alone in logs and under bark (Seevers 1957).

Table. 1. The host termites of three termitophilous staphylinid species associated with *Coptotermes formosanus* in Taiwan. The number of the termite colonies found with termitophilous staphylinid shows in the bracket.

Termitophilous staphylinids	Host termites in Taiwan
subfamily Aleocharinae	
tribe Termitohospitini	
genus ***Japanophilus***	*Coptotermes formosanus* (6)
genus ***Sinophilus***	*C. formosanus* (14)
tribe Mesoporini	
genus ***Anacyptus***	*C. formosanus* (8), *C. gestroi* (4), *Neotermes koshunensis* (2), *Neotermes* sp. (1)

Host dependence of rove beetles associated with *C. formosanus*

The survival times of *Japanophilus* and *Sinophilus* were not significantly different between the without termite and with dead termite treatments. However, when the living termite was present, the survival time of both species increased significantly. The results show *Japanophilus* and *Sinophilus* were highly dependent on living termites (Figure 1). Furthermore, they were not able to use the dead termite as the food sources, which support that they were highly integrating into the termite society.

Life history and interactive behavior of rove beetles associated with *C. formosanus*

Both genera, *Japanophilus* and *Sinophilus* showed integrated behavior with the termite worker, such as allogrooming between the beetles and termites. We did not complete the life history of *Japanophilus* sp. and *Sinophilus* sp. in the laboratory so far. On the contrary, no integrated behavior between *Anacyptu*s sp. with their host termites was observed and we had successfully reared *Anacyptus* sp. in the laboratory and completed their life cycle by feeding them dead termites or other insect corpses. This observation shows *Anacyptus* does not integrate into the termite society but it can use termite nests as a habitat.

Conclusions

The high degree of host specificity and dependence of *Japanophilus* and *Sinophilus* supports that they could be the good indicators when termitophiles are used for interpreting the native range of their host termite. Furthermore, not all of the termitophiles found in termite nests can serve as an indicator of termite native range. *Anacyptus,* is a staphylinid genus that is not a species-specific inhabit of termite nests. The termitophile-host relationship should be examined prior to use of termitophiles to interpret the native range of the termite.

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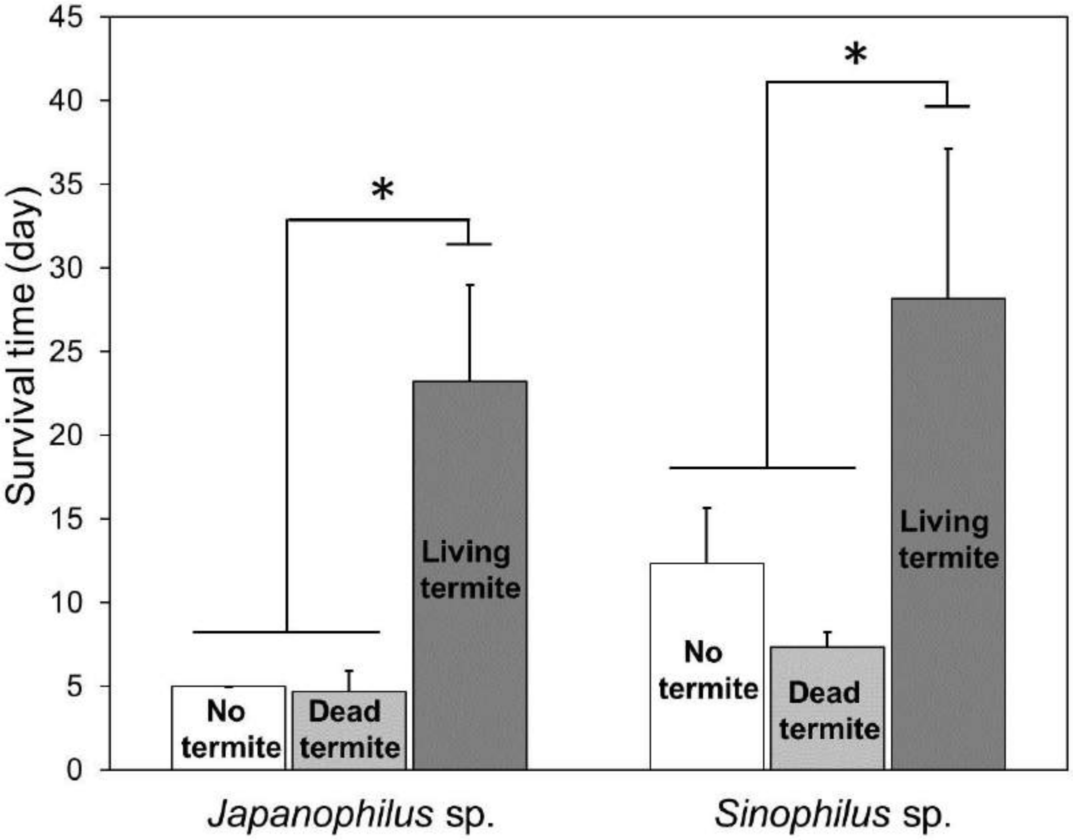

Figure 1. The survival time (day) of two termitophilous staphylinid species associated with *Coptotermes formosanus* among the three experimental treatments. *, significance (*p*<0.05, by Mann-Whitney U test)

Effects of lignin on the physiological activities of the lower termite, *Coptotermes formosanus*

by
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Abstract

The decomposition of polysaccharides by termites and their gut microbial symbionts has been relatively well documented. However, there is little information regarding the effects of lignin on the physiological activities of termites. Such studies would improve our understanding of lignocellulose-oriented carbon recycling in nature, and may also contribute to the development of sustainable technology to convert lignocellulosic biomass to valuable fuels and materials. In this paper, we summarized our recent studies regarding the effects of lignin on the physiological activities of the lower termite, *Coptotermes formosanus*.

Key words: lignocellulose, lignin, lower termite, physiological activities, symbiotic protists

Introduction

It has been well known that lignocellulosic materials are a nutrient source for termites, composed of cellulose, hemicelluloses, and lignin. Lignin, a heterogeneous aromatic polymer, encrusts cell-wall polysaccharides, i.e., cellulose and hemicelluloses, and provides resistance to enzymatic degradation (Boerjan et al., 2003, Jackson et al., 2008). Therefore, termites have to break down recalcitrant lignin barriers to digest the cell wall polysaccharides. Studies on polymeric lignin using conventional wet-chemical and spectroscopic approaches have detected no conclusive evidence for chemical modification of the polymers upon their passage through the gut of lower termites (Geib et al., 2008, Ke et al., 2013), also there has been no clear evidence that termites can utilize lignin as a nutrient source.

In this paper, we summarized recent studies conducted to investigate the decomposition of lignin polymers in the digestive system and the resulting nutritional effects on *C. formosanus*.

Materials and methods

Three lignocellulose samples, i.e., Japanese cedar (*Cryptomeria japonica*) (softwood), Japanese beech (*Fagus crenata*) (hardwood), and rice straw (*Oryza sativa* L. ssp. *japonica* cv. Nipponbare) (grass) were prepared to investigate the effects of lignin on a lower termite. The lignocelluloses were fed to *C. formosanus* workers, and their feces collected. Those feces and the original lignocellulose samples were subjected to NMR spectroscopy to investigate the decomposition of lignin polymers processed in the digestive system of *C. formosanus* (Tarmadi et al., 2018). In addition, lignin, holocellulose, and cellulose were isolated from J. cedar, J. beech, and rice straw and presented as single-source diets along with two artificial diets (holocellulose mixed with lignin, and cellulose mixed with lignin) were fed to *C. formosanus* workers and physiological activities such as survival, body mass, and gut protist profiles periodically observed (Tarmadi et al., 2017a, 2017b).

Results and discussion

We analyzed lignocellulose deconstructions with emphasis on lignin polymer decomposition after termite digestion using NMR analysis to investigate the effects of lignin digestion on *C. formosanus* (Tarmadi et al., 2018). High-resolution NMR structural data suggested preferential removal of syringyl aromatic units in hardwood lignin and non-acylated guaiacyl units as well as tricin end-units in grass lignin. These findings suggest that lignin modification/degradation in the lower termite digestive system varies the chemical structure of the lignin substrates (Tarmadi et al., 2018).

We also assessed isolated purified lignin from J, cedar, J. beech, and rice straw on the physiological activities (termite survival and body mass change) and hindgut protist profiles of *C. formosanus* workers in an effort to identify the nutritional effects of lignin polymers (Tarmadi et al, 2017a). The survival rates of *C. formosanus* workers fed the three lignins were similar in comparison with the starvation treatment after 3 weeks (Figure 2a). However, at the end of the 4th week, the survival rate of workers fed the three lignins were lower than the starvation treatment (Figure 2a). These results suggest that lignin from J. cedar, J. beech, and rice straw were not nutritious for *C. formosanus*, and rather somewhat detrimental as a sole dietary component (Tarmadi et al., 2017a). In addition, as shown in Figure 2b, the body mass of *C. formosanus* workers fed the three lignins was similar throughout the test. These results further support the notion that termites hardly use lignin as a nutrient source (Tarmadi et al., 2017a).

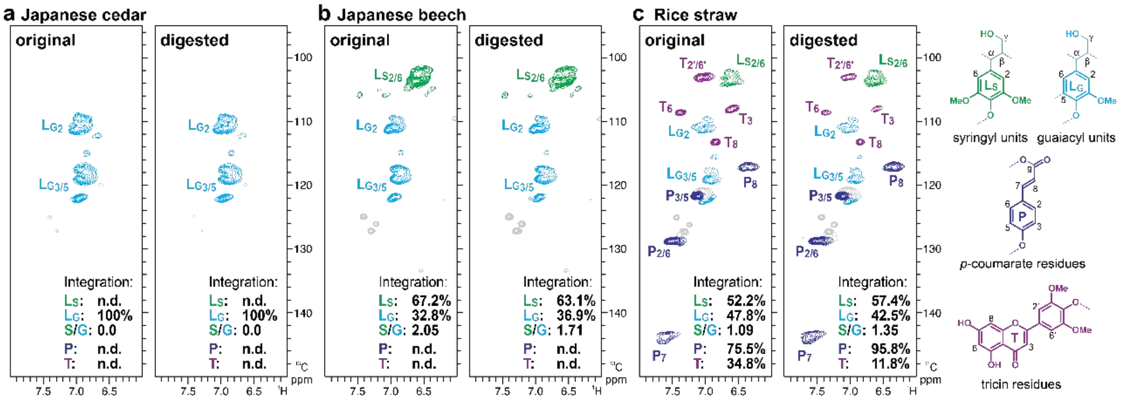

Fig 1. 2D ^{1}H–^{13}C correlation (HSQC) spectra of acetylated samples of lignin-enriched cell walls from original and digested lignocellulose diets fed to *C. formosanus* workers. Lignin aromatic sub-regions are shown for (a) Japanese cedar (softwood), (b) Japanese beech (hardwood), and (c) rice straw (grass) diets. Volume integrals are given for the major lignin aromatic units that are color-coded to match their assignments in the spectrum. The percentages noted in each spectrum are integrals relative to the sum of the syringyl and guaiacyl lignin unit signals (**L$_{S}$** + **L$_{G}$** = 100%) (Tarmadi et al., 2018).

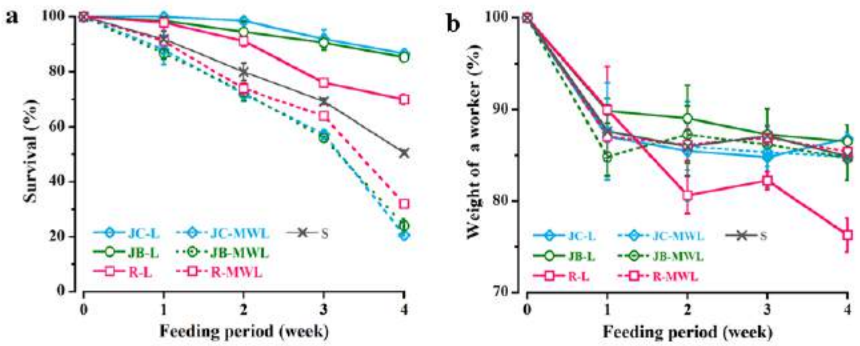

Figure 2. Changes in the survival (a) and body mass (b) of *C. formosanus* workers fed various lignocelluloses and isolated lignin (MWLs). Error bars represent standard deviation (*n*=3). JC-L, Japanese cedar lignocellulose; JB-L, Japanese beech lignocellulose; R-L, rice lignocellulose; JC-MWL, Japanese cedar MWL; JB-MWL, Japanese beech MWL; R-MWL, rice MWL; S, starvation (Tarmadi et al., 2017a).

Lastly, we conducted an experiment to assess the effects of lignin served with polysaccharides on *C. formosanus*. The survival of *C. formosanus* workers fed diets containing lignin from J. cedar, J. beech, and rice straw were higher than those of workers fed only polysaccharides (holocellulose or cellulose) (Figure 3) (Tarmadi et al., 2017b). In addition, we found the three lignins when served with polysaccharides had a positive effect on maintenance of two *C. formosanus* protists i.e., *Pseudotrichonympha grassii* and *Holomastigotoides hartmanni* (Tarmadi et al., 2017b), suggesting that lignin is crucial to maintaining the physiological activities of the lower termite, *C. formosanus* workers.

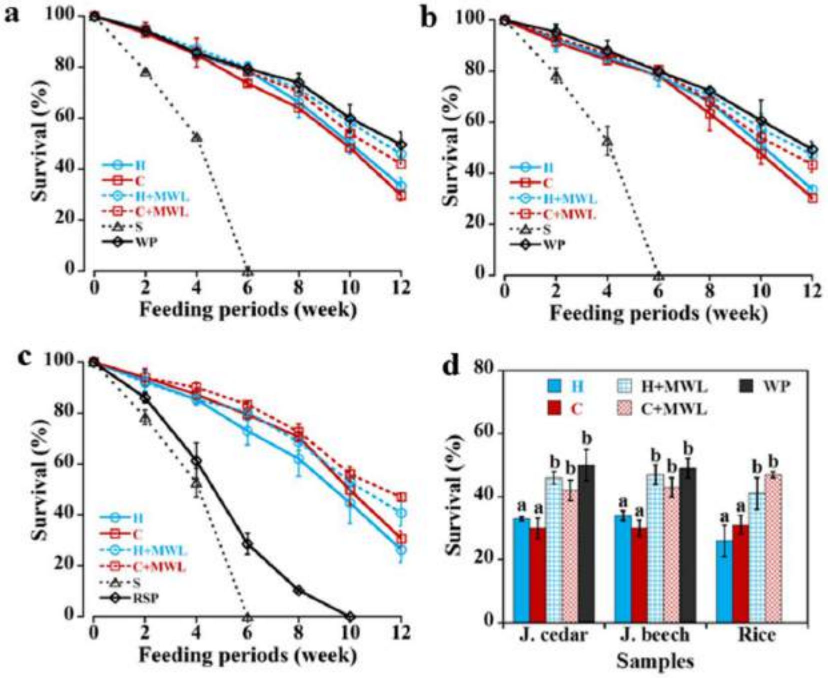

Figure 3. Periodic changes in survival rates of *C. formosanus* workers fed lignocellulose diets prepared from Japanese cedar (**a**), Japanese beech (**b**), and rice (**c**), and their comparison at the 12-week observation

(**d**). Error bars represent standard deviation (n = 3). WP, wood powder; RSP, rice straw powder; H, holocellulose; C, cellulose; H+MWL, holocellulose with lignin; C+MWL, cellulose with lignin; S, starvation control (Tarmadi et al., 2017b).

Conclusion

Based on our recent studies, *C. formosanus* has the ability to partially decompose lignin polymers during passage through the digestive system. In addition, lignin from softwood, hardwood, and grass gave marked positive effects on the survival of *C. formosanus* workers as well as maintenance of hindgut protists when served with polysaccharides. Our study has provided evidence that the presence of lignin is crucial to maintaining physiological activities and a wholesome hindgut digestive system of *C. formosanus* workers.

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Using a Pesticide Extracted from Leaves of Simaung (*Pangium edule* Reinw.) for Control of Soil Termite Pest (*Schedorhinotermes* sp.) as one of Solution Conservation

by
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Abstract

The use of plant extracts for pest control have been considered as an alternative to synthetic pesticides for effective pest control. The present studies examined the efficacy of simaung (*Pangium edule* Reinw) leaf extracts against *Schedorhinotermes* sp. in the Laboratory of the Forestry Faculty of Muhammadiyah University of West Sumatra. The experiment used a Completely Randomized Design with 2 factors, leaf condition, (young leaves or old leaves), and concentration (0 leaves/l (control), 15 g/l, 25 g/l, 35 g/l , and 45 g/l water) with 4 replication. Data were analyzed using ANOVA and DNMRT at P>0.05. *Lethal Concentration* and *Lethal Time* values were generated by probit analysis. The results showed that young leaves at concentration 45 gr/l water mortality value of 95 %, and it was not significantly different from the old leaf extract 45 gr/ l. The LC_{50}, LC_{95} young leaves was 3.82 gr/l water while old leaves provided a value of 10.15 gr/l water. The fastest *Lethal time* (LT_{50}) 1.3 days was obtained at 35 g/l for young leaves and, 1.4 days old leaves at 45 g/l concentration. Based on these results, using a concentration of young leaf extract (15 gr/l of water) applied to soil will effectively kill 80% of the termites test.

Keywords: *leaf extract pesticide, simaung, Schedorhinotermes sp., (LC), (LT)*

Introduction

Pesticides are one of the means to control pest attack. Along with the rapidly expanding technological developments, the increasing and urgent need for life, the use of chemical pesticides is an alternative to the control of plant pest organisms (OPT). This, given the easy chemical pesticides, and many found and sold on the market and is instant, live applications without a long and complicated work process. Natawiria, (1973) in Sari (2012) states that the use of chemical pesticides in Indonesia has destroyed 55% of pests and 72% of biological control agents. Unwise use of chemical pesticides will pose a risk of poisoning that leads to death especially to wildlife, birds, bees, fertilizing insects, and affect forest biodiversity. On the other hand also cause an impact on the quality of water, soil and air.

One solution to the negative effects of chemical pesticide use is to substitute it with vegetable pesticides. Vegetable pesticides are pesticides whose basic ingredients come from plants. Made from environmentally friendly and biodegradable materials. Utilization of one forest plant, which is well known by the community in Batu Hampar, District Akabiluru in West Sumatera as simaung (*Pangium edule* Reinw). The leaves and seeds contain natural chemicals that can be used in the eradication of insect pests.

Termites are social insects that live in a community consisting of several castes. In forests, termite have an important role to decompose plant remnants, especially dead wood and litter. But under certain conditions termites also become pests, especially for Industrial Plantation Forest (HTI).

The use of vegetable pesticides on termite control is not new, Tarmadi *et al*. (2006) usied bintaro (*Carbera odollum* Gaertn) and amethyst (*Brugmansia candida* Press). Papaya leaf extract (*Carica* *papaya* Linn.) the gastric poison method used against ground termites by Zulyusri *et al*., (2012), or the sour leaf extract (*Sambucus javanica* Reinws) by Zulyusri *et al*. (2013). But the leaf extract it self has not been tested in termite control, especially related to the condition/age of leaves. Therefore, this study aimed to determine the mortality and toxicity of simaung (*Pangium edule* Reinw) leaf extract as a pesticide against soil termite pest (*Schedorhinotermes* sp.).

Materials and Methods

This research was conducted in February until August 2017 located in Laboratory Faculty of Forestry University of Muhammadiyah West Sumatra.

The tool used in this research included a catter knife, blender, glass petridihess, measuring 20 ml and 100 ml, aluminum foil, Whatman filter paper, plastic beverage bottle (container of solution), 50 mesh filter, bucket or container, label paper, and stationary. The materials used included a simaung leaves taken from private gardens in the Nagari Batuhampar, District of Akabiluru, Lima Puluh Kota. Termites were collected from the remnants of wood material using a tissue, water, Whatman filter paper.

This research used a Completely Randomized Design (RAL) with 2 factors, namely: the conditions of young leaves and old leaves, and 4 concentrations plus a control as comparison with 4 replications. Each experimental unit consisted of 20 termites. The data obtained were analyzed by analysis of variance, and tested with Duncan's New Multiple Range Test (DNMRT) at the 5% level, and probit analysis was used to determine the value of toxicity. The observation parameters in this study were percentage mortality, leaf condition interaction and extract concentration, *lethal concentration* (LC_{50}, LC_{95}), *lethal time* ($L\square_{50}$)

Results and Discussion

Mortalitas (%)

The results of observation of mortality of test termites after analyzed by using variance showed that the treatment of some concentration of Simaung leaf extract with different leaf conditions gave a significant effect on the value of termite mortality in each treatment. DNMRT further test results at 5% level can be seen in Table 1 below.

Table 1. Mortality Value (%) of Termite by Treatment

Leaves Conditions	Concentration				
	0 gr/l	15 gr/l	25 gr/l	35 gr/l	45 gr/l
Young leaves (A1)	27,50 d	83,75 abc	85,00 abc	93,75 ab	95,00 a
Old Leaves (A2)	28,75 d	68,75 c	70,00 bc	73,75 abc	90.00 abc

Based on Table 1, the lowest mortality was obtained in the treatment using old leaves at a concentration of 15 g/l water that is 68.75%, but not significantly different from the concentrations of 25 gr/l (70.00%), 35 g/l (73.75%), and 45 gr/l (90.00) of old leaf extract, while the young leaf extract at 15 g/l concentration had a mortality of 83.75%, that also was not significantly different from 25 g/l concentration (85.00%), or 35 g/l (93.75%).

The highest mortality was in the treatment using young leaves at a with concentration of 45gr/l water, 95.00%. However, it was not significantly different with the treatment of young leaves at the concentrations of 15 g/l, 25 g/l and 35 g/l and old leaves at 35 g/l water, 45 g/l water. The above data also show higher concentrations of simaung leaf extract increased mortality of termites. These data are reinforced by Dewi (2010) which states that higher concentrations of extract will have higher influence.

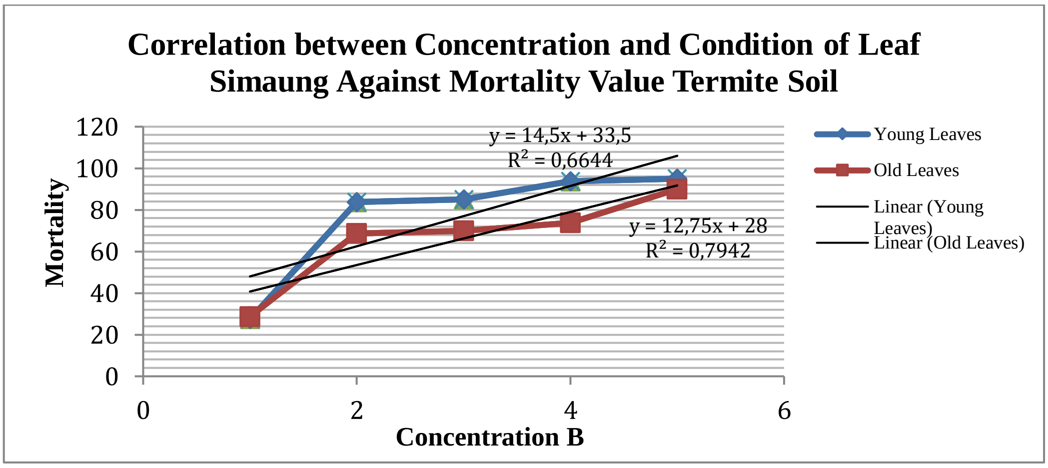

Figure 1. Correlation between concentration and condition of simaung leaves against termite mortality

Based on the regression R Square (R) or coefficient of determination for young leaf extracts 0.664, and old leaves 0.794, - indicates a good relationship between concentration and mortality. According Raharjo (2017), the value of the coefficient of determination (R Square) is only between 0-1. A small coefficient of determination (R Square), means the influence of independent variables on the dependent variable is weak. Conversely, if the R Square value is nearly 1, then the influence will be strong.

Leaf extract was effective at controlling *Schedorhinotermes* sp. pests. This is due to the concentration of simaung leaf extract 35 gr/l water that is capable of termite mortalities that ranged from 90.00% to 95.00%. This opinion is in accordance with the statement of Dadang and Prijono (2008) in Juliati *et al* (2016) which state that vegetable pesticide extracts are effective when mortality rates are greater than 80%.

Effect of Leaf Condition Interaction and Concentration on Soil Termite Mortality

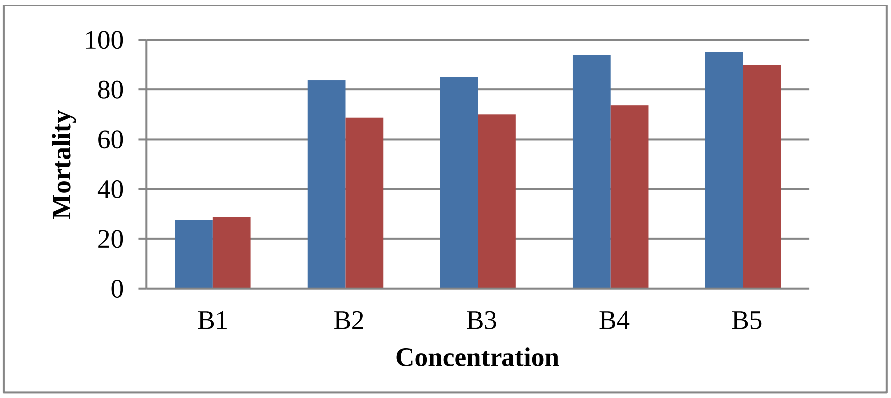

Figure 2. Influence of Leaf Condition and Concentration of Leaf Simaung on Soil Termite Mortality

Figure 2 shows that young simaung leaf and old leaf extract had different mortality values at the same concentration. Young leaf extracts tend to be superior to old leaf extracts. According Yuningsih *et al*. (2008) simaung leaf has the same cyanide content as young seeds which is about 500 ppm. The content of cyanide in the leaves of the simaung is influenced by soil conditions, season and seed structure. Cyanide is a deadly toxin if, not handled properly and wisely.

According Yuningsih *et al*. (2008) declared a python plant can be used as a vegetable pesticide, especially on the leaves. The anti-feedant properties contained in the leaf extract of this leaf, significantly affect termites, this is considering the condition of termite test food that the test paper alone without other options, the content of leaf extracts at various concentrations mixed on the test paper cause termites to be reluctant to eat it. This condition if left continuously lead to termite death.

Extractions obtained with two different leaf conditions have different results, this is indicated by the difference in color and odor of the resulting extract solution. Young green leaf extract solution is soft but smelly, while dark green leaves are dark and smell less.

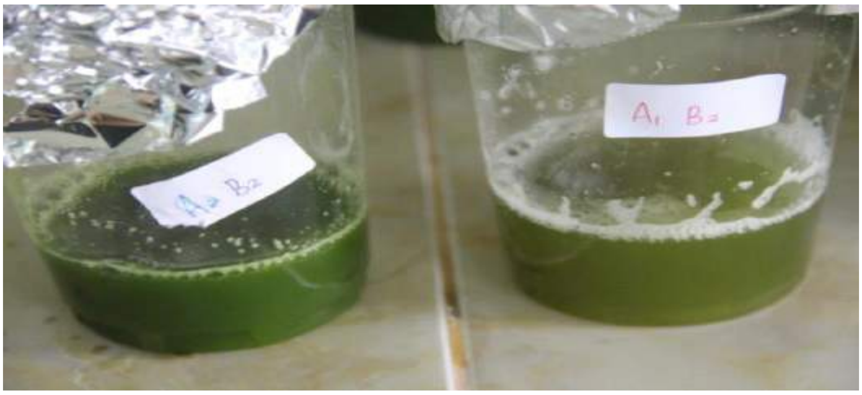

Figure 7. Result of Simaung Leaf Extract with Different Condition but Same Concentration

Differences in extracts produced between young leaves and old leaves can also be influenced by the age of the leaves. The older leaves tend to have a better nutrient content but are at a reduced phytotoxic compounds.

A. *Lethal Concentration* (LC_{50}, LC_{95})

Lethal concentration is the value of a certain concentration and it's ability to kill test insects. In this study the calculated *Lethal concentration* (LC_{50}, LC_{95}) as shown in Table 3 below.

Table 3. *Lethal Concentrations* (LC_{50}, LC_{95}) for Young Simaung Extract Leaf Extract and Old Extract.

Leaf condition	LC_{50},	LC_{95}
Young leaves	3.81 gr/l	49.07 gr/l
Old leaves	10.15 gr/l	165.58 gr/l

According to Table 3, young leaf extracts at concentrations of 3.81 g/l of water can cause 50% termite mortality. While the extract of old leaf leaves requires a concentration of 10.15 g/l of water. Meanwhile, to kill 95% of the test termites required young leaf extract of 49.07 gr/l water, and old leaf extract as much as 165.58 gr/l water. It takes a higher concentration of the old leaf extracts than young leaf extracts to cause death of termites. However, the mortality of each concentration indicates that the leaf extract is toxic certain concentrations.

According to Harbone (1987) in Heriyanto (2008) the toxic properties contained in simaung leaves are suspected bioactive compounds such as cyanide, cyanogen glycosides, alkaloids, flavanoids, and saponins. These compounds cause the presence of biological activity such as food-inhibition, antiparasite, and pesticide. The presence of toxic compounds in simaung leaf extract will provide a response by lowering the consumption rate or inhibiting termite feeding on the test paper and the effect their digestion and metabolism. This influence can be seen in the duration of the mortality period.

B. *Lethal Time (LT_{50})*

Provision of vegetable pesticides from simaung leaf significantly effected the mortality of termites. The *Lethal Time* (LT_{50}) aims to determine the time required for each treatment to kill 50% of the termites.

Tabel 4. *Lethal Time Value*(LT_{50}) Leaf Concentration of Simaung (gr/l) Each Treatment (Day)

Treatment	*Lethal time*(LT_{50})	
A1 B2 (Young leaf extract 15 gr/l water)	1,7 days	40,8 hours
A1 B3 (Young leaf extract 25 gr/l water)	1,8 days	43,2 hours
A1 B4 (Young leaf extract 35 gr/l water)	1,3 days	31,2 hours
A1 B5 (Young leaf extract 45 gr/l water)	1,4 days	33,6 hours
A2 B2 (Old leaf extract 15 gr/l water)	2,3 days	55,2 hours
A2 B3 (Old leaf extract 25 gr/l water)	2,2 days	52,8 hours
A2 B4 (Old leaf extract 35 gr/l water)	1,9 days	45,6 hours
A2 B5 (Old leaf extract 45 gr/l water)	1,4 days	33,6 hours

Table 4 shows that the treatment of different concentrations of simaung extracts differed with the time required to kill 50% of the test termites. The higher concentration of simaung leaf extracts showed an increase in the speed of killing termite pests. The above data show that the application of several treatments with different concentrations resulted in a range of LT_{50} values ranging from 1.3 days to 1.7 days for young leaf extracts and 1.4 days to 2.3 days for the old leaf extract.

Treatment using young leaf extract 15 gr/l water, provided LT_{50} values of 1.7 days or 40.8 hours (40 hours 48 minutes) after application. Meanwhile, treatment with old simaung leaf extract, *was* 2.33 days or 55.2 hours (55 hours 12 minutes). This shows that low concentrations of young leaf extract can cause faster mortality. It is suspected that the content of young leaf extract is superior to the old leaves so it does not take long in terminating test termites. According to Sa'diyah *et al*., (2013) concentration is directly proportional to the development, the higher the concentration, the development of test insects is increasingly hampered.

This is not different at the concentration of 25 g/l water, young leaf extract with LT_{50} termite of 1.8 days equivalent to 43.2 hours (43 hours 12 minutes) while the old leaf extract 2.2 day or 52.8 hours (52 hours 48 minutes) after application. The concentration of 35 gr/l of young leaf extract was 1.3 days or 31.2 hours (31 hours 12 minutes), 1.9 days old leaf extract or 45.6 hours (45 hours 36 minutes). The concentration of 45 gr / l young leaf extract was 1.4 days or 33.6 hours (33 hours 36 minutes), old leaf extract was 1.35 days or 33.6 hours (33 hours 36 minutes). Significant differences in each treatment are thought to be the result of more active compounds in the body of the termites tested and scattered in every part of the body will accelerate the time required for 50% mortality rate. The quick time to kill 50% of termites at a given concentration can be caused by higher concentrations. Because the higher concentration will cause more toxic compounds into the body of termites

The use of the old and young leaf extract are equally effective in controlling the termite pest at different levels of concentration. Young leaf extract is superior to the old leaf extract. Basically, the use of plant-based pesticides should not use conservative concentrations, this is because any concentration used in pest control will not last long in nature because of its biodegradability. In addition, the use of vegetable pesticides as an antifeedant that inhibits the rate of consumption, inhibits production activities, and breeding so as not to destroy pests. And the vegetable pesticide it self is part of conservation.

Given the effectiveness of leaf extracts in killing test termites, it is expected to become one of the vegetable pesticide materials that can reduce the use of chemical pesticides in termite control. Thus, pest control in the forestry sector does not contribute to environmental pollution that leads to climate change and the destruction of existing food and ecosystem chains.

The recommended concentration in the use of vegetable pesticides from young simaung leaf extract is at 15 g/l water concentration and for the old leaf extract is 45 g/l water. Leaves used are expected to be one solution for people whose areas are affected by termite pests, where the residence area there is a simaung tree. So that people do not need to look for other alternatives, or other toxins. However, the use of vegetable pesticides is not recommended when plants have not been attacked by pests, this is to avoid the risk of pesticide ineffectiveness when used.

Conclusions

1. The use of vegetable pesticides from leaves of simaung (*Pangium edule* Reinw.) has the potential to control soil pest infestation (*Schedorhinotermes* sp.), because it has a marked effect on mortality of *Schedorhinotermes* sp. during the trial.
2. Extract simaung (*Pangium edule* Reinw) leaf has a different termite mortality value between the condition of young leaves and old leaves used on *Schedorhinotermes* sp., young leaves with concentration 45 gr/l water had the highest mortality 95.00% . This was not significantly different from the use of young leaf extract at 15 gr/l, 25 g/l, 35 g/l and 25 g/l, 35 g/l and 25 g/l concentrations of old leaf extract.
3. The existence of interaction between condition of leaf of simaung (*Pangium edule* Reinw) leaves and concentration, showed that young simaung (*Pangium edule* Reinw) leaf extract equal concentrations had superior mortality and toxicity compared to old simaung (*Pangium edule* Reinw) leaf extract
4. Toxicity value of leaf extract of simaung varies between young leaf extract with old leaf extract where young leaf extract at 3.81 g/l water concentration was able to kill 50% termites while the old leaf extract at 10.15 g/l concentration of new water killed 50% of the test termites. While the fastest time required to kill 50% of termites using the young leaf extract requires a concentration of 35 gr/l of water that was 1.3 days or 33 hours 12 minutes, while the old leaf extract at 45 g/l water concentration was 1.4 days or 33 hours 36 minutes.

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Evaluation of Cocoboard Resistance against Philippine Termites

by
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Abstract

The resistance of cocoboard (CB) to wood-destroying insects was evaluated under laboratory and field conditions. The resistance of low density CB (LD-CB) (300 kg/m^3) and medium density (MD-CB)(700 kg/m^3) cocoboard was evaluated using subterranean termites, *Microcerotermes losbañosensis* Oshima and drywood termites, *Cryptotermes dudleyi* Light under laboratory conditions. Resistance was rated based on percent damage and weight loss incurred to boards after exposure to the test insect. Field tests on the resistance of CB was conducted above-ground as ceiling or walls at the FRPDI Graveyard Experimental Site. Marine plywood (MPW) samples were used for comparison and tested in the same conditions.

The LD-CB and MD-CB were resistant and highly resistant, respectively against *M. losbañosensis* under laboratory conditions with MPW non-resistant against the same termite species. Likewise, the LD-CB and MD-CB were resistant and highly resistant to *C. dudleyi* respectively, after the 12-m test period while 19.1 mm MPW and 12.7 mm MPW were resistant to slightly resistant and sustained 21.0% and 65.0% termite damage.

Field tests showed that resistance of CB has higher than MPW. The results conform with resistance results in the laboratory suggesting that CB is a suitable material for building construction in terms of natural resistance. Further exposure of board samples is recommended to obtain more data to classify the natural resistance of CB against wood destroying insects under field conditions.

Keywords: Cocoboard, resistance to wood destroying insects, resistance of cocoboard, *Microcerotermes, Cryptotermes, Dinoderus*

Introduction

The coconut tree is a versatile plant with various uses and diversified applications. The roots can be used as medicine and décor; the trunk for construction and structural materials; the leaves as mats and household materials and the fruit for food, drinks and household products.

Coconut husks, the waste material in copra and oil production, are sources of natural fibers that can be used as raw materials in the manufacture of yarn, rope, bags, door mats, flower pots and cocopeat. Recently, cocofiber board made from coconut fibers with tannin as a binder was developed and shows potential as a structural material.

The raw fiber material from organic coconut fibers is available in large quantities; 14.902 billion nuts/year from 329.9 million coconut trees (http:/www.gov.ph/index.php/2015-2015-10026-03-15-57/20152026-03-22-41#nut; http:/www.gov.ph/index.php/2015-2015-10026-03-15-57/20152026-03-22-41#prod) and would provide a sustainable source of coconut fibers for producing CFB as well as various, currently produced, products like cleaning brushes, doormats, carpets, bags, ropes, mattresses and as a barrier to soil erosion. The utilization of coconut fibers in the production of CFB will convert husk waste materials from copra production or other coconut-based activities into value-added products, generate livelihood security and provide additional income to coconut farmers.

Coir fibers are being tapped as raw materials in the production of coconut fiberboardsbonded with tannin-based adhesives (Niro et al. 2016). The physical-mechanical properties of this fiberboard is as good as other types of composite products made from lignocellulosic materials for structural products. However, the use of cellulosic-based forest products for building materials may be subject to insects and fungal deterioration. Insects which include subterranean termites can cause severe damage on wood panels, invading from the ground through earthen tunnels they construct to extend their attack to wood components of a structure. Drywood termites and powder post beetles attack wood panels directly and they do not have contact from the ground.

Gypsum fiber board, hardiflex fiber board and cement bonded board were found resistant to insect attack (Garcia et al. 2011; Sukaratana et al. 2000). In termite-infested houses, termites can invade gypsum board and hardiflex fiber board but not cause significant damage. Recent studies on cement bonded board showed they were resistant to subterranean termites *Coptotermes gestroi* (Sukartana et al. 2010), *Microcerotermes losbañosensis* Oshima, and drywood termites *Cryptotermes dudleyi* Banks (Garcia et al. 2011). The resistance of cement bonded products to termites was attributed to the cement-bonded protective shield on wood strands and reduced cellulose content (Garcia et al. 2011).

In this study, we tested the ability of CB to withstand attack from wood deteriorating insects for consideration in the selection of potential alternative materials for construction of houses and buildings. Objectives are:

1. To evaluate the resistance of the CB against subterranean termites and drywood termites.
2. To compare the resistance of CB with MPW against insect attack.

Materials and methods

Production of coco-fiberboard (CFB)

Coconut husks, the wastes from copra production in Bicol Region were used as materials in the production of cocoboard (CB). Low density (LD) and high density (HD) cocoboard was produced with the following parameters.

Low Density Cocoboard (LD-CB) with thickness of 40-mm had a density of 300 kg/m^3 compared with 700 kg/m^3 density in 10-mm Medium Density Cocoboard (MD-CB). The production of LD-CB and MD-CB had the same pressing temperature - 180 °C, pressing time - 10 min, moisture content - 10% and adhesive/resin content -10%.

Cocoboard resistance to wood-destroying insects under laboratory conditions

Experimental board samples of LD-CB (40 mm x 20 mm x 60 mm) and MD-CB (10 mm x 20 mm x 60 mm) were prepared for resistance tests against test insects. Marine plywood composites (19.1 mm thick x 25 mmx x 60 mm and 12.7 mm x 25 mm x 25 mm) were provided for comparison. A total of 60 pieces of LD-CB, MD-CB, 19.1 mm-thick and 12.7 mm-thick marine plywood (MPW) board samples were allocated for the test. These were conditioned to constant weights and individual pre-exposure weight (W1) was recorded prior to exposure to test insects. The individual post-exposure weight of individual board samples (W2) were recorded after the required exposure period and used for computation of percent weight loss of boards.

a) Resistance of CB to subterranean termites

A termite nest containing an active population of Los Baños termites (*Microcerotermes losbañosensis* Oshima*)* was collected from the field and established inside a half-sawn plastic chamber with soil prior to the resistance test. A total of 20 sample boards of LD- and MD-CB were placed on top of a concrete block inside the termite chamber with 19.1 mm- and 12.7 mm-thick MPW samples provided for comparison. The experimental set-up was kept at ambient temperature and distilled water occasionally sprinkled on the soil to supplement the moisture requirements of the termites.

Termite invasion of board samples was observed monthly for 4 months. All board samples were retrieved on the 4th month to determine the degree of termite damage and percent weight losses. Resistance was classified based on the termite damage rating in Table 1.

b) Resistance of CB to drywood termites

Workers of the drywood termite *C. dudleyi* were collected from infested wood material and stored at the insect culture chamber. Termites were placed in an enamel tray and conditioned overnight in the dark room prior to inclusing in the resistance test.

Individual board samples were placed in separate petri dishes or a termite chamber with 100 workers plus 2 soldiers. The set-up was covered with black cloth and kept inside the exposure room for 12 months. Untreated plywood boards were provided for comparison.

Table 1. Classification of resistance of CB against termite attack.

% Damage	Degree of termite damage	Classification
0	No evidence of termite attack	Highly resistant (HR)
1 – 25	Slightly attacked (from initial nibbling to almost ¼ of the board volume is lost	Resistant (R)
26 – 50	Moderately attacked (more than ¼ to ½ of the board volume is lost)	Moderately resistant (MR)
51 – 75	Severely attacked (more than 50% but less than 75% of the board volume is lost).	Slightly resistant (SR)
75 -100	Destroyed (more than (3/4 of the volume of wood is lost).	Non-resistant (NR)

The percent termite damage on board samples was monitored at quarterly intervals for 12 months. A new batch of drywood termites was introduced on the 6th month. All board samples were retrieved on the 12th month and the degree of termite damage and percent weight loss determined. The resistance of CBs for drywood termite damage rating was classified the same as used for subterranean termites.

Field Evaluation on Cocoboard Resistance against Wood Destroying Organisms (FPRDI Standard Procedure)

Exposure shed/house model

The resistance of the CB as insulation and perimeter wall components was evaluated using a house model - exposure shed - under field conditions. A total of 12 exposure sheds were constructed at the FPRDI Graveyard Experimental Site, Los Baños, Laguna, Philippines. Three exposure sheds were randomly allocated for four types of boards: 1) LD-CB as ceiling component; 2) 19.1 mm-thick MPW as ceiling component; 3) MD-CB as exterior wall; and 4) 12.7 mm-thick MPW as exterior wall (Table 2).

Table 2. Dimensions of CB and plywood samples for test of natural resistance to insect attack under field conditions.

Density of Boards	Dimension (mm)	Total number of boards/shed	No. of Sheds	Location of Exposure
1. LD- CB	40 x 300 x 300	36 pcs at 12 pcs/shed	3 units	Ceiling
2. MPW	19.1 x 300 x 300	36 pcs at 12 pcs/shed	3 units	Ceiling
3. MD-CB	10 x 300 x 300	42 pcs at 14 pcs/shed	3 units	Exterior wall
4. MPW	12.7 x 300 x 300	42 pcs at 14 pcs/shed	3 units	Exterior wall

Each exposure shed had a 10-cm thick concrete slab floor (90 cm x 120 cm) as well as a corrugated galvanized iron roof supported by 10.0 cm x 10.0 cm x 100 cm coco wood columns on four sides. An overhang of 50cm was provided to prevent boards from getting wet during the rainy season. The wood supports served as attractant and food source for termites and the established insect populations served as source to attack the board samples.

A total of 36 LD-CB test boards and 42 MD-CB measuring 40 mm x 300 mm x 300 mm and 10 mm x 300 mm x 300 mm respectively, were exposed under field conditions. Twelve samples of LD-CB were mounted on a fabricated steel rack about 30-cm from the roof system. On the other hand, 14 samples of MD-CB were set-up as a exterior perimeter wall per exposure shed. A similar number of plywood samples (12.7 mm- and 19.1mm-thick) were provided for comparison.

Collection of data

All board samples were monitored for the occurrence and degree of insect attack under field conditions. The degree of termite damage was monitored monthly for the first 3 months and quarterly thereafter for 1 year. Insects found on samples were collected and identified. The resistance of the boards was classified based on the termite damage rating used in the resistance test against termites under laboratory conditions.

Results and discussion

A. Cocoboard resistance to subterranean and drywood termites under laboratory conditions

1. Resistance of CB to Test Insects

Resistance of CB against subterranean termites

Both types of CB and MPW samples were 100% invaded by *M. losbanosensis* as early as 1 month after exposure. The active termite population in earthen tunnels originated from the ground and extended toward the concrete foundation and invaded the side and cut ends of sample boards. Active termite populations were noted until 4 months of testing.

M. losbanosensis attempted to attack but the foraging activity was discontinued in both types of CBs. The MD-CBs had initial signs of nibbling on the surface with 0% termite damage despite the early invasion of subterranean termites within the 4-month exposure period (Table 3). Termites discontinued their feeding on LD-CBs resulting in very slight damage (3.5%) and resistance to termite attack under controlled conditions. There were instances that termites abandoned the CBs suggesting that the substrate was unpalatable. The nibbling activity and slight damage caused by *M. losbanosensis* caused minimal weight losses (2.3% and 3.3%) to MD-CB and LD-CB, respectively, after 4 months exposure. Based on the degree of damage, the LD-CB and MD-CB were classified as resistant and highly resistant, respectively, to attack by subterranean termites.

Table 3. Resistance of CB to subterranean termites under laboratory conditions after 4 mos.

Types of Boards	Application	% of CFB samples invaded by *M.losbañosensis*				Damage (%)	Weight Loss (%)
		1mo	2mos	3mos	4mos		
T1-LD-CB	Ceiling	100	100	100	100	3.5 R	3.3
T2–MPW – 19.1 mm	Ceiling	100	100	100	100	100 NR	96.7
T3–MD-CB	Panel	100	100	100	100	0 HR	2.3
T4–MPW – 12.7 mm	Panel	100	100	100	100	100 NR	97.3

Note :

% of Termite Damage		Classification of Resistance
0	:	Highly Resistant (HR)

1 to 25 : Resistant (R)
25 to 50 : Moderately Resistant (MR)
51 to 75 : Slightly Resistant (SR)
76 to 100 : Not Resistant (NR)

The MPW samples appeared sound but were hollow inside. The mid veneer of the composite was totally consumed leaving only the thin shell of the veneers. Termites penetrated inside and consumed all the wood volume inside. Both thickness of MPW sustained 100% termite damage with remarkable weight losses (96.7% to 97.3%). Based on the degree of damage sustained in MPW, it was classified as non-resistant to attack by subterranean termites.

Resistance of CB against drywood termites

All the surface of the LD-CBs exhibited nibbling by *C. dudleyi* after 3 months (Table 4). Initial attack by drywood termites (0.3% to 6.0%) was noted on the surface of LD-CBs after 3 to 12 months, LD-CB was therefore classified as a resistant composite. Drywood termites invaded the MD-CBs but later discontinued their feeding activity which consequently resulted to the 0% damage to board samples through 12 months of testing. MD-CB was classified as highly resistant and no evidence of termite attack was noted.

Marine plywood samples of 19.1mm-thick had 1.0% to 11.0% termite damage after 3 to 9 months of exposure. Likewise, slight attack (2.2% to 18.5%) was noted in MPW 12.7 mm-thick within the test period. After 12 months, the degree of termite damage in MPW 19.1 mm-thick did not change remarkably and was classified as slight attack (21.0%). The composite was classified as a resistant material. In contrast, the 12.7-mm was severely damaged with about 65.0% of the volume of the composite material lost. Based on the degree of damage, MPW 12.7 mm-thick was slightly resistant to drywood termites.

Table 4. Percent damage of CB caused by drywood termites, *C. dudleyi* under laboratory conditions

Types of Boards	Application	% Termite Damage*			
		3 mos	6 mos	9 mos	12 mos
T1-LD-CB, 40 mm	Ceiling	0	0.3	1.5	6.0 R
T2–MPW - 19.1 mm	Ceiling	1.0	1.0	11	21.0 R
T3–MD-CB, 10 mm	Panel	0	0	0	0 HR
T4–MPW - 12.7 mm	Panel	2.2	3.0	18.5	65.0 SR

*Replicated 20 times.

Note

% of Termite Damage **Classification of Resistance**
0 : Highly Resistant (HR)
1 to 25 : Resistant (R)
25 to 50 : Moderately Resistant (MR)
51 to 75 : Slightly Resistant (SR)
76 to 100 : Not Resistant (NR)

2. **Resistance of CB to Test Insects**

The percent of columns in the exposure shed invaded by subterranean termites after 12 months is presented in Table 5. Results showed that 41.7% of the LD-CB columns were invaded by subterranean termites while coco-columns of MD-CB had no evidence of termite attack after 1 month of testing. About 58.63% of the MPW 19.1 mm-thick coco-columns in exposure sheds and 50.0% of the MPW 12.7 mm-thick samples were invaded by termites after 1 month. The percent occurrence of termites increased with longer exposure periods and 50.0% to 91.7% of the coco-columns of exposure sheds were invaded by 2.0 to 3.0 months.

Table 5. Percent of columns of exposure sheds of LD and MD-CB invaded by subterranean termites under field conditions.

Treatment	% of Columns Invaded by Termites					
	1 mo	2 mo	3 mos	6 mos	9 mos	12 mos
1. LD-CB 40 mm	41.7	91.7	91.7	91.7	91.7	91.7
2. MPW,19.1 mm	58.63	91.7	100	100	100	100
3. MD-CB 10 mm	0	50.0	50.0	58.3	75.0	83.3
4. MPW, 12.7 mm	50.0	75.0	83.3	83.3	100	100

LD-CB - Low Density Cocoboards; MPW - Marine Plywood; MD-CB - Medium Density Cocoboards

The percent of columns invaded by subterranean termites increased remarkably during the succeeding 6 to 12 months. About 58.3% to 100% of the LD-CB and MD-CB coco-column supports were invaded by subterranean termites within 12 months. The percent invasion of MPW 12.7 mm- and 19.1 mm-thick column supports in the exposure sheds ranged from 83.3% to 100 %. The presence of abundant natural termite populations at the experimental area indicates that the area is an ideal site for the evaluation of the resistance of CBs under field conditions.

There were two (2) species of subterranean termites that invaded the exposure sheds: the mound building termites (*Macrotermes gilvus)* and Los Banos termites (*Microcerotermes losbanosensis).* These subterranean termite species are considered two of the six major termite species that attack houses and buildings in the Philippines. Both species were observed to attack one column or one termite species was present in a single exposure shed. The attack originated from the ground, moved toward the concrete flooring and column supports which later extended the attack to the board specimens.

The percent of board samples invaded by termites increased with longer periods of exposure. The LD-CB remained free from termite attack from 1 to 6 months of exposure. About 11.1% of LD-CB boards were invaded by subterranean termites 9 to 12 months into the test. On the other hand, early termite invasion, within 1 month, of MD-CB, MPW 19.1mm-thick and MPW 12.7mm-thick was observed. About 4.8% of MD-CB samples were invaded on the 1st month and termite invasion spread to 28.5% of board samples in 3 months (Table 6). Percent invasion increased from 35.7% to 52.3% in 6 to 12 months of exposure. On the other hand, 33.3% of MPW 12.7 mm-thick and 38.1 % of the MPW 19.1 mm-thick, respectively were invaded by *M. gilvus* and *M. losbanosensis* as early as 1 month after exposure. The percent termite invasion of board samples increased from 41.7% to 66.7% after 2 to 12 months.

Active populations of subterranean termites were seen during the retrieval of CB and MPW samples at the end of the year-long test. The LD-CBs despite the presence of termite invasion had only termite nibbling on the surface. There was no evidence of termite damage and it was therefore classified as highly resistant against termite attack. MD-CB had slight termite damage that ranged from initial nibbling to 7.2% and was classified as resistant to termites.

Table 6. % LD-CB and MD-CB samples invaded by subterranean termites under field conditions.

Treatment	% of CB Invaded by Termites						% Damage
	1 mo	2 mos	3 mos	6 mos	9 mos	12 mos	
1. LD-CB 40 mm	0	0	0	0	11.1	11.1	0 HR
2. MPW,19.1 mm	33.3	41.7	66.7	66.7	66.7	66.7	6.4 R
3. MD-CB 10 mm	4.8	21.4	28.5	35.7	52.3	52.3	7.2 R
4.MPW, 12.7 mm	38.1	54.7	59.5	59.5	59.5	66.7	27.2 MR

Note

% of Termite Damage		**Classification of Resistance**
0	:	Highly Resistant (HR)
1 to 25	:	Resistant (R)
25 to 50	:	Moderately Resistant (MR)
51 to 75	:	Slightly Resistant (SR)
76 to 100	:	Not Resistant (NR)

Conclusions and Recommendations

- The LD-CBs are resistant, and MD-CBs highly resistant to attack by the subterranean termite *M. losbañosensis* and drywood termites under laboratory conditions. MPW, regardless of thickness, are not resistant to attack by *M. losbanosensis* but resistant to slightly resistant to *C. dudleyi.*
- The LD-CB and MD-CB are resistant and highly resistant to drywood termites after 12 months. MPW 19.1 mm-thick was resistant while MPW 12.7mm-thick is slightly resistant to drywoods.
- Under field conditions, columns made from coconut materials in all exposure sheds were invaded by two species of subterranean termites (*M. losbanosensis* and *M. gilvus)* and the percent of columns invaded per shed varied from 0% to 100% in 12 mos.
- There were 11.1% and 52.3% of the LD-CB and MD-CB, respectively and 66.7% of MPW, regardless of thickness, invaded by subterranean termites in 12 months.
- MD-CB and LD-CB are resistant and highly resistant, respectively to termite attack with 7.2% and 0% damage after 12 months. MPW 19.1 mm-thick is resistant with slight attack (6.4%) while MPW 12.7 mm-thick is highly resistant with moderate attack after 12 months. In contrast, the CBs had termite damage of 0 to 7.2% compared to termite attack of 6.4% to 27.2% for MPW.
- Further exposure of board samples is necessary to obtain concrete data on the natural resistance of CB against wood destroying insects under field conditions.

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Polystyrene Impregnated Wood Resistance to Marine Borer Attack

by
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Abstract

Timbers from plantation forest on a short cutting cycle are inferior in terms of resistance to bio-deterioration, because the lumber has rich sapwood and less poisonous extractives. Polystyrene impregnation is a way to improve wood resistance to bio-deterioration. Three timber types, Indonesian pine *(Pinus merkusii),* rubber wood (*Hevea brasiliensis*), and sengon (*Falcataria moluccana*) were impregnated with polystyrene. All three timber types had wood specimens (2.5-X 5-X 30-cm; W:T:L) tested against marine borers in the Jakarta sea over three months at which time all the pine was consumed and rubber-wood and sengon were tested for eight months while the rubber wood ran for one year because all the sengon was consumed after the 8-month check. Untreated wood and impralite-CCB preserved wood were also prepared for comparison and replicated five times for each treatment. At the end of the test, each sample was split through the thickness into two parts to determine marine borer infestation. The purpose of this study was to determine the resistance of polystyrene impregnated wood to marine borer. The results showed that the three wood species could be be made into polystyrene wood with polymer loading ranging 72–163%. Wood species significantly affected resistance to marine borer attack, the lowest wood density being most susceptible to attack. The polystyrene impregnated wood was more resistant than untreated wood, but was still less resistant than impralit-CCB preserved wood.

Key words: Polystyrene impregnated wood, impralite-CCB preserved wood, attack intensity, marine borer attack

Introduction

Indonesian logs supplied, in 2016, 37.5 million m^3, and 85% of those logs were from plantation forests (Ministry of Environment and Forestry 2017). The logs from plantation forests were mostly from fast growing tree species cut at 6-10 years old, and that ‘juvenile’ timber is dominated by sap wood. That type of young, fast-grown wood has inferior physical–mechanical properties as well as being less resistant to bio-deterioration, including marine borer, when compared with mature wood (Fajriani et al. 2013).

The service life of wood could be lengthened through preservation processes that impregnate poisonous chemicals. The impregnated wood may also be poisonous to human beings and hazardous to the environment. Chemical modification aimed at preservation is a challenge to solve, and polystyrene wood is a choice that could improve physical and mechanical properties as well durability.

Chemical modification using polystyrene has been studied, and results show that the products have better physical-mechanical properties, and also resistance to bio-deterioration. There are a number of least-toxic options for wood preservation, including acetylation of particle board and fiberboard improve the resistance to bio-deterioration (Hadi et al. 1995, Rowell et al. 1997), polystyrene impregnated wood (Hadi et al. 1998, Abdul Khalil et al. 2014), furfuryl alcohol wood (Hadi et al. 2005, Esteves et al. 2011), smoked wood (Hadi et al. 2010a, 2010b, 2012), methyl methacrylate impregnated wood (Kartal et al. 2004, Hisham and Anwar 2005, Hadi et al. 2015), and polyurethane resins for non-biocidal wood preservation (Mubarok et al. 2016).

Hadi et al. (1998) mentioned that polystyrene impregnated wood had better resistance to bio-deterioration compared to untreated wood. Devi et al. (2003) found that the biodegradability resistance of polystyrene wood was improved on treatment with styrene/styrene–glycidyl methacrylate, and Hadi et al. (2016) mentioned that polystyrene impregnated wood provided better resistant to termite attack compared to untreated wood in a ground test.

Penetration and retention of wood preservatives, and weight gain in polystyrene processing are affected by anatomy and density of the wood. In general softwood is more easily penetrated by chemicals than hardwood. In this experiment samples were Indonesian pine (*Pinus merkusii*), rubber-wood (*Hevea brasiliensis*), and sengon (*Paraserianthes moluccana*).

The purpose of this study was to determine the resistance of polystyrene-impregnated wood, conventional wood preservation (impralit-CCB) and untreated wood to marine borers using small clear specimens.

Materials and methods

Wood preparation

Wood species tested were Indonesian pine, rubber-wood, and sengon. Small clear specimens sized 2.5- X 5- X30-cm (W:T:L) were used for the test. The wood samples were dried to reach 12% moisture content, and put in a tank with 20-mm Hg vacuum for two hours, and during vacuum release styrene monomer was streamed into the tank, followed with 10 kg/cm^2 pressure for one hour and the samples were immersed in styrene monomer for 24 hours. Terbutyl-hydroperoxide was used as the catalyst at 0.5% to styrene volume based. Furthermore, wood samples were taken out, wrapped in aluminum foil, put in an oven at 60° C for 24 hours, and conditioning for two weeks.

Untreated and impralit-CCB preserved woods were prepared for comparison. The wood preservation process was applied to the sample with 12% moisture content, and initiated with 20-mm Hg vacuum for 30 minutes. During vacuum release 3% impralite-CCB was streamed into the tank, followed with 10 kg/cm^2 pressure for 1 hour and immersion for 24 hours.

Marine borer test

A one-centimeter diameter hole was drilled through the center of each 5 X 30-cm sample board. Boards were fixed together with plastic cord and arranged into rafts. Rafts were exposed to the sea on Rambut Island near Jakarta, Indonesia, the three wood species were tested for three months, frubber wood and sengon for eight months because the pine was lost, and rubber wood for one year test because the other two samples were lost. At the end of each test period wood samples were cleaned of marine organisms, and split into two parts to determine marine borer infestation. Marine borers were identified from the traces of boring holes, form of cutting and pallet on the infested samples.

Data analysis

In this research, the investigated factors were (1) wood species consisting of pine, rubber-wood, and sengon, and (2) treatment consisting of untreated wood, polystyrene wood, and preserved wood (impralit-CCB). There were five replicates using a 3 x 3 completely randomized factorial designed for statistical purposes. Duncan's test was used for further analysis significant differences determined at $p \leq 0.05$.

Results and discussion

Pine is a conifer, while rubber-wood and sengon are broadleaf species. The initial density of each wood was 0.63 g/cm^3 for pine, 0.61 g/cm^3 for rubber-wood, and 0.32 g/cm^3 for sengon, and the durability class was V for all three wood species (Martawijaya *et al*. 1989 and Hadi *et al*. 2016). The durability class rankings range from I to V, with I being very resistant and V very susceptible.

After the polymerization process with heat, the average polystyrene loading for pine was126%; rubber-wood 72%; and sengon 163%. Impralite-CCB retention was 6.9 kg/m^3 for pine, 5.8 kg/m^3 for rubber-wood, and 7.3 kg/m^3 for sengon. Pine and rubber-wood had almost the same density, but pine resulted in a higher polymer loading and retention. Sengon had the highest polymer loading and retention because it has low density.

The average attack intensities of marine borer after each test period are shown in Table 1, while analysis of variance is in Table 2, and the multiple range test results shown in Table 3. From these tables, it is clear that untreated sengon wood was the most susceptible to marine borers because the wood had the lowest density reflecting easier feeding. Pine and rubber-wood had almost the same density, and attack intensities were almost the same. The three wood species display very low resistant to bio-deterioration, and the results showed that the untreated wood was attacked at a higher rate than treated wood.

Table 1. Marine borer attack intensity (%)

Test Period	Wood species	Untreated	Polystyrene	Impralite-CCB
Three-month	Pine	8.0 (1.6)	1.4 (0.5)	0 (0)
	Rubber-wood	6.2 (1.9)	2.6 (0.5)	0 (0)
	Sengon	18.8 (2.6)	6.4 (2.1)	0 (0)
Eight-month	Rubber-wood	30.4 (5.1)	6.4 (2.1)	0 (0)
	Sengon	66.0 (11.2)	8.2 (1.9)	0 (0)
One-year	Rubber-wood	89.0 (4.2)	42.3 (3.3)	0 (0)

Table 2. Resume of analysis of variance

No	Test period	Wood species	Treatment
1.	Three months	*	*
2.	Eight months	*	*
3.	One year	-	*

Table 3. Duncan's multiple range tests.

No	Period	Pine	Rubber	Sengon	Untreated	Polytyrene	CCB
1.	3-month	3.1 a	2.9 a	8.4 b	11.0 c	3.5 d	0 e
2.	8-month	-	12.3 a	24.7 b	48.2 c	7.3 d	0 e
3.	1-year	-	32.0	-	89.0 a	42.3 b	0 c

Wood species highly affected resistance to marine borer attack with pine and rubber-wood not significantly different and related to initial densities (Tables 2 & 3). During three-month and eight-month test periods sengon wood had the highest attack intensity because sengon had very low density, it seemed easier attacked for feeding. The three wood species were susceptible to bio-deterioration organisms because they were cut at young age and dominantly consisting of sap wood and juvenile wood resulting less extractive content,.

Treatment effects wood were highly significant affecting wood resistance to marine borer attack in all test periods (three-month, eight-month, and one-year). Untreated wood always had the highest attack intensity compared to treated wood in each test period, followed by polystyrene wood, and impralite-CCB preserved wood. Impralite-CCB preserved wood was very resistant and had no attack even after one-year, because the wood contained a toxin that assisted in preventing attack by the marine organisms.

Polystyrene wood showed better performance than untreated wood in all test periods indicating chemical occupation of the void was effective in reducing attack by marine organisms. Polystyrene has no poison but it presented a barrier for organism feeding. The degree of resistance in treated wood

(36.5%, polystyrene wood 11.4%, and impralite-CCB 0%) could be explained by the average attack intensities relative to the untreated wood. In other words, the attack intensity of polystyrene wood was about one-third of untreated wood, this is in-line with Hadi et al. (2016) who found that weight loss of polystyrene wood was one-fifth that of untreated wood tested against subterranean termites in a ground test. The impralite-CCB preserved wood showed the most resistantance and was not attacked even after one-year, and polystyrene wood was better than untreated wood.

According to the hole characteristics based on the form of cutting and pallet on infested samples, the marine organisms that attacked the wood were *Dicyathifer manni*, and *Bankia ceiba* from Teredinidae, and *Martesia striata* from Pholadidae.

Conclusions

It can be concluded that the three wood species are suitable for making polystyrene wood with polymer loadings of 72-163 %. Polystyrene impregnated wood was more resistant than untreated wood, but it was still less resistant than impralit-CCB preserved wood for protection from marine borers. The wood species tested with polystyrene treatments were not significantly different one each other. Organisms that attacked the wood were Dicyathifer manni, Bankia ceiba, and Martesia striata.

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Development of Bait Formulations from Official Waste Paper Supplemented by Soybean Boiling Water for Termite

by
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Abstract

Baits that display high consumption are necessary for achieving efficient and effective termite control. The use of a bait supplement may become a way to stimulate the feeding and tunneling behavior of termites. This study determined the effect of a bait supplement using soybean boiling waste water on the consumption rates of the subterranean termite, *Coptotermes formosanus* Shiraki. Soybean boiling waste water was added to different food sources, which were paper disks (filter paper) and paper matrices. The paper matrices were made from blended waste office paper which were formed into a cube (2 x 2 x 1-cm) with the target density of 0.75 g cm^{-3}. No-choice and pair choice tests were conducted with five replications. Results showed that consumption signicantly increased in all samples supplemented by soybean boiling water solution. Termite consumption was 22.94% higher in the supplemented paper disks and 278.69% higher in the supplemented paper matrices compared to untreated controls. This study indicates that soybean boiling waste water is promising as an effective bait supplement for *C. formosanus* subterranean termites.

Key words: Termite feeding behavior, termite baits, bait supplement, *C. formosanus*.

Introduction

Baiting technology for termite control is developing along various approaches. Baiting methods employ food sources containing slow-acting toxins that are readily consumed by termites with a lethal effect to the entire colony. The effectiveness of this technology depends on the availability of baits with the characteristics of being easily detected, recruited to, and fed upon by termites. In the application of no-toxic baits, or monitoring stations, are generally placed at certain intervals in the ground around a structure to being protected from termite attack. Bait matrices containing a slow-accting toxin are used to simply replace any of the stations with the sign of termite activity. However, previous studies showed that termites did not easily detect the presence of food sources in the soil or had very low discovery rates (Rust et al. 1996; Jones, 2003; Paysen et al. 2004). The discovery rate of termite baits needs to be increased for effective control. It is, therefore, important to search for potential subtances that stimulate termites to move toward a bait.

Some effort has been made to employ certain semiochemicals in the soil and found that they potentially stimulate termite tunneling toward a bait station (Castillo et al, 2013; Cornelius, 2005; Cornelius et al, 2009). The impregnation of wood with extracts of decayed wood increased termite orientation toward treated wood (Su, 2005). The role of fungal extracts in increasing termite tunneling activities were also recorded in decayed sawdust (Cornelius et al. 2002). In addition, increased feeding by the subterranean termites *Coptotermes curvignathus* and *Coptotermes gestroi* was observed in bait matrices supplemented with various sugars, amino acids, and cassava (Castillo et al. 2013). In a field test of bait supplements including decayed material, a sports drink, and the combination of an aqueous solution of a commercial food source, it was found that only the aqueous solution caused a significant increase in the termite discovery rate (Cornelius et al. 2009). These findings indicate the value of bait supplements, especially in an aqueous solution that have the potential to diffuse into the soil and decrease the time required for termites to discover the associated baits. Bait formulations with soybean boiling water were noted to be easily converted to biogenic production by termites (Indrayani et al, 2016; Muin and Arif, 2016). This study employed soybean boiling water as a supplement in a food source for subterranean termites *Coptotermes formosanus* Shiraki. This aqueous solution was used as it was thought to contain substances and fermented compounds capable of attracting termites.

Materials and methods

Preparation

Samples were prepared using paper disks (filter paper) for no-choice and paper matrices in case of paired choice-tests. Filter paper disks were treated with soybean boiling water or water only with five replications. The matrices were made from HVS waste office paper by blending for fiber separation and formed into cubes (2 cm x 2 cm x 1-cm) with the density of 0.75g/cm^3. All samples were conditioned at 60°C for 48 hours and weighted to determine initial weights. The addition of soybean boiling water solution (60% of the matrix oven-dry weight) to the bait was performed just before testing. In this study, the soybean boiling water solution was obtained by boiling 200 g of soybean in 3000 ml of water for about one hour and allowed to fermentate for four days before used as supplement for the bait samples.

Laboratory tests

No-choice and paired-choice tests were performed in laboratory. No-choice test was conducted using a Petri dish measuring 8-cm (ID) with 50 *C. formosanus* workers and 5 soldiers collected from Deterioration Organisms Laboratory, Kyoto University. Filter papers at the bottom of each dish were moistened with 200µl distilled water or soybean boiling water. In case of pair-choice test, a pair of treated and untreated baits were introduced into a Petri dish with 0.5 cm of sand moisten with 20% distilled water. The bioassays were conducted in the dark at 28±2°C and 80% relative humidity for three weeks for no-choice test and two weeks for the paired-choice test. Weight loss of all samples was determined at the end of the observation period, while mortality of termites was calculated for no-choice test only. Treated and untreated data obtained from this study were compared by Dunnett`s test.

Results and Discussion

No-choice test

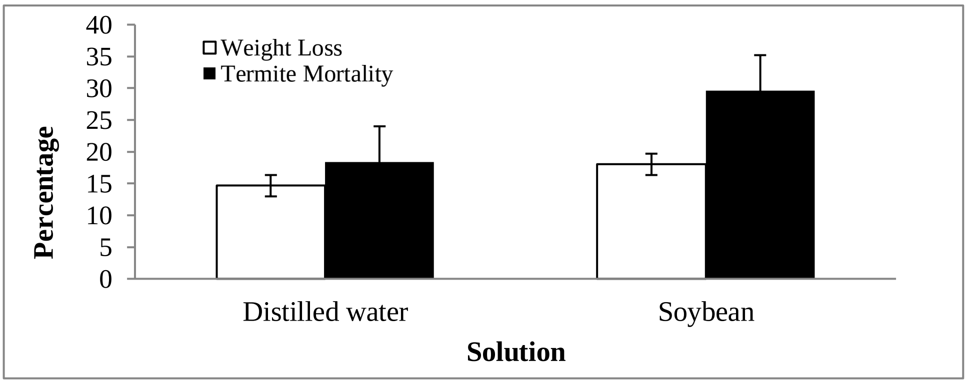


Figure 1. Percentage of weight loss and mortality of *C. formosanus* in no-choice test

Weight loss of filter paper treated with soybean boiling water after three weeks exposure to C. formosanus, were 22.94% higher than controls (treated with distilled water) (Figure1). This finding was supported by Indrayani et al (2016) who state that materials enriched with soybean boiling water were preferred by termites. Mortality of C. formosanus after three weeks exposure to filter paper treated with soybean boiling water was to 60.87% higher than control (Figure1). These results indicated that soybean boiling water be an effective supplement for subterranean termite C. formosanus.

Pair-choice test

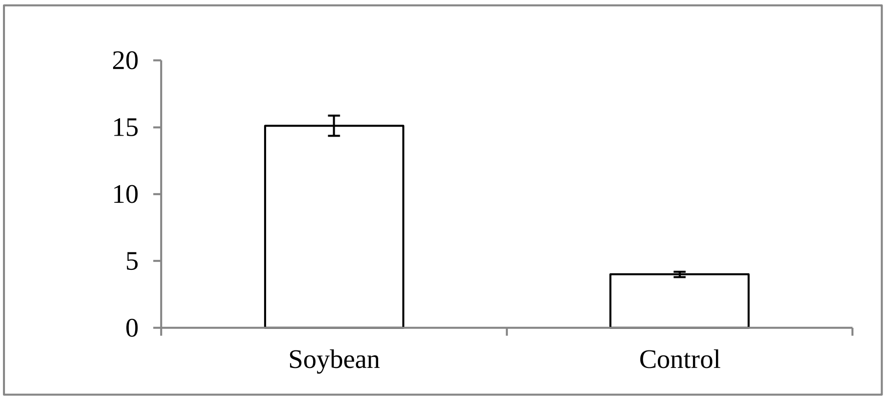
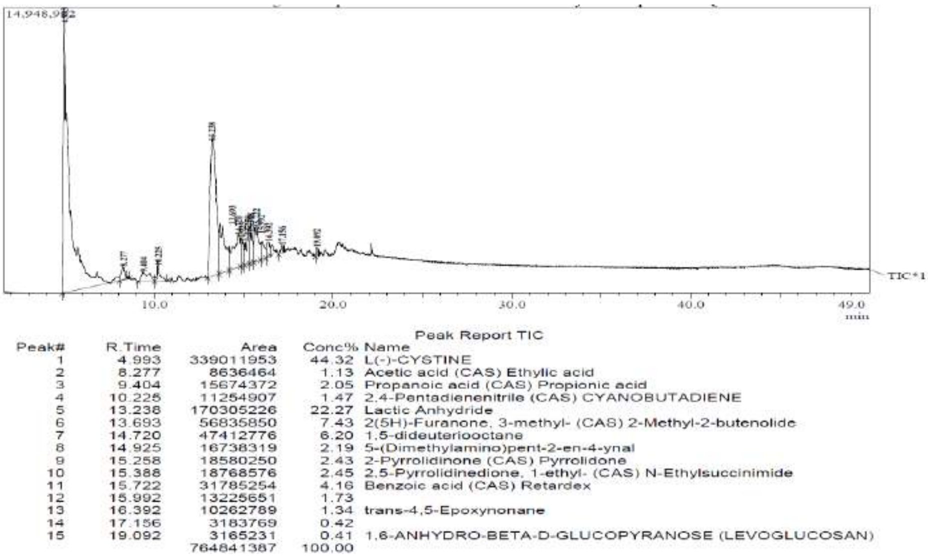


Figure 2. Percentage of weight loss of bait after two weeks exposure to *C. formosanus*

There was a significant difference in weight loss between treated and untreated bait at $p<0.5$ (Figure 2). Weight loss of treated bait was 278.69% higher than untreated bait. This finding demonstrates that soybean boiling water was preferred over the paper cubes treated with water only. This might be caused by soybean boiling water contain 44.32% L-cystine, which is the most abundant amino acid in the solution (Figure 3).



```
                              Peak Report TIC
Peak#    R.Time        Area     Conc% Name
    1     4.993   339011953     44.32 L(-)-CYSTINE
    2     8.277     8636464      1.13 Acetic acid (CAS) Ethylic acid
    3     9.404    15674372      2.05 Propanoic acid (CAS) Propionic acid
    4    10.225    11254907      1.47 2,4-Pentadienenitrile (CAS) CYANOBUTADIENE
    5    13.238   170305226     22.27 Lactic Anhydride
    6    13.693    56835850      7.43 2(5H)-Furanone, 3-methyl- (CAS) 2-Methyl-2-butenolide
    7    14.720    47412776      6.20 1,5-dideuteriooctane
    8    14.925    16738319      2.19 5-(Dimethylamino)pent-2-en-4-ynal
    9    15.258    18580250      2.43 2-Pyrrolidinone (CAS) Pyrrolidone
   10    15.388    18768576      2.45 2,5-Pyrrolidinedione, 1-ethyl- (CAS) N-Ethylsuccinimide
   11    15.722    31785254      4.16 Benzoic acid (CAS) Retardex
   12    15.992    13225651      1.73
   13    16.392    10262789      1.34 trans-4,5-Epoxynonane
   14    17.156     3183769      0.42
   15    19.092     3165231      0.41 1,6-ANHYDRO-BETA-D-GLUCOPYRANOSE (LEVOGLUCOSAN)
                  764841387    100.00
```

Figure 3. Identification of soybean boiling water using Gas Chromatography Mass Spectrometry (GCMS)

L-cystine is a proteinogenic amino acid contributing to constructing proteins. Protein is needed by termites for its growth. Termites obtained protein by eating fungi growing in the nest. This is evidenced by the content of protein in termite's workers more than 80% while, protein in termite's reproductive more than 85% as state by Paul and Dey (2011). This results also accordance with Arquette et al., (2012) who found that termite feces contain high protein compared to wood.

Conclusions

The results demonstrate that adding soybean boiling water to a paper-based bait matrix can increase consumption by termites with concomitant low toxicity as indicated by the no-choice test. Similarly, in the paired-choice test, soybean boiling water resulted in a higher rate of consumption by termites. It can say that adding soybean boiling water as a supplement to a termite bait formulation should increase consumption of the bait.

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Research on using *Xylaria* to determine the main chamber of Odontotermes nest on dikes

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Abstract

The pesticides Lenfos 50EC and BDM 08 bait were used to control 40 termite nests in dikes and we found a relationship between the *Xylaria* and dead termite nests:

- There were 4 kinds of *Xylaria* growing in dead termite nests,
- It takes at least 10 days for *Xylaria* to grow in termite nests after we controlled them,
- BDM 08 was the pesticide with a higher proportion of *Xylaria* growth than Lenfos 50EC.
- Termite chambers were found under the *Xylaria* and the main chamber was usually located under the site where the most *Xylaria* was found.

Introduction

Treatment of *Odontotermes* termites on dikes at present consists of two steps: determine the main chamber of the termite nest, spray chemicals to exterminate the termites and fill up the termite nest clay with clay. The current method of determining the main chamber of the termite nest is to use exploratory geophysical equipment, however, this method is costly. Research has shown that *Xylaria* fungus often grows from dead *Odontotermes* termite nests. Therefore, the Institute of Ecology and Works Protection has carried out research entitled "Research on using *Xylaria* to determine the location of the main chamber of *Odontotermes* nest on dikes". We tested the extermination of 40 *Odontotermes* nests on dikes using two different chemicals and monitored the appearance and development of *Xylaria* fungus on these nests, in order to determine the relationship between *Xylaria* and the various chambers, especially the main chamber of *Odontotermes* nests on dikes.

Materials and method

Research location, period

- The research was conducted on the sections K25 to K40 of Day Left dike in Hanoi.
- The research period was March 2017 to August 2017.

Research materials

- Odontotermes termite nests on Day Left dike from K25 to K40.
- Bait BDM08 produced by the Institute of Ecology and Works protection.
- Lenfos 50 EC produced by Hockley international Ltd. (UK) pumped into termite nests using a water pump 750w

Research method

Method of determining areas with Odontotermes termite nests on dikes through biological and ecological signs

Surveyed a section of dike section starting at beginning of March. The position of 56 flying castles was marked to temporarily determine the position of termite nests.

Method of treating termite nests with bait BDM 08

The termite bait BDM08 in the form of rigid bar was put into 20 termite nests through holes, the amount of bait used depended on the size of the nest (directly proportional to the number of flying castles), with the average of 10-15g/nest (the largest nest recieved 30g).

Method of treating termite nests with lenfos 50EC

Lenfos was mixed at a ratio of 2/1000 and sprayed into 20 nests through holes. The chemical solution was applied into a hole until overflow appeared on the dike surface to ensure that the nest was full of chemical.

Method of anatomy, observation and recording data

The anatomy of 16 termite nests was measured and illustrative photos taken upon excavation of the nest.

Results and discussion

Some characteristics of Xylaria species on Odontotermes termite nests

Through the test four types of *Xylaria* was observed on 32 of the dead *Odontotermes* nests (photo 1). When comparing this result with the research by Yu-Ming Ju et al (2007) who found 9 species of *Xylaria* on termite nests in Taiwan, we found fewer species. Among the 4 types of fungus found on nests in Vietnam, two species N1 and N2 were found on all nests and two others N3 and N4 only on some nests.

1 2

3 4

Figure 1. Four types of *Xylaria* in the dead termite nest (Note: 1=N1, 2=N2, 3=N3, 4=N4)

Observing the surface-growing structures from dead nests, we found fungus N1 has a long cylindrical, relatively tough and firm on top while new growth was bright yellow, and the root contacting with soil was black. Later on, the upper yellow part turned a darker yellow, to ash and finally black. Along with this change in color was a change in the size of the hyphae. The survival time of N1 on termite nests was the longest, lasting up to 1 month or more (until we performed the final nest anatomy on 8 June, still some N1fungus growing).

Fungus N2 was more filamentous, longer, fragile than N1. The upper hyphae was milky white, then turned to ash gray. The root-hyphae near the soil was black. This is the first fungus type to grow on the dead termite nests. The survival time of N2 was shorter than N1, ranging from 15 to 20 days.

Fungus N3 had white new growth and a thicker body than N2 but smaller than N1. The different characteristics compared to the other types of *Xylaria* is that N3 had hyphae that divided into many lobes. Also like the other fungus types, the root-hyphae at the soil was black and the top also became darker over time. The survival time of N3 type was similar to that of N2, about 15 to 20 days.

Fungus N4 was a light yellow, thin, flattened, and spiraled like a spring, with quite elastic hyphae. This fungus type appears last on dead termite nests compared to other fungus types.

We found that these *Xylaria* fungus originated from the termite fungus garden nest chambers. The hyphaes grew straight from the termite nest chamber through the soil to the surface but it also grew along the air cave lines to the surface. The hyphae that grow in the air cave line usually have a larger root size. The large exposed root hyphae at the soil surface are formed by many branched hyphae just below the soil surface.

A special characteristic of all four types of fungus is that the initial growth rate is very fast and much slower later. We measured 10 N1 fungi and 10 N2 from the first day we observed these fungi in termite nests with long and large hyphae. For example, N1: 9cm, N2: 12cm, N3: 6cm, N4: 10cm. However, in the following days, the height of these fungus types did not increase significantly. After one day, the increased average height of N1 and N2 was 0.9 cm and 1.78 cm, respectively. After 2 days, the increased average height compared to the previous two days at 1.1 cm and 0.73, respectively. The following days we noticed that the height of the hyphae did not increase and the diameter of hyphae N2 remained almost unchanged, only fungus N1 provided visible changes in diameter.

*The relation between weather conditions (rainfall) and growth of Xylaria fungus***.**

The 40 termite nests were divided into 5 different phases, one week apart from each other. We found that the time of occurrence of the *Xylaria* on the surface of the treated nests was closely related to the rainfall after treatment.

Table 1 shows that there was no difference in the rate of growth of *Xylaria* (the time of growth) between the different termite treatment methods. In fact, the research shows that termite nests treated according to the different methods that the growth of *Xylaria* was from the beginning, there were a few nests (02 nests) where the growth was delayed a few days.

There were 3 treatment dates, every 7 days, however, *Xylaria* on 3/5/2017. Rainfall was the deciding factor on when we observed fungus growth. After the treatment phases 1 and 2, weather conditions was hot with no rain, that started 29/4 lasting for 3 days, so although the termite nest treated during phase 1 conducted from 10/4 and phase 2 on 17/4 we saw *Xylaria* growing only after rain.

Table 1. The time of appearing *Xylaria* fungus on the treatment termite nests

TT	Treatment phase	Date of treatment	No. of nests treated	First day of appearing *Xylaria*	Days after treatment when the fungus appeared	Weather conditions
1	1	10/4/2017	12	3/5/2017	23	The average outdoor temperature was 24 – 33^0C from 10/4 to 29/4/2017. Starting from 29/4 to 2/5 it rained.
2	2	17/4/2017	8	3/5/2017	16	
3	3	23/4/2017	6	3/5/2017	10	
4	4	29/4/2017	8	9/5/2017	10	The average outdoor temperature was 21 – 30^0C, it rained on 2nd, 3rd, and 7th – 9th May
5	5	6/5/2017	6	17/5/2017	11	It rained on 14th, 15th and 16th, May.

The following nest treatments had more favorable conditions. At this time, the soil on the dikes was no longer dry and hard, as during the first phase treatments because of the previous rains. At the same time, after those termite treatments, there was rain, and the time of *Xylaria* growth after treatment was 10 and 11 days respectively and equivalent to the time of appearance of *Xylaria* for the third treatment phase.

It can be seen that although the conditions of soil moisture as well as time and rainfall were favorable for treatment phases 4 and 5, the time for *Xylaria* to appear on the dead termite nests was not shorter than the third treatment phase. The shortest time possible for the *Xylaria* to grow on the termite nests is 10 days after treatment.

The relation between the treatment method and the rate of growing Xylaria on termite nests

Termite nests treated with bait BDM08 had a higher rate of growth and amount of *Xylaria* on dead termite nests compared to Lenfos 50EC (Table 2).

Table 2. Relation between the number of nests growing *Xylaria* and the treatment method

No.	**Type of treatment chemical**	**No. of nests to be treated**	**No of nests growing *Xylaria***	**Remark**
1	Lenfos 50EC	20	12	4/12 nests growing *Xylaria* were found to grow many funguses, other nests only grow less
2	BDM08 bait	20	20	17/20 nests growing *Xylaria* were found to grow many funguses and divided into the centralized areas

Among the 12 nests treated with Lenfos 50EC, only 4/12 nests grew significant amounts of *Xylaria*, the remaining nests provided but a small amount. When digging some treated nests we found that all termites dead but the fungus garden growing a green mold, but no sign of *Xylaria*.

Nests treated with BDM08 bait, the proportion growing *Xylaria* was 100% (20/20 nests). At the same time, most of the nests growing *Xylaria* had large amounts of *Xylaria*, divided into distinct areas (17/20 nests had these conditions).

Thus, for the nests treated with chemical, it can be seen that although the termites died, they still did not grow *Xylaria*, while nests treated with bait did. Previous research shows that, *Xylaria* exists with *Termitomyces* fungus in the fungus garden, when meeting with suitable conditions such as the termite nest dies or *Termitomyces* fungus cannot develop, the *Xylaria* develop strongly (Batra and Batra 1979). However, treatment of termite nests with a large volume of pesticide solution dramatically changes the moisture conditions in the nest chambers, and is new condition in moisture is not suitable for *Xylaria*. The formulation itself may also contribute to inhibition of *Xylaria* growth.

The relation between the density of Xylaria and the main chamber of the termite nests

Our main objective when implementing this topic was to determine the location of the main chamber of *Odontotermes* nests on dikes through indication of *Xylaria* fruiting bodies, hence our interest in the fugal morphologies, size of hyphae, time of appearance, and density of the four *Xylaria* types. The end point was to define some common points to identify the position of the main chamber of the termite nest. Finally, we conducted excavations on the anatomy for termite nests to verify our judgments to see whether it is suitable method for locating the main chamber.

Through monitoring 32 termite nests growing *Xylaria*, there were the following questions:

- For many termite nests we realize that the place growing *Xylaria* did not coincide with the position the hole through which the bait was applied with most nests have a greater distribution of *Xylaria* growth than the distribution/position of holes. Where was one nest with two holes but *Xylaria* growth was mainly in the area between the two holes (nest 17); we also noticed that *Xylaria* appeared far from (2m) a nest through a road section spread with concrete (nest 40). The question was is the appearance of *Xylaria* fruiting bodies related to the termite nest or not?

- Many nests had the phenomenon that the N1 fungus grew later, larger, and longer than fungus that grew first. Immediately after growing out of the ground, these fungi had an average length of about 12 cm - 13 cm and as long as 16.5 cm (average length of this fungus was 8 - 11cm). This phenomenon was only acknowledged with the N1. The question is whether these large-sized fungi originated from the main chamber, because the main chamber lies deeper than the fungus gardens. If so, the position of the fungus would determine the main chamber of the termite nest.
- Most termite nests are found to have an area growing many more concentrated fruiting bodies than other areas, however, these areas have fungus growing from the first day of fungal appearance and do not coincide with the area where the big N1 that grows later. So, the position of concentrated *Xylaria* fruiting bodies may alternatively indicate the main chamber.

Figure5. A big *Xylaria* fruiting body that grew later and the distance from the main chamber to that fruiting body

To answer the above questions, we selected 16 termite nests growing *Xylaria* with different characteristics to perform an excavation to examine the anatomy in order to answer the above questions.

- The position of *Xylaria* fruiting bodies coincide with a fungus garden chamber in the termite nest. The concentration of fruiting bodies is related to the size of the nest chamber – the more fruiting bodies means a larger chamber. At nest 17, after digging the area where the *Xylaria* grows between the flying castles, we found that the main chamber was located 60cm underground. At nest 35, the area where the *Xylaria* grew was 2m away from the bait application hole, there also were three auxiliary chambers with fungus gardens in that area.
- At the nests with many large N1 *Xylaria* fruiting bodies we found that the position growing these fungi did not coincide with the position of the main chamber. The main chamber of the termite nest lay under the position with concentrated fungal growing right from the beginning. However, these fungi are also related to the main chamber of the termite nest. They originated from main chambers and grow obliquely along roads leading to the ground surface, so the positions of these fungi is quite far from the main chamber, for example at nest No. 37, the distance between the main chamber to the position of the large N1 fruiting body was 85 cm.

Through the anatomy of 16 termite nests, there were 14 nests where we found the main chamber of the termite nest coincident with the position of the densest sign of the *Xylaria*. Most of the nests have a main chamber lying far from the center of the treatment holes. At the same time, we found that the difference between the growth rate of *Xylaria* and depth of the termite nest chamber was not significant. *Xylaria* grow from the deep main chamber and from the auxiliary chambers near to the surface appear on the same day (only a few fungus N1 from the main chamber grew later).

Thus, it can be affirmed that the places where there is a conspicuous, large concentration of *Xylaria* fungus fruiting bodies there is the main chamber of an *Odontotermes* nest in dikes.

Conclusion

- There are four types of *Xylaria* growing on dead *Odontotermes* nests in dikes in Vietnam.
- The shortest time for *Xylaria* fungus fruiting bodies to grow on a dead termite nest is 10 days after treatment.
- The use of the termite bait BDM08 to exterminate *Odontotermes* termite provided a higher rate of growing *Xylaria* on the dead nests than when using Lenfos 50EC.
- The place where *Xylaria* growth is most concentrated is the main chamber of an *Odontotermes* nest in a dike.

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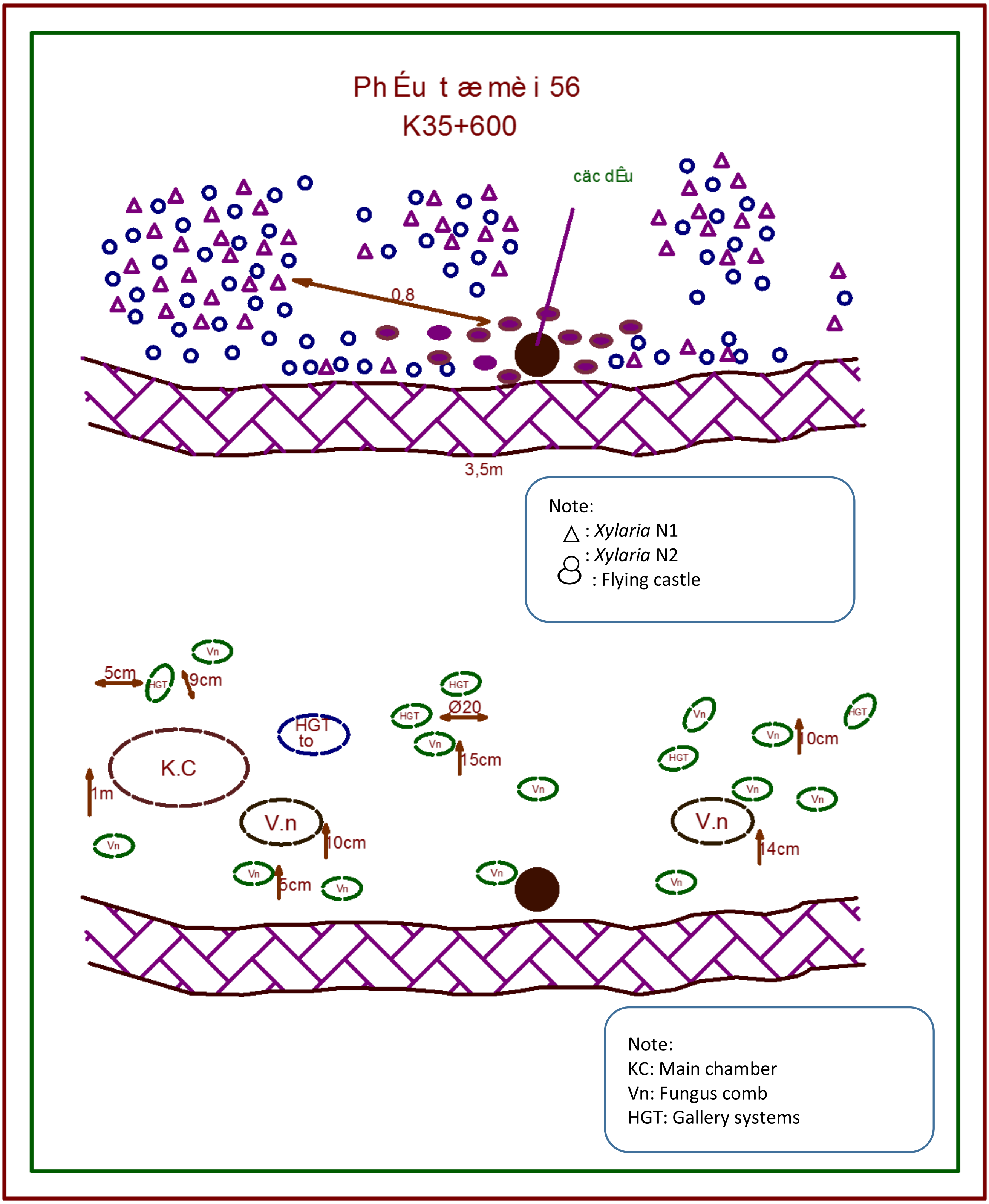

Figure 6 Diagram of flying castles, *Xylaria* and chambers in the nest treated with BDM08

Efficacy of hexafluamuron bait on control of *Coptotermes* in Vietnam

by
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Abstract

Mobahex - C16 bait has been studied for successful control of subterranean termites (*Coptotermes*) that represents an improvement from the precursor BDM10. Mobahex – C16 contains powder of A fungus culture medium, bagasse powder, LG bark powder, sugar and hexafluamuron (0.75%), which are compressed into a 100 x 50 x 2.5 mm stick with dividing lines on 1 surface. The efficacy of Mobahex C16 and BDM10 were compared for this paper. The effectiveness of Mobahex – C16 to eliminate a colony was 100% within 17 days. The LT_{50} of Mobahex - C16 termite at 0.3g/300 individuals was 198 hours (range from 175-231 hours). Re-infestation was not recorded in all field trials (100%). The average consumption/removal of bait was 226 g/construction and the average time to elimination was 39 days.

Keywords: *Coptotermes*, termite bait, Mobahex-C16, hexafluamuron.

Introduction

Subterranean termites (*Coptotermes*) are major destructive pests to building and structures (Su, 1994; Kirton and Brown, 2003; Lee 2007; Nguyen et al. 2016). Baiting is an effective method for the control of these species that has been studied for more than 20 years (Buczkowski, 2014). In Vietnam, BDM10 bait was investigated to measure the success in controlling *Coptotermes* in 2010 (Trinh et al., 2014).

BDM10 bait contains two components that is a bait matrix (bagasse) and active ingredient (chitin synthesis inhibitor) (Tran et al, 2011). The hard bait is in a form with the size 50 x 10 x 2 mm. The form of this bait is suitable for applying into cracks, gaps, wood blocks, etc. The quantity of bait needed to treat one termite colony (*Coptotermes*) is from 200 g to 400 g with control achieved in 30 to 40 days (Tran et al. 2011). However, BDM10 was subject to becoming moldy due to its components, which affected the baiting process when a big quantity of bait was installed and not consumed over a long time frame. In addition, there was difficulty in the manufacturing process of this bait. Therefore, we carried out to improve the BDM10 bait. In this paper, we present the results of our work with Mobahex – C16 in the laboratory and field trial.

Materials and Methods

Materials

- Termites (*Coptotermes*) were collected from a field colony from infested structures in Hanoi. Termites were kept under laboratory conditions for 24-hours before carrying out experiment at the Institute of Ecology and Works Protection. Field trials were carried out in 13 infested structures in Hanoi.
- Mobahex-C16 bait comprises: A fungus culture medium powder, bagasses powder, LG bark powder, sugar and hexafluamuron (0.75%). Characteristics of Mobahex – C16 bait are shown in Figure 1 (bar form with size of 100 x 50 x 2.5 mm and lines divided in 1 surface).

Figure 1. Form of Mobahex-C16 bait (two surfaces of bait)

Methods

Laboratory experiment on eliminate of termites by Mobahex – C16

- 300 *Coptotermes* workers, maintained in a petri dish with moist filter paper under laboratory conditions for 24 hours, were released to plastic containers (58 x 43 x 50mm) containing 50g sterile sand and 15% moisture. Baits were cut into small pieces (0,3g) and added in each plastic container with termites. Mobahex -C16 bait, BDM10 bait and a control (pine wood) were tested under laboratory conditions with a mean temperature of 27^{0}C (±2^{0}C). The number of dead termites was recorded after 1, 3, 5, 7, 9, 11, 13, 15 and 17 days. Three replicates were performed for the experiment.

- Abbott's formula was used to evaluate bait effectiveness and SPSS 16 used to determine LT_{50} values.

Field trail

A wood box (pine wood) monitoring station was used to lure termites. Two bars of wood (200 x 30 x 15 mm) were weighted and added in the wood monitor. Monitoring stations were checked 2 times/weeks. Bars of wood was taken out after 1 month after termites were first observed in a monitoring stations and cleaned and dried before weighing.

Termite activity index (TAI 1) was calculated using the following formula:

$$\text{TAI 1} = \frac{\text{Initial weight of wood – weight of wood after week } 4^{th}}{\text{Initial weight of wood bars}} \times 100\%$$

Bait evaluation in each station was take placed after determining of termite activity. The stations were checked 2 times/weeks. Bait was replenished if necessary and based on the amount of bait remaining until termites stopped eating or dead termites or a large proportion of soldiers were observed.

New weighed wood bars were added and the station checked 2 times/week for 1 additional month. The activity of termites inside the stations were observed (yes/no). Wood was taken out and weighted after drying and cleaning. We calculated the activity index of termites (TAI 2) similar as formula 1.

The effectiveness of termite infestations eliminated was calculated using the formula 2:

$$E\ (\%) = \frac{(1 - \text{TAI } 2)}{\text{TAI } 1} \times 100\%$$

in which E is effectiveness of bait (calculated by %)

Results and Discussion

The efficacy of baits under laboratory conditions

Efficacy of improved Mobahex-C16 and BDM10 bait under laboratory in Table 1.

Table 1. Effectiveness of Mobahex-C16 and BDM10 bait in the laboratory

Checked time (day)	**Efficacy (%)**		**Rate of termite dead in control (%)**
	Mobahex-C16	**BDM10**	
1	0,6 ± 0,4	1,3 ±0,9	0
3	6,7 ±0,4	5,3 ±2,3	0,6
5	17,2 ±2,1	19,0 ±1,6	1,6
7	27,6 ±0,9	28,8 ±2,8	1,6
9	53,4 ±0,7	58,8 ±4,3	2,3
11	74,7 ±2,4	73,6 ±4,8	3,0
13	87,3 ±2,1	89,1 ±2,9	5,6
15	97,7 ±0,5	98,0 ±1,0	9,3
17	100	100	12,3

The effectiveness of both Mobahex -C16 and BDM10 bait slowly increased during the baiting process and reached 100% at Day 17 (Table 1). The results showed that normal activity of termites was found at early stages of baiting. However, termite activity slowed by the 7th and 9th day, was significantly weaker after the 11th and 13th day and no alive termites were found by Day 15 while the control termites showed normal activity.

The resulting test showed that the LT_{50} values for both Mobahex-C16 and BDM10 bait were not significantly different at 198 hours and 203 hours, respectively (Table 2).

Table 2. Lethal time 50% of each bait at 0.3 g/300 termites

Type of bait	**LT 50% (hours)**
BDM10	203 (179 – 226)
Mobahex-C16	198 (175 – 231)

However, molding was observed in BDM10 after 3 days in bioassay while it took 16 days with Mobahex-C16. Mobahex-C16 bait also had less mold than BDM10 at 13 days, which can reduce the time required for bait replenishment and minimize the treatment duration.

3.2. Field trail result

The field trial results showed that no termite activity was found in 13 (100%) structures treated with Mobahex – C16 (table 3). However, the treatment duration, as well as, the amount of bait were different in each construction, which may be due to of colony size and colony activity area (Table 4).

Table 3. Termite activity index in each of structure

Number	Structure	TAI 1(%)	TAI 2 (%)	Effectiveness (%)
1	CT1	50,0	0	100
2	CT2	53,8	0	100
3	CT3	57,1	0	100
4	CT4	52,9	0	100
5	CT5	46,1	0	100
6	CT6	46,6	0	100
7	CT7	56,2	0	100
8	CT8	33,3	0	100
9	CT9	57,1	0	100
10	CT10	42,8	0	100
11	CT11	64,2	0	100
12	CT12	66,6	0	100
13	CT13	52,3		
Average		**52,2**	**0**	**100**

Table 4. Amount of bait exploited and duration before no termite actitivy in each structure

Number	Structure	Quantity of Mobahex-C16 (g)				Date of no termite activity
		1st	2nd	Sum	Consumed amount	
1	CT1	314		314	288	49
2	CT2	360		360	336	49
3	CT3	146		146	104,9	35
4	CT4	120		120	98,6	31
5	CT5	193		193	160	45
6	CT6	248	173	421	386,4	29
7	CT7	250	70	320	299,7	42
8	CT8	227		227	192	44
9	CT9	188	98	286	267,3	30
10	CT10	118		118	98,4	28
11	CT11	217		217	197	38
12	CT12	209		230	209	42
13	CT13	316		316	300	52

The average number of days until termite eliminate was 39 days with the shortest at 28 days (CT 10) and the longest 52 days (CT 13).

The Table 4 shows that the amount of Mobahex C16 bait used at each site was from 118g to 421g and the duration to elimination was 28 to 52 days. The amount of Mobahex – C16 bait used ranged from 98,4g - 386,4g (226g/structure) before colony elimination (no alive termites or only a small number of soldiers) was observed. Termites actively exploited bait after 7 to 14 days after addition of bait to a station. Only 3/13 structures needed to have bait replenished a second time. In additional to the inspection of activity of termites and bait replenishment, we also assessed the molding of bait. Molding was found in 3 out of 13 structures on left over bait (CT6; CT7; CT11). The earliest appearance of mold found was between the 21th to 28th days (CT6) and the lastest between the 28th to 35th day (CT7) .

In comparison with the efficacy of BDM10 (Tran et al. 2011), the consumption bait by termites was the same. However, Mobahex –C16 reduced of molding and reduced the number of times that bait had to be replenished which reduced termite treatment times. In addition, the manufacture procedure is easier and offers a cost reduction with the new bait form.

Conclusion

- The eliminate effectiveness of the bait Mobahex – C16 was about 100% whithin 17 days. The lethal time for 50% mortality with Mobahex – C16 at 0.3g/300 termites was 198 hours (range from 175-231 hours).
- 100% of the field trials reported no return of termites. The average bait used was 226 g/structure. The average of treatment was 39 days.

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Field Efficacy of Termidor® HE High-Efficiency Termiticide

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Abstract

A total of 166 termite-infested structures were treated with Termidor® HE High-Efficiency termiticide using the Exterior Perimeter/Limited Interior application method described in the Termidor label. Compared to traditional liquid treatments, Termidor HE was applied using half the amount of water per meter (2.5 l/m instead of 5 l/m), smaller trenches (5 x 10 cm instead of 15 x 15 cm), wider rod spacing (45 cm instead of 30 cm), and half the maximum rodding depth for deep foundation footers (0.6 m instead of 1.2 m). Treated structures were inspected at 1-, 3-, 6-, 12-, 18-, 24-, 36-, 48-, and 60-month intervals. Fewer than 3% of the sites had termite activity after one month and no sites had activity after 18 months. Reduced labor involved in Termidor HE applications result in approximately a 57% time savings for an average sized home when compared to a conventional SC treatment. Bioassays were also conducted using field-collected soil from the treated zones around a subset of 30 structures at 12 and 24 months. Exposure of *Reticulitermes flavipes* workers to the treated soil resulted in 100% mortality within 24 hours in the lab.

Key words: soil treatment, structural protection, fipronil, **T**ermidor, subterranean termite

Introduction

Fipronil has proven itself to be an extremely effective insecticide and is the active ingredient in multiple termiticides, including the most prevalent termite control product in the US – Termidor® SC termiticide/insecticide (Curl, 2017). Because of its nonrepellent properties at toxic doses (Ibrahim, et. al., 2002) and ability to be transferred among colony members (Bagnères, et. al., 2009), BASF has formulated fipronil into a variety of products, including a soluble granule (Termidor WG), a dry powder (Termidor® DRY), and a ready-to-use foam (Termidor® FOAM termiticide/insecticide), the latter two of which are specialized products used to target localized termite infestations using spot treatments.

As a liquid formulation, Termidor® SC termiticide/insecticide (Termidor SC) has been on the market in the US for more than a decade and has proven itself to be an extremely efficacious product that controls termites, protects structures, and greatly reduces callbacks (Potter and Hillery, 2000). Because of the success of the product, BASF sought to enhance both the chemistry of the termiticide and the application process. Fipronil binds readily to soil, and therefore a significant amount ends up bound in the upper few centimeters of soil when using conventional liquid formulations. The Termidor® HE High-Efficiency Termiticide (Termidor HE) formulation enables more of the active ingredient to pass through the upper few centimeters and distribute more evenly within the targeted treatment zone (approximately 18 cm – the region of soil where termites are most likely to be active) relative to Termidor SC and other liquid fipronil-based termiticides. The Termidor HE formulation requires relatively less water to achieve distribution throughout the target zone (2.5 l/m finished dilution instead of 5 l/m). Reduced volume allows a Pest Management Professional/Termite Control Operator (PMP/TCO) to spend significantly less time preparing for and making the application. In addition, the traditional trench created around a structure (15 x 15 cm) can be reduced to 5 x 10 cm, the rodding spacing can be increased from 30 to 45 cm without losing treatment continuity, and the maximum rodding depth for treatments – even for structures with basements – can be reduced from 1.2 to 0.6 m. Moving less soil, drilling and patching fewer holes, and spending less time applying material all contribute to a significant time savings, allowing the PMP/TCO to complete more applications or devote the extra time to other tasks. Using less water also has environmental implications and though fipronil is not necessarily at high risk for leaching when applied properly, using less water to apply it reduces the risk even further.

Materials and methods

To test the efficacy of Termidor HE, BASF worked with the US Environmental Protection Agency (US EPA) to develop a protocol that would demonstrate its ability to control termites and provide structural protection. The protocol dictated that structures had to have termite activity (one of the primary subterranean termite genera – *Coptotermes*, *Heterotermes*, or *Reticulitermes*) at the time of treatment (i.e., curative treatments as opposed to preventative treatments). A variety of foundation types (e.g., slab, crawl space, basement) were represented, as were a variety of EPA Regions, which assured variation in soil types and climactic conditions. All structures were to treated utilizing the differences in trench size, rod/drill spacing, and application depth according to the Exterior Perimeter/Localized Interior section of the proposed Termidor HE label, which involves treating the entire exterior perimeter and any areas within the structure where termites are found. Structures were inspected initially and at three, six, twelve, eighteen, twenty-four, thirty-six, forty-eight, and sixty months. All inspections were full exterior and interior inspections that utilized at least some aspect of advanced technology (moisture meter, acoustic emission detector, etc.). All applications were performed by a licensed PMP/TCO using traditional termiticide application equipment (mixing tank, hose, t-handle & rod, etc.) that was calibrated before every treatment. Structural inspections and data collection were performed by termite experts (e.g., university faculty, experienced urban entomologist, research contractors with experience in termite control), who, along with at least one BASF representative, were also present for the application. Data collected included application details such as structural features, location(s) of infestations and treatments, conducive conditions, and volumes/amounts of product applied. Diagrams for each structure were also created with location(s) of infestations and treatments designated. Time intervals for applications were recorded for Termidor HE treatments to compare to times collected from conventional treatments.

In addition to the initial applications and structural inspections, soil samples from the treated zones were collected at twelve months and twenty-four months for efficacy bioassays in the laboratory, where field-collected termites were exposed to field-aged, treated soil. Core samples were taken from each side of a subset (thirty) of the treated structures. The samples were kept frozen until the bioassay was performed, at which time the top 7.5 cm layer was ground/fractured and homogenized. Approximately 2.5 g of the homogenized soil was then added to a 60 mm Petri dish lined with agar as a moisture source and supplied with a piece of filter paper as a food source. Fifteen *Reticulitermes flavipes* workers (third instar or larger) were introduced to the soil in the dish and were observed for mortality at one and two days after introduction. Due to the small amounts of soil, three replicates were performed for each treatment. Untreated sandy soil (NC Dark: 90% sand, 6% silt, 4% clay) was used for the control.

Results and discussion

A total of one hundred sixty-six active structures in seven different EPA Regions (nineteen states) were treated using Termidor HE during 2009-2011 (Table 1). *Reticulitermes* was the most prevalent termite genus and was encountered at 74% of the sites, followed by *Heterotermes* at 15% and *Coptotermes* at 11% (Figure 1). The set of treated structures included all the major foundation types, but primarily monolithic, floating, and supported slabs, crawl spaces, and basements (Table 2).

No termite activity was found at 97.6% of the structures one month after treatment (Figure 2). Termite activity was detected at one structure in Hawaii six months after the initial application. This activity was in an area that had not been previously treated – there had been no signs of activity in that area at the time of application and since it was an interior location, there was therefore no reason to treat it. The area was subsequently treated and no termites were found during any later inspections. Activity was detected in one structure one year after treatment. A site in MS had a moisture issue that was addressed and a foam and liquid Termidor HE treatment was made to the area. No termites were found during subsequent inspections, and no termite activity was detected at any site at the eighteen-month, or the two-, three-, four-, or five-year inspections.

Bioassays using the one-year-old and two-year-old samples of treated soil resulted in 100% mortality for all samples and all replicates after 24 hours (Table 3).

PMPs/TCOs saved significant time during both the trenching and product application stages of Termidor HE applications (59% and 65%, respectively). The estimated time saved on an average-sized home in the U.S. is approximately one hour seventeen minutes, meaning that a treatment using Termidor HE is 57% faster than a treatment using Termidor SC or other termiticide applied in a similar method (Table 4).

Table 1. EPA Regions where treatments took place

EPA Region (States)	**Number of structures**
Region 2 (NJ)	8
Region 3 (MD, PA)	3
Region 4 (FL, KY, MS, NC, SC, TN)	42
Region 5 (OH, IN)	8
Region 6 (LA, OK, TX)	48
Region 7 (KS, MO)	14
Region 9 (AZ, CA, HI)	43
Total	166

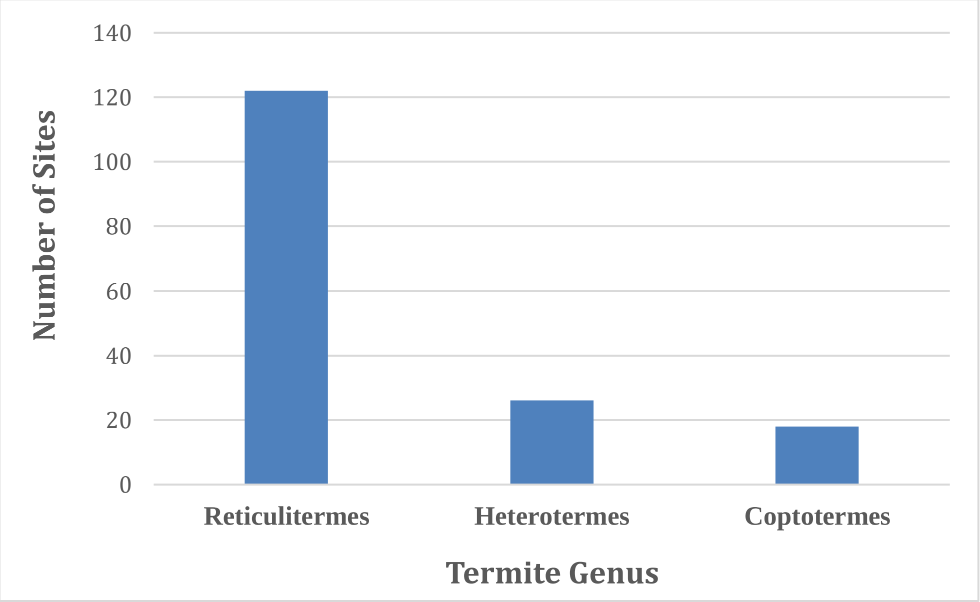

Figure 1. Number of sites where each termite genus was found.

Table 2. Foundation types of structures treated with Termidor HE

Foundation type	**Number of structures**
Monolithic Slab	48

Basement	40
Floating Slab	36
Crawl Space	33
Supported slab	9
Total	166

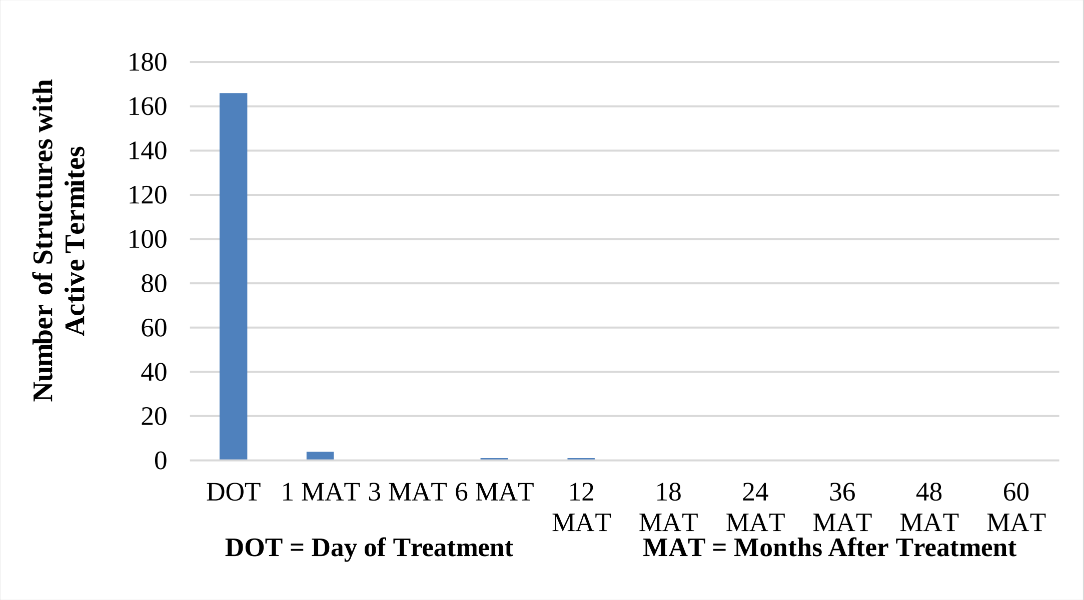

Figure 2. Number of structures with termite activity at each inspection interval after the structure was treated with Termidor HE.

Table 3. Bioactivity of field-collected Termidor HE against *Reticulitermes flavipes* via soil exposure assay.

Collection interval	% mortality at 24 h
1 year after treatment	100
Control	0
2 years after treatment	100
Control	0

Table 4. Calculated times each major element of a termiticide treatment requires for a conventional (Termidor SC) treatment compared to a Termidor HE treatment.

Treatment	Trenching	Treating	Total	Total hours for an average (61 m) home
Termidor SC	13.7	27.5	41.2	2:17
Termidor HE	5.7	12.2	17.9	0:59
Difference	8.0	15.3	23.3	1:17

Conclusions

Termidor HE successfully controlled termites and protected the structures involved in this study for five years. No structures had activity after one year, and the few structures with activity after one month had specific circumstances that allowed termites to persist (moisture issues, previously undetected and untreated activity). Once these conditions were addressed (with a supplemental treatment, if necessary), termites were not detected again. These results indicate the following: a 45 cm drill spacing is sufficient for applying Termidor HE, a trench of 5 x 10 cm is acceptable for Termidor HE applications of 2.5 l/m, rodding to 0.6 m instead of 1.2 m still delivers Termidor HE to the area where termites are most active in sufficient quantities that the structure is protected. Furthermore, the bioassay illustrates that even the soil with the highest risk of breakdown of the active ingredient (the upper 7.5 cm) showed that two years after application the treatment was efficacious against termites. Finally, Termidor HE treatments save PMPs/TCOs valuable time and resources when compared to traditional termiticide applications.

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Poster Session

Termite Fauna in Semi-natural Landscapes of the Sundaic Region and Papua, Indonesia: Preliminary Survey Around Sumatra, West Java Regions and Asmat Regency

by

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Abstract

Termite diversity is an interesting topic, especially in archipelagic countries sharing different geographical origins such as Indonesia. Apart from the tropical forest on Java Island, other islands in Indonesia have many natural areas yet to be explored in regards to termite diversity. Many natural areas have been converted into urban landscapes yet semi-natural landscapes, defined as an area modified by anthropogenic activities in which many natural features are still preserved were sampled for this study. The areas included two botanical gardens in West Java, one botanical garden in Batam Island and two adjacent islands, Karimun Besar and Karimun Kecil located in the Sundaic Region as well as outside the Sundaic Region from Asmat Regency on Papua. A standardized sampling protocol was used with a belt transect of 100 x 2-m (3-4 transects per location). Casual collecting was done in Simeulue, both Karimun islands, and the Asmat Regency. Termite samples were taken from all possible microhabitats. These collections provided 3 families, 6 subfamilies and 22 genera from all the locations. Three groups were constructed based on the biogeographic background. The first biogeographic group, Sumatra (Batam, both Karimun and Simeulue Islands) was dominated by members of the Nasutitermitinae (44.21%) followed by the Termitinae (23.14%), Rhinotermitinae (10.33%), other subfamilies namely Coptotermitinae, Macrotermitinae, Prorhinotermitinae and a family Kalotermitidae (<10% respectively). The second biogeographic group included 7 districts in the Asmat Regency of Papua, where we collected mostly members of the Nasutitermitinae (63.33%) followed by Coptotermitinae, Termitinae and Rhinotermitinae (15.00%, 15.00% and 6.67%, respectively). The West Java group (Kuningan and Baturaden) largely consisted of Termitinae (34.42%) followed by members of the Macrotermitinae, Rhinotermitinae and Nasutitermitinae (25.13%, 21.86%, 18.59%, respectively).

Keywords: *termite, semi-natural landscape, Botanical Garden, Sundaic Region, Papua*

Identification and Development of Polymorphic Microsatellite Markers from the Invasive Termite, *Coptotermes curvignathus* in Malaysian Oil Palm Plantations

by
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Abstract

The subterranean termite, *Coptotermes curvignathus* is an invasive pest species native to Southeast Asia, which often causes structural damage in the built environment. *C. curvignathus* is among the few termite species that prefers to feed on living plant tissues making it an important pest in agriculture. This xylophagous species is now considered a major pest of oil palm plantations in Malaysia and Indonesia due to their ability to thrive in peat soil environment, leading to palm death and hence severe economic losses. In the current study, we isolated and characterize novel polymorphic microsatellite markers from the genome of *C. curvignathus* collected from oil palm plantations in order to understand their population genetic structure and breeding strategies to further enhance our knowledge on the infestation dynamics of this pest. A modest volume of 454 next generation pyrosequencing generated 47,462 sequence reads whereby 1,996 (4.2%) contained microsatellites with di-, tri- and tetra- nucleotide repeat motifs. Sixty primer pairs were randomly selected for a preliminary test of polymorphism across five individual *C. curvignathus* specimens collected from several geographically distinct oil palm estates in Sarawak, Malaysia. Ten of the 30 primer sets were found to be polymorphic with 4-15 alleles per locus and these were subsequently assigned to four multiplex groups for future population genetic studies. Observed and expected heterozygosities ranged between 0.19 to 0.86 and 0.44 to 0.92, respectively. No linkage disequilibrium was found between any pair of loci and all loci did not deviate from Hardy-Weinberg equlibrium. The high degree of polymorphism among these 10 microsatellite loci will be useful as a tool to investigate colony and population genetic structure of *C. curvignathus*.

Molecular Detection of Ectoparasitic fungi *Laboulbeniopsis termitarius* Thaxt and *Antennopsis gallica* Buchli and Heim on Termite Colonies

by
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Abstract

The ectoparasitic fungi *Laboulbeniopsis termitarius* Thaxt and *Antennopsis gallica* Buchli and Heim are two of the most commonly found ectoparasitic fungi on the body surface of termites. The two fungi have a unique shape, small thallus size, and it can be found all around the world with a variety of termite species as their hosts. Visual observation under a dissecting microscope is a common method for screening the fungi, generally requires observation of hundreds of termites and is thus very time consuming. DNA-based methods are also commonly used to detect fungal infection. In this study, a fast and efficient assay was developed to detect fungal infection in a termite colony. A polymerase chain reaction (PCR) was conducted with species-specific primers, based on 18s rRNA sequence data from the two fungi. We then developed a multiplex nested PCR assay using those species-specific primers to detect these fungi in a robust yet economic manner. Results suggested that both fungi could be successfully detected, even in cases where *L. termitarius* was at low titer (e.g., a single thallus per termite). The new assay is recommended for future surveys of the two fungi, as the method is more sensitive, species-specific, and faster than visual observation, and could facilitate better understanding of the fungi and their dynamics in host populations.

Key words: *multiplex nested–pcr, nested–pcr, termite, ectoparasitic fungi*

Microcerotermes sp. (Termitidae) attack on *Eucalyptus pellita* trees in alimantan Timur, Indonesia

by
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Abstract

Termites are wood-feeding insects that can be severe pests in urban and rural environments damaging wooden materials or even living trees. Species from the genus *Coptotermes* are commonly found attacking living trees and wooden products in both environments. In the other hand, *Microcerotermes* are known as wooden products pests in Southeast Asia but there are only few reports associating this genus with forest plantations, especially *Eucalyptus*. Thus, the aim of this study was to report the occurrence of *Microcerotermes* sp. attacking commercial plantations of *Eucalyptus pellita* in Kalimantan Timur, Indonesia. *Eucalyptus pellita* trees showing signs of termite attack were assessed for the presence and activity of these insects. Additionally, soldiers were collected for species determination. Incidence assessment was conducted by using the transect method in three compartments where termites were found. Attacked trees were 15 months old and showed symptoms of dried leaves, termite galleries and holes in the bark. Termites identified as *Microcerotermes* sp. (Termitidae: Termitinae) was found, on average, in 14.9% of the trees with a range from 9.3 to 24% between compartments. It was observed that compartments with higher incidence of termites had more wood debris from previous rotations. In contrast, cleaner compartments had lower incidence of this pest attack. Termites were found nesting in the wood debris and availability of debris in compartments likely influenced the incidence of these pests. Further experiments must be carried out to confirm this assumption as well as provide more information on the biology of *Microcerotermes* species. The information generated in the present study increases the knowledge on termites associated with forest plantations in Southeast Asia and provides better guidance on *Microcerotermes* prevention.

Keywords: *forest entomology, integrated pest management, monitoring, sampling*

A Review of Termite Management Efforts: Performance of Termite Control Company in Bandung City

by
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Abstract

Development and implementation of environmentally friendly termite control programs has increased due to public concern on environment health. The purpose of this study was to conduct a review of termite control efforts in Bandung City. Questionnaires were sent to registered pest control companies in Bandung in order to understand the level of termite control services conducted in Bandung. There were 17 pest control companies officially registered in DPD ASPPHAMI West Java in 2014 and about 10 companies offer termite control services. The service of antitermite contributes about 25% of the total revenue with the average number of jobs per year reaching 99.6 unit-jobs per company. The potential antitermite jobs in Bandung City reach only approximately 1/3 of the estimated number of termite-infested buildings in Bandung City in 2012 (1% of all buildings). The estimated of value of antitermite jobs reached Rp13.5 billion in 2012, and is projected to increase to Rp45 billion in 2017, Rp99 billion in 2022, and Rp180 billion in 2027.

Key words: *performance, termite control company, bandung city, ASPPHAMI*