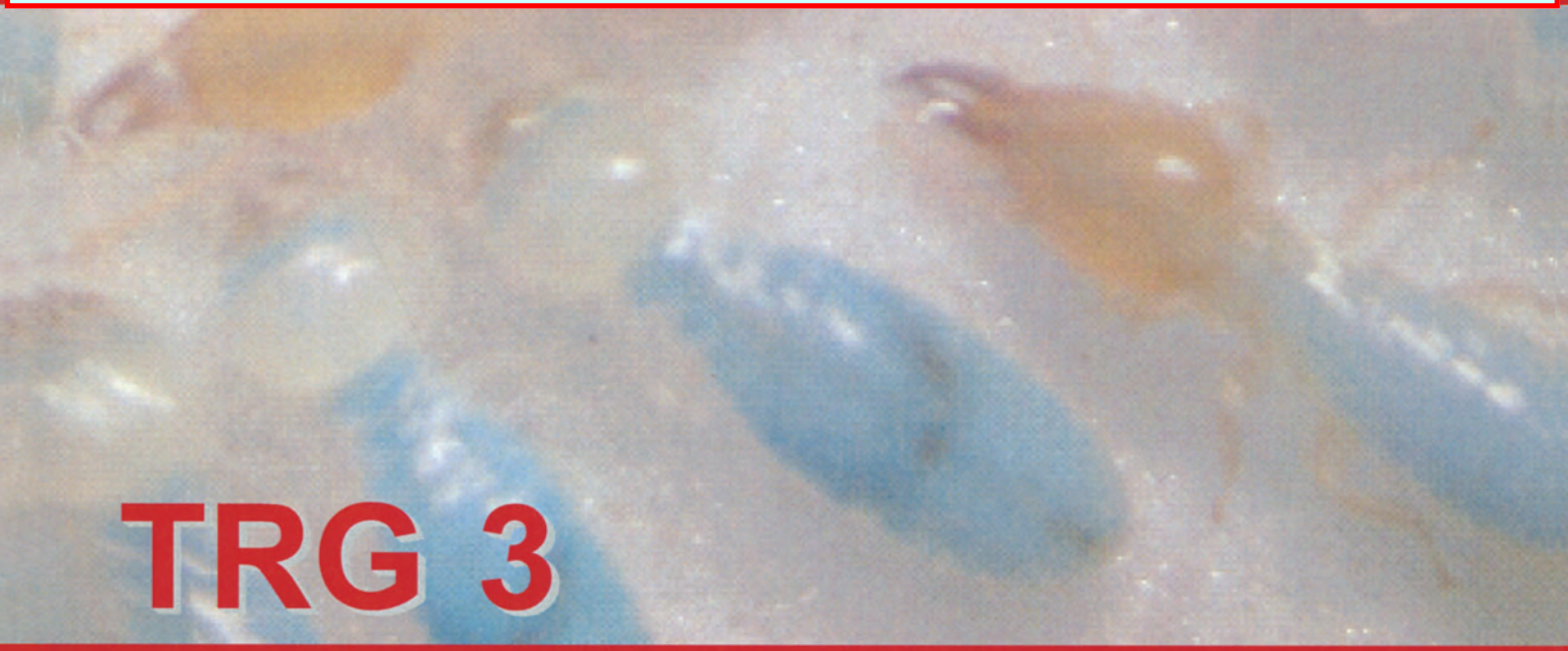

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Contents

Termite Damage and Detection: an American Perspective

by

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Abstract

Worldwide, there are more than 2,300 different species of termites now recognized. Much of this diversity can be lumped into four distinct ecological groups that include dampwood, drywood, subterranean, and arboreal/mound builders. Fortunately, less than 15% of this diversity poses any threat to landscapes managed by humans that include urban, agriculture, forest plantations, and wild lands. Global estimates of damage from termites are difficult to find in the published literature, but certainly are in the billions of dollars (US$) each year. However, their benefits to the world's ecosystems far exceed the damage they cause. In this paper I will review important termite pest groups by continent. I will also review methodologies and strategies currently used for termite detection and some new innovations planned for in the near future.

Key words: termites, Isoptera, detection, inspection, damage, global

Introduction

Americas. Termite diversity in North America (NA) is low, less than 50 species are currently recognized. For many hundreds of years, termite diversity in NA fell into three distinct ecological groups: dampwood, drywood, and subterranean. However, recently an arboreal species was recently introduced into Florida (Scheffrahn et al. 2002). Dampwood termites (genus *Zootermopsis*, Family Termopsidae) are very restricted in NA, confined primarily to the coniferous forests of the Pacific States and desert southwest. Drywood termites (important genera include *Incisitermes, Neotermes*, Family Kalotermitidae) occupy a narrow band from costal Northern California, through Arizona and New Mexico, including costal areas of five Gulf States, and finally costal areas for several Southeastern Atlantic States. In nature, they prefer hardwood forests and scrubs at elevations < 1,500 m. Subterranean termites (important genera include *Reticulitermes, Heterotermes*, *Amitermes*, and introduced species of *Coptotermes*, Family Rhinotermitidae) are the most diverse and widespread termites in NA. There are > 24 species and they occur from below sea level to ~ 3,000 m. These termites nest below ground and have large (tens of thousands or more) and diffuse colonies. In general, all termite species in NA prefers dead or decaying wood, rarely are tissues of living plants attacked. Estimates of damage attributed by termites to structures in NA exceed several billion (US$) annually (Su and Scheffrahn 2002).

In South America (SA), at least 400 termite species are recognized and many more yet to be described. Mound species and arboreal species are common in SA. Termites in SA occupy many ecological zones and habitats. Important termite genera include *Cryptotermes* and *Neotermes* (Family Kalotermitidae), *Coptotermes* and *Heterotermes* (Family Rhinotermitidae), and *Nasutitermes* (Family Termitidae). Not much has been reported estimating the damage caused to habitats by termites from SA. Unfortunately, not much is known on termite biology and ecology for the Caribbean. Similarly, for Central America and Mexico much of the termite diversity and ecology is poorly known.

European continent. Termite diversity in Europe is the least compared to other populated continents (Eggleton 2000). Fewer than 10 species naturally occur. The most important genus is *Reticulitermes*. This genus currently contains five species. Although native species of *Reticulitermes* exist in Europe, there are still questions about their origins and relatedness with species from eastern coastline of NA. In parts of France *Reticultermes* kills living trees, an unusual occurrence in NA. Damage to structures by termites is increasing in some European countries; enough so that France has mandated buildings inspections prior to sell. I also saw a severe

drywood termite infestation (*C. brevis*) in a multi-unit living building while in Rome, Italy. Estimates of damage and costs exceed 500 million (Euros) annually (Clément 2000).

Africa. Termite diversity in Africa reflects this continent's topological and climatological diversity. Termite diversity is tremendous, more than 1,000 of the > 2,300 recognized species occur on the African continent. Mound species of termites occur throughout most of the African landscape. Termite diversity for northern Africa is low, about 11 species, represented by subterranean and drywood termite groups. The important genera are *Anacanthotermes* (Family Hodotermitidae), *Psammotermes* and *Reticulitermes* (Family Rhinotermitidae), *Amitermes,* and *Microcerotermes* (Family Termitidae), and several species of Kalotermitidae. Termite diversity is great in eastern Africa, especially among the abundant Macrotermitidae. The important genera include *Macrotermes* (Family Termitidae), *Hodotermes* (Family Hodotermitidae), and *Schedorhinotermes* (Family Rhinotermitidae). Termite diversity in western Africa is similar to eastern Africa; mound species dominate the landscape, although subterranean and drywood species also occur. Important genera include *Ancistrotermes, Macrotermes, Odontotermes, Microtermes,* and *Cubitermes* (Termitidae). Termites have been transported over much of Africa over the millennia due to commerce and nomadic migrations. Little information is available on the tropical forests of central Africa and savanna and deserts of southern Africa. These areas also contain much termite diversity and ecology. Termite ecological groups for many African habitats include straw feeders, structure infesting, and agricultural pests; although monetary estimates of damage and crop loss are underreported.

Asia. Termite diversity in China is especially great, with more than 435 species described. Most termite ecological groups, subterranean, drywood, harvester, and mound builders, are found in China. Common and important genera include *Coptotermes, Reticulitermes* (Family Rhinotermitidae), *Macrotermes* and *Odontotermes* (Termitidae), as well as members of the *Cryptotermes* (Kalotermitidae) and Hodotermitidae. Termite species occur in many environmental habitats throughout the provinces of China; natural, forests, agricultural, dikes, and urban. There is much termite diversity and ecology yet to be discovered in China. During my travels through Asia that have included Japan, China, Singapore, and Pakistan, I have seen many examples of damage to structures by termites. Estimates of damage and crop loss caused by termites are few for most of Asia.

Australia. More than 360 species of termites have been described from Australia. All termite ecological groups (dampwood, subterranean, drywood, harvester, and mound builders) are represented in the Australian region. However, the Australian termite fauna is most known for its relict, primitive genera *Mastotermes, Porotemes,* and *Stolotemes*. In depth understanding of the biology and ecology of termites is restricted to 5 to 15% of the described species. Structural and agricultural pest species occur in Australia and severity is highly dependent on locality. Estimates of damage to structures and crops exceed 100 million (Aus$) annually (Lenz 2000).

Detection

There are at least seven detection devices and methods proposed as alternatives to visual searches. They include optical borescopes, dogs, electronic odor detectors, microwaves, acoustic emission devices, infrared, and X-ray. They all claim high levels of successful detection of termites; however, few have been scientifically tested (Lewis 1997, Lewis 2003a, Lewis et al. 2004, 2005).

Optical borescopes use visible light passing through a hollow tube as a means to view termites and damage hidden away behind walls. A small hole must be drilled into walls to allow viewing. Fire blocking, insulation, and viewing through a fish-eye lens may impede the inspector's view. Optical borescopes are currently marketed; however, their efficiency in the detection of termites has yet to be scientifically tested.

Canines. Several breeds of dogs have been trained, beagles being the mostly frequently used breed (Lewis et al. 1997). The mode-of-action used by dogs in finding termites, audition, olfaction, or both still needs further research (Lewis et al. 1997). For California, the effectiveness of beagles was mixed but only included subterranean termites. Drywood termites were not included in the laboratory investigations (Lewis et al. 1997). However, drywood termites were included in laboratory trials conducted in Florida and the success rate for dogs (beagle and German

shepherd) in identifying plastic containers containing drywood termites (*Cryptotermes cavifrons* Banks and *Incisitermes snyderi* (Light)) was 88.8% (Brooks 2001). False positives, canines response to containers without drywood termites was < 1% (Brooks 2001). Currently, there are few commercial firms that train and provide dogs to assist with termite inspections.

Odor detection. Electronic odor detectors are another method of detection of termites. Their mode-of-action includes detecting methane gas, commonly produced by termites (Lewis et al. 1997). One device (Termitect II) was tested on subterranean termites and produce highly variable detection rates, 20 to 100% (Lewis et al. 1997). There have been no reports on the use of electronic odor detectors in successfully identifying drywood termites.

Acoustic emissions. Termites produce vibrations in wood while feeding and by alarm calls from the head banging of soldiers. These sounds are produced during feeding (Matsuoka et al. 1996) and by vibratory movements of workers (Leis et al. 1992, Maistrello and Sbrenna 1996). The earliest commercial audible listening device for termite feeding was an INSECTA-SCOPE. However, no data are available on its performance. Newer technology that amplifies and records termite-feeding vibrations is acoustic emission (AE). Surface and subsurface probes are available and successfully detection of termites in laboratory settings is at least 80% (Fujii et al. 1989, Fujii et al. 1990, Scheffrahn et al. 1993, 1997, Lewis and Haverty 1996, Lewis et al. 2004, 2005). Wall covering can impede sensor and AE performance. Distance in detection is limited to ≈80 cm along the length of a board and < 8 cm across the grain (Scheffrahn et al. 1993). Excessive background noise can also result in false positive results for active termites. AE detection equipment is commercially available now on several continents, although availability locally is very limited.

Microwaves. Recently, portable microwave detection devices have been marketed in North America and Australia (Lewis 1997, Evans 2002, Peters and Creffield 2002). Published papers using microwaves have been reported for several species of termites (Peters and Creffield 2002, Evans 2002). Success in detection of drywood termites (*Cryptotermes brevis* (Walker)) using microwaves (TERM_A_TRAC™) was 86% based on laboratory studies (Peters and Creffield 2002). Detection distance was 35 mm along the long axis of test boards and 25 mm deep below the surface. However, water in wood, wall coverings, and excessive wind and motion can lead to false positives results for live termites.

Infrared. This portion of the electromagnetic spectrum is nearest red in the visible range. Although invisible to eye, infrared energy has a penetrating heating effect and is easily felt when encountered. Most objects, living or not, give off infrared heat, whether internally generated or reflected. There are many uses for infrared and include measurement devices, binoculars, night viewing for hunting, etc. In structures, infrared devices have been used to find faulty electrical connections and heat and water leaks in walls and roofs (Tobiasson 1994, Maldague and Moore 2001). A more recent use includes termite detection (Lewis 2003a). Commercial termite detection models are available. Some testimonials and demonstrations on the termite detection ability of infrared exist (Anon. 2000); however, the effectiveness in finding termite infestations has not been scientifically tested.

X-ray. These penetrating rays are part of the electromagnetic spectrum that is nearest ultraviolet rays. There are many commercial applications for X-rays and include dental, medical, military, security, and nondestructive evaluation of materials (Martz et al. 2002). These penetrating rays have also been used to nondestructively view insects in hidden locations (Fisher and Tasker 1939; Berryman and Stark 1962a,b; Berryman 1964; Davies et al. 1988; Kim and Schatzki 2001), and internal structures of insects (Westneat et al. 2003). For viewing insects hidden in wood, X-rays have been used for at least seven decades (Fischer and Tasker 1939, Kim and Schatzki 2001). X-rays have also been used to view several structural infesting beetle pests (Fisher and Tasker 1939, Suomi and Akre 1992). Only recently has the potential use of X-ray in detecting drywood termite infestations been explored (Lewis et al. 2005).

Concluding remarks. Because of proceedings paper format, I will not be reviewing control/management for termites. The topic is too vast for the space allotted. For more comprehensive regional and global reviews on termite management, see Su and Scheffrahn 2000; Pearce 1997; and Lewis 2003b. Excellent reviews on termite diversity and management are also contained in a United Nations website at http://www.chem.unep.ch/pops/termites/termite_toc.htm.

Lastly, recent excellent reviews on the positive contributions of termites to global ecologies of soils, air, carbon cycles, as well as invertebrates and vertebrates can be found Abe et al. 2000 and the United Nations Environmental Program (UNEP) and Food and Agriculture Organization (FAO) at http://www.chem.unep.ch/pops/newlayout/repdocs.html.

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Termites 'Down Under' – Comments on Termite Fauna and Current Areas of Termite Research in Australia

By

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The described Australian termite fauna comprises about 370 species and the level of generic endemism is relatively low. The most distinctive feature of the fauna is the presence of relict primitive genera in the Mastotermitidae and Termopsidae (*Porotermes, Stolotermes*) the latter surviving from Gondwana. The most famous endemic is the basal *Mastotermes darwiniensis*, the only living species in this termite family. Absent from the fauna are Hodotermitdae and fungus-growing termites. The fauna of grass- and litter-feeding termites is rich, notably among the Termitidae in the genera *Amitermes* and the endemic *Drepanotermes*.

This prevalence of grass and litter-feeders is linked to the prevailing climatic conditions of Australia which lies between the southern latitudes of 10° and 44°. It is a rather flat continent except for the Eastern ranges, and is dominated by shrub and grassland. Most of Australia is dry and receives less than 500mm of rain annually. Forests and woodlands are restricted to areas of higher rainfall along the eastern, northern and south-western coasts. Summer temperatures are hot. Eastern and south-eastern Australia receive rainfall throughout the year, but in northern Australia rainfall is monsoonal and falls during summer.

Among the wood-feeding termites only about 18 species are considered major pests of structural timber and other materials. The key groups are *Mastotermes, Coptotermes* and *Schedorhinotermes* and other Rhinotermitidae and *Nasutitermes* among the Termitidae.

In contrast to the richness of the termite fauna, there is a notable paucity of termite researchers in Australia. Much of the termite research is carried out by Federal and State Government agencies, such as CSIRO (Commonwealth Scientific and Industrial Research Organisation) and State Forest or Agricultural Departments (New South Wales, Queensland, Northern Territory, Western Australia). At Australian universities termites have only a limited role as research topics, in stark contrast to many other countries.

Current topics of research in termite biology encompass: taxonomy (notably of *Coptotermes*, including molecular studies; Quarantine intercepts); population genetics (*Mastotermes, Coptotermes*); foraging biology, correlations between food resources and reproductive strategies; the role of vibro-acoustic signals in termite communication; termites as soil engineers (nutrient recycling, water transport, land management, physical structure of soil and associations with roots); the role of the bacterium *Wolbachia pipientis* in termites; and differences between invasive and non-invasive species of *Coptotermes* (reproductive strategies, feeding biology).

Current topics of research in termite management include development of laboratory and field methods of assessment of the termite resistance/efficacy of products; development of termite-resistant materials (woody, non-woody and composite products; environmentally more acceptable wood preservatives and glue-line additives for wood composites, novel methods of treatment of wood products, evaluation of a wide range of termite barrier materials such as repellent and non-repellent soil termiticides and sheet materials; physical barriers); discrepancies between laboratory and field data on the efficacy of boron treatments; surveillance regime on the level of chemical preservatives in exposed timber components of shipping containers; protection of timber power poles and bait technology.

Using Neural Networks to Predict Subterranean Termite Hazard in China

by
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Abstract

This paper describes the rationale and methodology used to predict the hazard of termite attack in the different geo-climatic zones of China using Neural Network technology. Existing geo-climatic information of different locations in Japan, the United States and Australia was linked with previously developed survey-based hazard maps for the three countries. This matrix was used to train a Neural Network, to accurately predict the hazard of the two most economically important Termite genera in China, *Reticulitermes* & *Coptotermes*. The resulting map identifies three hazard zones, High, Medium-low and Negligible. The development of the map using a Neural Network reduces the effort of performing extensive surveys and provides important information for designers, developers and researchers.

Keywords: Hazard Map, Subterranean Termites, Neural Network, China

Introduction

Termite hazard has always been an issue of concern in China. Various documents state that there are around 470 termite species in China (Zhang, 2000; Zhong and Liu., 2004), but only 10 are considered of economical importance to man made wood structures. The economic importance of these termites is mostly due to attack on wooden infrastructure like dams, utility poles, forestry plantations and orchards (UNIDO, 2003). Termites of the *Rhinotermitidae* family, belonging to the subterranean termites have become less of a problem in urban areas than formerly (Zhang, 2000). This is because wood is not a common structural material in urban areas anymore and a paved environment with predominantly masonry, concrete and steel buildings provides little ground for subterranean termite spread. Nevertheless in Guangdong province and Hainan about 90% of residential buildings are infested with *Coptoermes formosanus* Shiraki (Formosan subterranean termites) and in Guanxi, Hunan, Fujian, Hubei, Zhejiang provinces about 60% are infested (Zhong and Liu 2001). Presumably the termites are feeding on non-structural wood products and other cellulosic materials.

The necessity to learn more about termite hazard in China arises because light wood frame houses are being introduced in new developments in the outskirts of some major Chinese cities. Although light wood frame construction has a long record of excellent performance in Hawaii, where Formosan termites have been present for around 150 years, this form of construction is very new to China. Information on termite distribution is necessary to determine appropriate termite management measures for different regions.

Also, China is one of the signatory countries at the international treaty on the use of Persistent Organic Pollutants POPs (Stockholm Convention). This means, that after its ratification (May 2004) China is under obligation to comply with the agreement. Pest control is one of the activities in China that still uses POPs as treatment methods. Alternative measures to traditional (POP) treatments are sought and a hazard map will be needed to help implement any new pest management system.

Mapping of termite hazard requires normally extensive surveys and a good amount of educated guessing. This is especially difficult in such an extensive country as China. The only termite map we were able to obtain for China shows the northern limits of all termite activity (Li, 2002; UNIDO, 2003; Zhong and Liu., 2004). Descriptions of locations where *C. formosanus* are found (Zhong and Liu 1994, 2002, 2004, Zhang 2000) suggested it should also be possible to define a northern boundary for this species.

The effort of mapping termite distribution and assigning termite hazard zones has already been done in Japan, North America and Australia. The mapping was made for the subterranean Termites of the *Rhinotermitidae* family. In Japan and North America *Reticulitermes* and *C. formosanus* were mapped and other species of *Coptotermes* and *Mastotermes Darwiniensis* were mapped in Australia. At this stage it is worth investigating whether this information can be used to predict termite distribution in other parts of the world like China. Suggestions of identifying simple climatic boundaries (Morris 2000) have not always been successful possibly due to human introductions beyond the natural range and the heat island effect of large cities. Matching the myriad characteristics of locations with and without specific termites in one part of the world to the characteristics of comparable locations in other parts of the world would be a daunting task if only a formal regression based approach was attempted. Alternatively the Neural Network approach offers a powerful method to address this issue without needing apriori knowledge of the functional relationship between the various factors. This technology has been used for example in hand written digital recognition or to classify land-cover in satellite imaging and in many other tasks involving pattern recognition of complex input (Jain and Fanelli, 2000).

Materials

The subterranean termite hazard map for Japan is the 2004 version of a distribution map for the two major subterranean termites in Japan: *Reticulitermes speratus* Kolbe and *C. formosanus*. The map was generated with the information gathered by the Japan Termite Control Association (JTCA) from a recent questionnaire survey collected from Pest Control Organizations (PCOs) all over the country.

The North American termite hazard map can be found in the "termites and wood" section of the wood durability website of Forintek Canada Corp. (http://www.durable-wood.com/termites/index.php) and has been generated using different published sources and experts opinions. The map shows the different hazard zones for the native subterranean termites such as *Reticulitermes flavipes* Kollar and *Reticulitermes hesperus* Banks. The occurrence of *C. formosanus* is, for the purpose of this study assumed to be coincident with the high hazard zone for the native subterranean termite.

The Australian termite Hazard map was developed by CSIRO Australia and can be found in a CSIRO web site. (http://www.csiro.au/index.asp?type=mediaRelease&id=Termite&stylesheet=mediaRelease). Six hazard zones are found in this map.

The Weatherbase® is a free source of climatological information for more than sixteen thousand locations in the world that is gathered from different public domain sources and normalized for easy of access. The information gathered in this database has undergone the same process of normalization for all different locations in all four different countries included in this research; therefore, it is considered the best source of climatic information available.

The commercially available Neural Network software NeuroSolutions® was used as the platform for network training and classification production.

Methodology

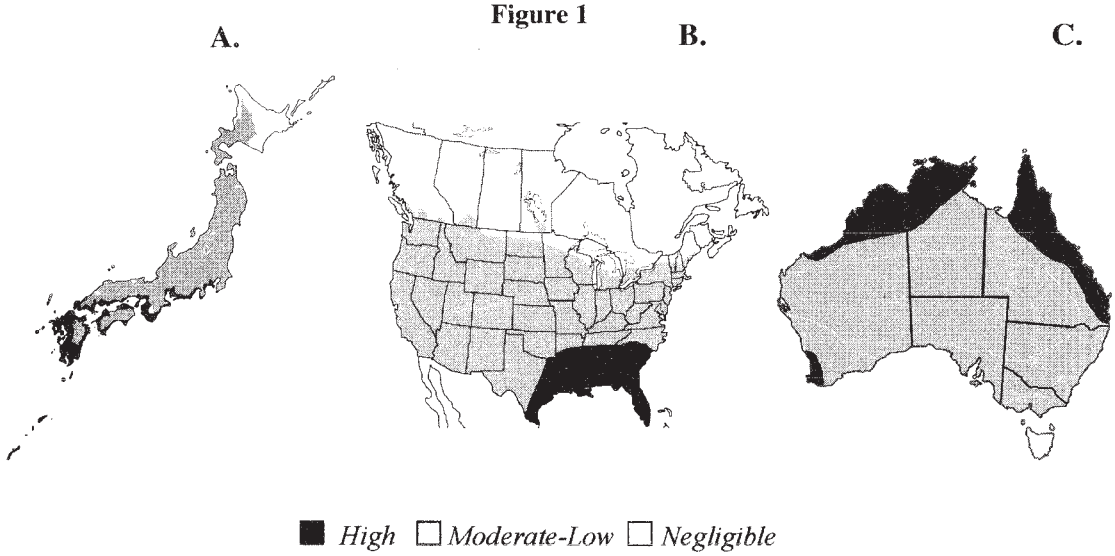
For the purpose of comparison, in this study the hazard zones in all maps were normalized and reduced to three levels:

1. High
2. Moderate-Low
3. Negligible

Also taken into consideration is that the limits that are of most economical importance are the northern limits of the distribution for both termite genera, *Reticulitermes* and *Coptotermes*.

The map used in this study for Japan (Figure 1.A.) is the overlapping of both termite species hazard maps. The map for North America (Figure 1.B.) is the result of joining the moderate and low hazard zones into one big zone. The intention was to include only North America and Japan in this study because the same termite species are mapped for both countries. The Australian map (Figure 1.C.) is a simplified version of the one developed by CSIRO and it was conceived, as explained later, considering that it only served as reference and that the data gathered from this map was not included in the final network.

Figure 1



The criteria used for the selection of the locations included in this study are as follow:

1. Relevance of location (major cities)
2. Sufficient information to assess climate
3. Climatic information format. (for comparability)

Major cities were selected at the beginning to define the formatting of the climatic information sought. Not all information found in the Weatherbase® database is displayed in the same format and also many locations lack sufficient information to match the requirements set to assess climate. Many times the information was presented in a different format than that of the major cities and could not be compared with the data already collected. The selection of the parameters needed to assess climate was based on a literature review on climate and termite behavior and adapted to fit the database format (Marais, 1939; Snyder, 1948-49-56; Skaife, 1955; Roonwall, 1970; Lee *et al.*, 1971; Ebeling, 1975; Wood, 1977; Mathews *et al.*, 1978; Edwards *et al.*, 1986; Mampe, 1990; Lenz, 1994; Pearce, 1997; Li, 2002; Tsunoda *et al.*, 2002; Shelton *et al.*, 2003). Ninety-one locations were found in North America, but it was not possible to find enough locations that met these criteria for Japan. Most locations did not provide enough information, thus making it impossible to provide a realistic sample-set to train the network. This is why Australia had to be incorporated into the training sample-set. The subterranean termite species found in Australia are different from the ones found in Japan and North America, but as mentioned before and explained later, the data gathered from the Australian locations was only used as a reference for the consolidation of the final network.

Table 2

Categories and Parameters used to assess Climate

Category	Unit	Parameter
Elevation	m	1. Elevation
Latitude	D° M'	2. Latitude
Temperature	° C	3. Year average temperature
		4. Monthly average temperature standard deviation over year
		5. Maximum monthly average temperature
		6. Minimum monthly average temperature
		7. Number of days over 32° C over year
		8. Number of days under 0° C over year
Precipitation	cm	9. Year average precipitation
		10. Monthly average precipitation standard deviation over year
		11. Maximum monthly average precipitation over year
		12. Minimum monthly average precipitation over year
Relative Humidity	%	13. Year average morning relative humidity (MRH)
		14. Monthly average MRH standard deviation over year
		15. Maximum monthly MRH over year
		16. Minimum monthly MRH over year
		17. Year average evening relative humidity (ERH)
		18. Monthly average ERH standard deviation over year
		19. Maximum monthly ERH over year
		20. Minimum monthly ERH over year
Dew Point	° C	21. Year average dew point
		22. Monthly average dew point standard deviation over year
		23. Maximum monthly dew point over year
		24. Minimum monthly dew point over year

Sixty locations were found in Australia to meet the criteria to be included in the network training sample-set. The inclusion of the Australian locations permitted the generation of a consistent network with a total of 151 samples. Training of the Network was performed using 75% of the samples as the training-set and 25% as the testing-set. The samples were re-randomized 20 times, creating each time a new network, in which the testing and training-set were always different. Since all three maps are hand drawn and the limits represent an estimated graphical representation of the hazard areas, there are many locations that fall in an uncertainty area, where the hazard factor is not well defined. Since the Neural Network software can be used to plot values to classify the locations in either one of the categories, it is necessary to eliminate the locations that confuse the network results because they lay close to or right on the virtual border. This process was named "cleaning" of the sample-set. The assumption was made that for the purpose of classification, using locations that lay well within the different hazard areas is better because, this will provide the Network with more reliable information. This process also allows trends to be identified easier by using samples that are well defined within the different areas.

The network was "cleaned" two times, using the same process described above and eliminating the locations that met the border condition and were recurrently misclassified. After the "cleaning process the classification achieved an average linear correlation coefficient (r) of 96%. In order to identify which parameters had a higher relevancy in the network outcome, the "clean" samples-set was used to

run networks including and also excluding only one parameter at a time. None of the parameters showed a particularly low degree of relevancy when excluded from the network. Most r values continued to be over 90% on average. But considering the parameters individually shows that relative humidity, as a category, and altitude were surprisingly not of particular relevance, dropping the r levels close to 0. All parameters related to relative humidity and altitude were therefore eliminated from the network. Tests were performed to confirm that the network still had a high average linear correlation coefficient.

Eliminating these parameters permitted the inclusion of more locations in Japan into the network sample set. A total of 62 locations in Japan were included in the network sample set, and the locations in Australia were eliminated, coming to a total of 153 samples between North America and Japan. The network was "cleaned" again, following the same process described previously. Twenty nine locations were eliminated from the network due to proximity to the borders and recurrent misclassification. The final average linear correlation coefficient for this network was 90%.

This sample set was used as the training sample set for classifying 274 locations in China. All locations that fell within a 75% certainty were classified within that zone. Meaning that the outcome value plotted by the network was higher than 0.75 for that particular location in either of the hazard zones. All locations that did not meet the 75% certainty requirement were declared unclassified.

From the 274 locations, 76 fell within the high hazard zone, 99 within moderate-low, 68 within negligible and 31 locations were declared unclassified.

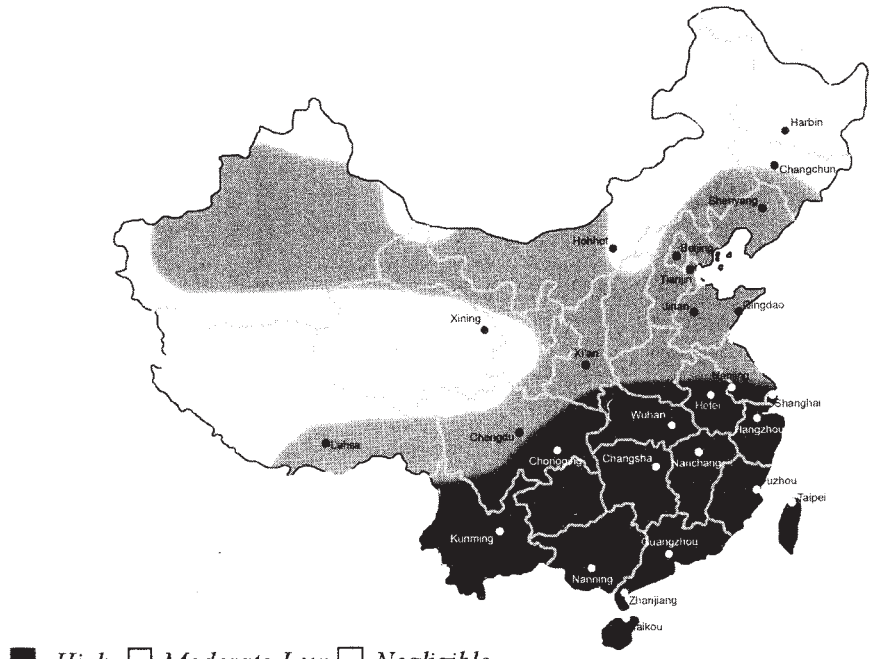
Results and Discussion

The map shown in Figure 2 is the result of the plotted network outcome. The northern boundary for the moderate–low hazard zone coincides with previous maps and descriptions of termite distribution in China (Li, 2002; UNIDO, 2003; Zhong and Liu., 2004) Although found to be slightly north of previous representations, the line that winds down from the southern Jilin region passing just north of Beijing and then curving south to pass just north of Xi'an and then curving west into Tibet (Xizang) has been described and drawn many times before. Something new to consider in this map though is the extension of the moderate-low limit into the Xinjiang region, considered outside of the recognized termite hazard zone outlined by Li (2002), the UNIDO report (2003), and Zhong and Liu (2004). This may suggest that conditions only need to change slightly for termites to spread into that region or that their absence is due to a natural barrier that could be breached by human transport of infested material. The northern limit for *C. formosanus* also coincides with previous descriptions (Zhong and Liu., 1994 - 02 - 04; Zhang, 2000).

It is important to remember that this map is a representation of hazard areas, which should not be interpreted as risk zones. The first condition for this map to apply is the observed presence of termites in the region, which could have been prevented by geographical or microclimatic barriers. The map represents a rough approximation of the areas where it is more or less likely that *Reticulitermes* as a Genus and *C. formosanus* might occur.

The intention of this study is to prove the effectiveness of the method used. The method and the outcome map are subject to improvements. Also the accuracy of the outcome will depend on the quality of the input information and further studies, including new parameters, such as soil properties, degree of urbanization, forested areas and others could be performed later to generate a more precise forecasting tool. Also similar exercises could be carried out for other species of termites.

Figure 2



Conclusion

This study used Neural Network to innovatively develop a termite hazard map for China. The hazard map for *Reticulitermes* and *C. Formosanus* generated by the trained Neural Network may help in the implementation of a successful termite management plan for China. Developers that plan to use wood as the main structural material in their projects can also benefit from this map. Strategies could be implemented in accordance to the different hazard areas to address the termite issue.

Using the Neural Network technique to map termite hazard provides little information to evaluate which climatic parameters or their interaction might influence most termite distribution. However, the little information it provides could be further analyzed to be used as reference in future studies.

Finally, this Neural Network approach to mapping termite hazard can also be used to generate termite hazard maps for other regions in the world. Perhaps even the North American map, which is based on experts' opinions could be improved by using the same method based on a hazard map for China if one could be developed.

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Biology and Extermination of the Main Economically Significant Termite Species in China

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The major representative termite genera in China are *Cryptotermes, Reticulitermes, Coptotermes, Odontotermes and Macrotermes,* the most destructive species being *Coptotermes formosanus* Shiraki and *Odontotermes formosanus* (Shiraki)

(I) Biology of *Coptotermes formosanus* Shiraki and its extermination

1. Biology of the Formosan subterranean termite (*Coptotermes formsanus* Shiraki)

Coptotermes formosanus is a serious cosmopolitan pest widely distributed in south of the Yangtze River in China, Taiwan, Hong Kong, Macao and Xisha Archipelago of Hainan Province; it causes damage to buildings, subterranean power lines and landscaping trees.

1) Constructing large centralized nests

With full expansion of a colony, *C. formosanus* can form large centralized termite nests. The nest is composed mainly of numerous wood fiber laminae, including mud, feces, sand and excretions to form a honeycombed and laminated structure called a multi-laminated nest. The site of the nest may be above ground, under ground or in trees, and with continued growth of the colony, secondary nests may be set up, which are interconnected to the primary nest by communication channels. Isotope tracing has determined that foraging activity may take place within a 50 to 100-meter range, and termites from secondary nest can return to the primary nest within 4 hours; there is only one primary nest, but secondary nests may be one or more depending on necessity (Li et al, 1989) .

2) External characteristics of the nest

(1) Excrement (voidings): these are brown-colored pieces of mud carried by the workers in their mouth from inside the wall and deposited in the surroundings, but in case of wooden nests or tree nests, what is brought outside is processed fibrous material.

(2) Dispersal flight slits (swarming slits): these are openings where winged reproductive termites emerge from the nest during the mating season to disperse and set up new colonies.

(3) Ventilation holes: these are small with a pore size of only 1 mm near the primary nest, used by the termite colony to regulate air and temperature in the nest.

3) Winged adults carry out dispersal swarming flights when the colony is fully developed

Swarming time is closely connected to temperature and moisture conditions, which coincide with the rainy season in April to June in the Guangdong region and May to July in Hunan; Swarming may be divided into initial phase, peak phase and terminal phase, and the ratio of female termites to males is usually 1.2 to 1.3 (Li, 2002).

4) Initial establishment of the colony

Male and female pair and build a new nest site, immediate copulation occurs. Eggs are laid one week later and hatch out in another one month afterwards. For the first half-year individuals number 50, after 1 year about 80-243, after 1.5 year 300, after 2 years 418-956, after 2.5 years 1000-1500, after 3.5 years 2732, after 4 years 5000, and after 5 years maybe only 10000, but after 6-7 years colony growth accelerates; the number of individuals in the colony varying with its age, increasing with colony age; colonies that have not produced winged adults are not considered mature (Li, 2002)..

The rate at which colony size develop depends primarily on ecological conditions, particularly on temperature, moisture and availability of foodstuff. Mated adult pairs were introduced into incubators with constant temperature (30°C), moisture (85%) and adequate foodstuff, and it was found that 15 months after mating, the colony size had reached 1039 individuals, among which workers numbered 915 (88%), soldiers 124 (12%), and besides there was a pile of eggs present (Li et al, 1989).

5) Development and maturation of the nested colony

Observations on mated pairs cultivated indoor and those transferred to underground sites outdoors, have revealed that the nested colony became mature after 8 years, as shown by the production of winged individuals, and when colony size was estimated at 150 to 200 thousand individuals (Li et al, 1989). The US scientist Higgs (1981) performed experimental observations on single pair transfer of swarming termites in Hawaii, and achieved winged adults after 11 years, when it was estimated that the colony contained over 100 thousand individuals (Krishna and Weesner 1969).

From the time the matured colony produced the first batch of winged adults to the decline of the colony, it is judged to take 30 to 40 years, over which the number of winged individuals would increase under normal conditions each year until a stable level is reached, and then gradual retrogression take over until natural death of the colony occurs.

Table 1 Changes in the size of *Coptotermes formosanus* soldiers at different colony age

Colony age (years)	Width of head (mm)	Width of sclerite on prothorax (mm)	Antennular segments (number)
0.5	0.80~1.00	0.63~0.68	12~13
1.5~4	0.93~1.07	0.68~0.75	12~14
8	1.07~1.18	0.75~0.82	14~15
11~19	1.10~1.24	0.75~0.85	14~15
21~22	1.11~1.25	0.85~0.90	15

Table 2 Changes in the number of individuals in relation to colony age

Colony age (years)	Number of colonies (nest)	Colony population (individuals)	Average (individuals)	Workers (average)	Soldiers (average)	Proportion of soldiers (%)
0.5	18	20~102	50.2	42.8	7.4	14.7
1	10	80~243	158	137.5	20.5	13.0
1.5	4	177~600	300	267	33	11.6
2	8	418~956	483	432	51	10.6
2.5	1	1492	1492	1357	135	9.1
3.5	3	2506~2967	2732	2568	164	6.0
4	1	5006	5006	4741	265	5.3
8	1②	87041	87041	83215	3801	4.4
9	1③	323438	323438	273087	50300	15.56①
20 (est.)	1④	395766	395766	378738	17028	4.3

N.B.: ① Development of the colony somewhat abnormal ② 25 nymphs found ③51 young termite nymphs found ④Count from a 80 kg nest dug out from under a stairway in 1970

As to the proportion of soldiers, young colonies may reach as high as 6%-15%, the proportion in the primary nest is higher than secondary nests, 4.3%-7.8%(av.6.5%) in the primary nest versus 1%-3% (av. 2.3%) in secondary nests.

2. Extermination of *Coptotermes formosanus*

(1) Killing with dust poisons

There are two kinds of dust poisons in common use, one with arsenious acid as principal component (acute poison) and the other hexachlorocyclopentadiene series pesticide (product name Mirex) (chronic poison).

1) Arsenious acid (poison) 80%; talcum powder (bulking agent) 15%; ferric oxide (colorant) 5%

2) Arsenious acid 70%; Sulphur (potentiator) 15%; Talcum powder 10%; Ferric oxide 5%

3) Mirex powder 50%; Sugarcane bagasse, or cellulose powder 50%

4) Hexaflumuron 0.25-0.5%; sugarcane bagasse or cellulose powder 99.5-99.75%

The powder should be dry, forming a uniformly dispersed mist when sprayed. The efficacy of this pesticide is good, yielding ideal result in a slightly moist environment.

Since the seventies of the 20th century, the application of Mirex in termite extermination has

been found to exhibit good efficacy and, its toxicity being lower than the conventional arsenic agent, it has afforded a new powerful addition to dust preparation for termite eradication. This kind of dust preparation is slow-acting, transmissibility good, and can cause death of the entire colony.

（2） Luring method

①Simple luring method: the common method used outdoors is to dig a trench where termites frequent or beside a tree known to harbor them; the dimensions of the trench should be approximately 30-40 cm in depth, length and width, and should not accumulate water. Material such as pine wood or sugarcane bagasse, preferably with a small amount of pine pollen, that termites love to eat is laid in the trench, and finally covered with pine branches, fern leaves or gunny bag and plastic membrane. Indoors, a luring box made of wood or cardboard is placed where termite activity is observed, and within 3-4 days or at most over 20-odd days or so, tens of thousands of termites would be lured to concentrate there and the luring box may even become a nest if more time is allowed. Spraying termite extermination dust after gently lifting the lid of the box and any pieces of board present can achieve 100% death of the colony. Even when the termite nest cannot be found, one can still realize extermination of the entire colony.

②"Termite lure and kill preparation" in which pharmaceutical product is combined with luring: In recent years this has become the predominant method of termite extermination in China. For bait, different regions use pine wood shavings, sugarcane bagasse, toilet paper or corn cob, depending on the feeding habits of diverse termite species. For termicide, Mirex, which is suitable for poison in termite baits is selected, and the popularization of this drug in recent years for termite extermination can be claimed a major breakthrough in our country.

(II) Biology of *Odontotermes formosanus* and its extermination

1. Biology of the black-winged Taiwan subterraneous termite *Odontotermes formosanus*

Odontotermes formosanus is a highly evolved fungus-growing termite, very stringent in its ecological requirements and very difficult to cultivate indoors, no success having been achieved worldwide in feeding them artificially to colony maturity with winged adults emerging. It is a great pest of dykes, dams and forest crops, serious and widespread in the southern provinces of China.

1) Primary nest (royal fungus gardens) and secondary nest (satellite fungus garden)

What is called primary nest is where the termite king and queen reside, and where the royal fungus garden is located. The king and queen live seclusively in a "royal cell" made of mud specially processed by the workers, and which is smooth on the exterior and solid in construction. Within a range of 1-10 meters around the primary nest, there are studded numerous secondary nests, fungus gardens and empty cavities; there is only one primary nest but secondary nests (fungus gardens) may number several dozens to over a hundred. Most of the satellite fungus gardens lie within 1 meter, but 8-10 meters away may still be found scattered distribution of fungus gardens.

2) Mature nests (mature colonies) produce winged adults for swarming

(1) Swarming holes: In swarming seasons, the termites build swarming holes distributed on the soil around their nest; these are small irregular mounds which when pried open with a screw driver show flat-bottomed, convex-topped chambers 3-5 cm in diameter at the bottom and 3-4 cm in height. Swarming holes may number at least 3-5, generally a dozen or so, but may reach several dozens.

(2) Preswarming chamber: By digging 3-30 cm below a swarming hole, one may see many crescent-like or flat chambers; these are the "preswarming chambers" in which winged adults congregate in preparation for dispersion flight. The preswarming chamber is 3-5 cm wide, 1-5 cm high, a few to 20 cm long, tortuous and winding, and sometimes multi-layered; the preswarming chambers connect upwards to the swarming holes, and downwards the main termite tunnel and directly to the nest.

(3) Swarming season: wing development and swarming occur in the same year, the swarming season usually takes place during April to May, varying with latitude and weather, already started by mid-March in Hainan Island, but still going on late June in Hubei and Henan Provinces. In a thriving mature colony, each nest may have several dispersing flights during one swarming season of the same year.

(4) Number and sexual ratio of winged adults (alates)

Luring and catching winged adults outdoors to determine the number of alates is difficult,

luring with lights cannot ascertain the number of nests, and sometimes light do not become effective before dark. Counts based on nest excavation place the number of winged adults at from nearly a thousand to a few thousand to 20 thousand.

Sex ratio of alates: in a normally developed colony the ratio of male to female winged adults is about 1:1.

3. Establishment of the initial colony

1) Overwintering at ambient temperature is difficult

A total of 30 mated pairs were introduced into culture apparatus, glass panes, flower pots and glass jars, when it was found that 25 pairs (83.3%) survived to lay eggs 2 months later. The survival rate after 3-4 months was 36-40%, and 6 months later (i.e., November of the same year to March of the following year when room temperature dropped to 20-10°C) the colonies had difficulty in overwintering, the survival rate being only 6.6%. Mated pairs after settling down start to lay eggs in about 6-8 days, laying 4-6 eggs each day and 30-40 eggs in the first batch, with 26-40 days incubation time. After several molts (requiring about 60-80 days after hatching) the larvae develop into small workers, which start to perform duties such as carrying mud and constructing pathways.

2) Oviposition and incubation

Within 9 days after oviposition commenced, 28-45 (av. 35) eggs were laid in the first batch, then a pause occurred for some time, after which the number of eggs laid gradually increased, 60-103 (av.87.3) eggs being observed in initial nests of 26 mated pairs within 64 days. From hatching to the first appearance of workers required 14-19 (av.16.7) days, whereas it took 20-26 (av.23.81) days for a soldier to develop after hatching.

3) Influence of temperature and foodstuff on young colonies

Optimum temperature is 25-28 °C, oviposition cease at 15°C, and temperature above 33°C is also unsuitable (see Table 3). Cultivated colonies initiated with mated pairs, besides a suitable temperature of 25-30°C and relative humidity of 80%, also need sufficient foodstuff: powdered robusta bark, sugarcane bagasse, pine pollen, fungus bed dry powder (or fresh fungus bed), tissue paper (filter paper) underlining etc.; when there is deficiency of foodstuff the parent termites would devour eggs or larvae.

4) Formation, development and decline of the colony

Seven basic forms could be observed during the development, maturation and decline of the termite colony, where it progressed from single to multiple chambers and from simple to complex structures.

1) Young nest phase

(1) Single cavity empty nest (no fungus bed - initial stage of fungus garden): Here can be seen mated winged adults that have entered the soil to make a nest living in a small chamber 1.0 x 1 x 0.5 cm in size, a few to 20 cm under the surface, which after 3 months would have reached 10-39 cm underneath and the chamber 1.5 cm x 1-1.5 cm x 0.8-1.6 cm in dimension; when colony composition would be workers (25-35), larvae (17-54), eggs (32-50), termites and eggs totaling 74-135. Fungus garden building starts from the 4th month, but at six months there is still no completed fungus garden.

(2) Single cavity fungus garden nest (full-blown fungus garden present in the cavity): The small cavity nest is located 30-70 cm deep, and individuals in the colony number 300-500, requiring 1-2 years to reach that stage.

(3) Multicavity fungus gardens nest (many fungus gardens and many empty cavities present, but no swarming holes or preswarming chamber seen as yet)(age of nest 3-4 years). The primary nest is located 35-90 cm below ground, the royal fungus garden is 20 cm x 20 cm x 12 cm in dimension, there are 3-14 satellite nests with fungus gardens, and 3-13 empty cavities, with colony inhabitants numbering 1020-5000.

2) Mature nest phase (the main indications are the production of winged adults, swarming holes on the ground outside the nest and preswarming chambers inside): age of the nest may be anywhere from 5-6 years up to 7-8 years.

(1) Early mature stage nest (stacked multicavity nest): Within 1-2 years after production of the first batch of winged adults, of swarming holes there may be a small number, from a few to 20 of them, of alates from a few hundred to a few thousand, and of individuals in the colony from a few tens of thousand to 200 thousand. Principal features: framework of the primary nest not very

developed, its fungus gardens only divided into a few layers, large cap-like fungus beds present in the nest cavities, the royal cell generally located at the upper third of the fungus garden, length of primary nest 35-50 cm, with 30-50 satellite fungus gardens and a few empty cavities, all of which are mainly situated 0.85-1.95 m deep underground.

(2) Thriving mature nest (block-shaped multicavity nest): Three to five years after the appearance of winged adults, the colony would reach the most flourishing phase of development, where there appear from several dozen to more than a hundred swarming holes which may be arranged into patches of 3-5, 8 or 10; The number of alates may be from 10 thousand to several tens of thousand, and that of the individuals in the whole colony several hundred thousand to over a million.

Principal features: Framework of the primary nest well developed, the nest divided into several small chambers by mud sheets, each chamber containing a full-blown fungus garden, patches of fungus beds seen at the top of the royal fungus garden, and the royal cell located at the lower third of the royal fungus garden.

3) Declining nest phase (shriveled multicavity nest): The process from young to mature to declining nest phase may take 20 years, after which colony size gradually decrease, and no winged adults are produced to carry out swarming. Many empty cavities appear, numbering more than the fungus gardens, most of the latter not full, half empty or only one-third full, most of the fungus beds in the royal fungus gardens of the primary nest have shrunken, creating a large empty cavity at the top and exposing the framework. It is estimated that 3-5 years later, natural death of the whole colony would take.

5. Extermination of *Odontotermes formosanus*

The extermination of Odontotermes formosanus in China has progressed from crude to refined methods with constant improvement in technique. At present the most widely used and found to be most effective is using poisoned bait made of Mirex poison plus luring agents.

Formulation of bait using Mirex

1) Formula for making Mirex poisoned bait bars:

Sugarcane bagasse or Eucalyptus robusta 54% (bait)
Mirex 2% (poison ingredient)
White (brown) sugar 22% (enticing agent)
Wheat flour 22% (cementing agent)

Adding a small quantity of pine pollen and preservative can lure termites more quickly as well as prevent the bait from becoming moldy.

Method of preparation: Grind the sugarcane bagasse or Eucalyptus robusta bark to a powder, dissolve the sugar in a small quantity of water and, into the resulting syrup, mix in wheat flour to make a paste, then the powdered bagasse or eucalyptus bark is stirred in uniformly, and the mixture shaped into 6 cm x 1 cm x 1cm bars, which are dried in an oven at (60-70°C) temperature. Package well and be careful to avoid wetting.

2) Formula for Mirex poison bait powder:

Bait (sugarcane bagasse, Eucalyptus robusta bark, pine wood shavings, cellulose powder, cassava flour) powder, which should pass through 100 mesh pore size 60%.

Mirex (poison ingredient) powder, which should pass through 100 mesh pore size 40%. The poisoned bait should be kept dry and able to make a uniform spray, which has been found to be especially efficacious when applied in a luring box.

Application of poisoned bait:

Mirex poisoned bait can be applied to termite tunnels, mud cover, mud tracks, and swarming holes where termites are active, preferably in main channels where the insects pass to and fro. The bait once placed should be covered with tile or tree branches and sealed with wet soil, thus preventing ants from eating it. As long as the termites ingest the bait, the whole colony will be killed.

Searching for termite nest indicator Black Candle-snuff and pouring concrete to fill in the nest cavity.

Ingestion of the poisoned bait will lead to death of the entire colony in 93-99.3% of cases, and within 1-3 months Black Candle-snuff (Xylaria nigripes) would appear on the surface of nests less than 3 m deep below the surface. Concrete is poured into cavities found in the area indicated by the growth of the fungus to reinforce the infested dams.

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Termite Diversity and Some Important Wood Destroying Termites in Thailand

by
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Abstract

Identification of more than 4,000 specimens from the random survey through out Thailand revealed totally 160 species of 39 genera, 10 subfamilies and 4 families. One species of genus *Archotermopsis*, one species of genus *Incisitermes*, two species of genus *Parrhinotermes*, two species of genus *Reticulitermes*, one species of genus *Prohamitermes*, one species of genus *Synhamitermes*, two species of genus *Angulitermes*, one species of genus *Homallotermes*, one species of genus *Longipeditermes* and two species of genus *Lacessititermes* were reported as new species recorded from Thailand. *Coptotermes gestroi* and *C. havilandi* are the most important wood destroying termites in urban and rural area, while *Microcerotermes crassus* and *Globitermes sulphureus* are the main species infested in rural area.

Key words: termite diversity, newly recorded, wood destroying termite

Introduction

Termites are abundant soil animals in the tropical and subtropical regions (Lee and Wood, 1971; Wilson, 1990). They and the number of species and their biomass are especially large in the tropical zone (Pearce, 1997). In the wide range of tropical climates, from savanna to rain forest, termites are believed to play an important role on the turnover of organic matter and the maintenance and improvement of soil fertility (Krishna and Weesner, 1969; 1970; Lee and Wood, 1971; Wood and Sands, 1978).

Termites are also considered as "ecosystem engineers" (Jones *et.al.*, 1994). They are large-bodied organisms that move through soils and mix organic and mineral materials forming organomineral complexes with medium to long-term persistence, and are therefore major determinants of soil structure, important conservators of fertility and even landscape architects (Martius, 1994; Eggleton *et.al.*, 1995).

Thailand is a tropical country that is governed by monsoon; consisted of various types of forest ecosystem that is suitable for termite growth and development. However, only limited information on termite in Thailand has been published, especially on the ecological intervention of termites in various forest ecosystems. In Thailand, Ahmad (1965) described 74 species of termites over the whole area of the country. Morimoto (1973) presented paper deals with 90 species, of which 4 are new to science and 13 are new to Thailand. Intanai (1987) recorded 25 species of termites in rubber plantation of Chanthaburi and Trat Provinces which 2 species were new record of Thailand. Chutibhapakorn (2002) reported 42 species were surveyed in Moist Evergreen Forest and Dry Evergreen Forest in Chanthaburi Province which *Angulitermes* sp. was new record of Thailand. The main objective of this paper is to update termite species and their habitat that was recorded from Thailand until 2005.

Materials and methods

Observation in sampling plot

The size of sampling plots was 100m x 100m for each forest type. Sixty sample plots or 60% of the study area were randomly surveyed in each forest type.

Random survey

In the forest is not made a sampling plot, the observation were made by random survey along the walking trial.

Intensive observations were made on ground, trees, branches, twigs and dead stumps for presence of termites. Tunnels, foraging trails on ground, trees or under leaves and debris were also searched. Information in relevant to the specimen collected were recorded in writing and also photographed. Specimens were preserved in 80 % alcohol.

Results and discussion

More than 4,000 specimens from the survey are preserved at Forest Economic and Forest Products Research Office, Royal Forest Department and all of these specimens were reported by Ahmad (1965), Morimoto (1973) and Intanai (1987) revealed that totally 160 species belong to 37 genera, 9 subfamilies, 4 families (Table 1). Ninety species was reported in this paper has already been described by Ahmad (1965) and Morimoto (1973) the remaining 70 species were still undetermined.

Table 1 List of termite genera and species.

	Termite species	Distribution						Nest habitat	Food habitat
		C	N	E	W	NE	S		
1	**F. Kalotermitidae (6G, 18SP)**								
1.1	**SF. Kalotermitinae**								
1	*Cryptotermes thailandis*[1]			✓		✓		IW	W
2	*Cryptotermes domesticus*[2]							IW	W
3	*Cryptotermes bengalensis*[2]							IW	W
4	*Glyptotermes brevicaudatus*[1]						✓	IW	W
5	*Glyptotermes kachongensis*[1]						✓	IW	W
6	*Glyptotermes pinangae*[1]		✓	✓				IW	W
7	*Glyptotermes thailandis*[2]							IW	W
8	*Glyptotermes* sp.1			✓				IW	W
9	*Glyptotermes* sp.2			✓	✓			IW	W
10	*Glyptotermes* sp.3			✓				IW	W
11	*Glyptotermes* sp.		✓					IW	W
12	*Neotermes* sp.1			✓				IW	W
13	*Neotermes* sp.2			✓				IW	W
14	*Neotermes* sp.3			✓			✓	IW	W
15	*Neotermes* sp.4			✓			✓	IW	W
16	*Incisitermes* sp.*					✓	✓	IW	W
17	*Postelectrotermes tongyaii*[1]						✓	IW	W
18	*Bifiditermes indicus*[1]	✓						IW	W
2	**Termopsidae (1G, 1 SP)**								
2.1	**Termopsinae (1G, 1 SP)**								
19	*Archotermopsis* sp.*		✓					IW	W
3	**F. Rhinotermitidae (5G, 16SP)**								
3.1	**SF Rhinotermitinae (2G, 6SP)**								
20	*Schedorhinotermes medioobscurus*[1]		✓	✓	✓	✓	✓	S	W
21	*Schedorhinotermes rectangularis*[1]			✓			✓	S	W
22	*Schedorhinotermes sarawakensis*[1]		✓				✓	S	W
23	*Schedorhinotermes* sp.						✓	S	W
24	*Parrhinotermes* sp.1*						✓	S	W
25	*Parrhinotermes* sp.2*						✓	S	W
3.2	**SF. Prorhinotermitinae (1G, 2SP)**								
26	*Prorhinotermes tibiaoensiformis*[1]	✓	✓				✓	S	W

27	*Prorhinotermes* sp.			✓			✓	S	W
3.2	**SF. Heterotermitinae (1G, 2SP)**								
28	*Reticulitermes khaoyainensis**		✓			✓		S	W
29	*Reticulitermes* sp.*		✓			✓		S	W
3.3	**SF. Coptotermitinae (1G, 6SP)**								
30	*Coptotermes gestroi*[1]	✓	✓	✓	✓	✓	✓	S	W
31	*Coptotermes havilandi*[1]	✓	✓	✓	✓		✓	S	W
32	*Coptotermes premrasmii*[1]						✓	S	W
33	*Coptotermes curvignathus*[1]		✓	✓			✓	S	W
34	*Coptotermes kalshoveni*[1]						✓	S	
Termite species		Distribution						Nest habitat	Food habitat
		C	N	E	W	NE	S		
35	*Coptotermes* sp.1	✓	✓	✓			✓	S	W
4	**F. Termitidae (27G, 125SP)**								
4.1	**SF Macrotermitinae (5G, 40SP)**								
36	*Macrotermes annandalei*[1]		✓	✓	✓	✓	✓	E	W&L
37	*Macrotermes gilvus*[1]	✓	✓	✓	✓	✓	✓	E	W&L
38	*Macrotermes chaiglomi*[1]	✓						E	W&L
39	*Macrotermes maesodensis*[1]		✓	✓	✓		✓	E	W&L
40	*Macrotermes malaccensis*[1]						✓	E	W&L
41	*Macrotermes carbonarius*[1]		✓	✓			✓	E	W&L
42	*Macrotermes* sp.		✓		✓			E	W&L
43	*Microtermes obesi*[1]	✓	✓	✓	✓	✓	✓	S	W&L
44	*Ancistrotermes pakistanicus*[1]	✓	✓	✓	✓	✓	✓	S	W&L
45	*Hypotermes makhamensis*[1]	✓	✓	✓	✓	✓	✓	S	W&L
46	*Hypotermes xenotermitis*[1]		✓				✓	S	W&L
47	*Hypotermes obcuriceps*[3]			✓				S	W&L
48	*Odontotermes* sp.1	✓	✓	✓	✓		✓	S	W&L
49	*Odontotermes* sp.2		✓	✓	✓			S	W&L
50	*Odontotermes* sp.3		✓	✓	✓			S	W&L
51	*Odontotermes* sp.4					✓		S	W&L
52	*Odontotermes* sp.5		✓		✓			S	W&L
53	*Odontotermes* sp.6		✓					S	W&L
54	*Odontotermes* sp.7		✓					S	W&L
55	*Odontotermes* sp.8		✓		✓			S	W&L
56	*Odontotermes* sp.9		✓					S	W&L
57	*Odontotermes* sp.10				✓			S	W&L
58	*Odontotermes* sp.11	✓			✓			S	W&L
59	*Odontotermes* sp.12	✓			✓			S	W&L
60	*Odontotermes* sp.13	✓			✓			S	W&L
61	*Odontotermes* sp.14	✓			✓			S	W&L
62	*Odontotermes* sp.15	✓			✓			S	W&L
63	*Odontotermes* sp.16		✓		✓			S	W&L
64	*Odontotermes* sp.17				✓			S	W&L
65	*Odontotermes proformosanus*[1]	✓	✓	✓	✓	✓	✓	S	W&L
66	*Odontotermes formosanus*[1]	✓	✓		✓	✓		S	W&L
67	*Odontotermes longignathus*[1]		✓			✓	✓	S, E	W&L
68	*Odontotermes feae*[1]			✓	✓	✓	✓	S, E	W&L
69	*Odontotermes takensis*[1]		✓	✓	✓			S	W&L
70	*Odontotermes maesodensis*[1]		✓					S	W&L
71	*Odontotermes oblongathus*[1]						✓	S	W&L
72	*Odontotermes paraoblongathus*[1]					✓		S	W&L

73	*Odontotermes sarawakensis*[1]		✓					S	W&L
74	*Odontotermes javanicus*[2]							S	W&L
75	*Odontotermes prodives*			✓			✓	S	W&L
4.2	**SF Termitinae (12G, 37SP)**								
76	*Amitermes dentatus*[1]			✓			✓	A	S&H
77	*Amitermes longignathus*[1]			✓			✓	A	S&H
78	*Microcerotermes crassus*[1]	✓	✓	✓	✓	✓	✓	A	W
79	*Microcerotermes annandalei*[1]		✓	✓	✓			A	W
80	*Microcerotermes minutus*[1]	✓	✓	✓	✓		✓	A	W
81	*Microcerotermes paracelebensis*[1]		✓				✓	A	W
82	*Microcerotermes distans*[2]			✓				A	W
83	*Microcerotermes* sp.		✓					A	W
Termite species		Distribution						Nest habitat	Food habitat
		C	N	E	W	NE	S		
84	*Globitermes sulphureus*[1]	✓	✓	✓	✓	✓	✓	E	W
85	*Prohamitermes* sp.*						✓	S	W
86	*Synhamitermes* sp.*						✓	S	W
87	*Dicuspiditermes garthwaitei*[1]		✓	✓	✓			A	S&H
88	*Dicuspiditermes makhamensis*[1]		✓	✓				A	S&H
89	*Dicuspiditermes* sp.1			✓			✓	A	S&H
90	*Termes huayangensis*[1]						✓	A	S&H
91	*Termes cosmis*[1]		✓	✓			✓	A	S&H
92	*Termes propinquus*[1]		✓				✓	A	S&H
93	*Termes major*[2]		✓					A	S&H
94	*Pericapritermes semarangi*[1]		✓	✓			✓	S	S&H
95	*Pericapritermes latignathus*[1]		✓	✓	✓		✓	S	S&H
96	*Pericapritermes* sp.B			✓				S	S&H
97	*Pericapritermes* sp.C			✓				S	S&H
98	*Pericapritermes* sp. D		✓					S	S&H
99	*Pericapritermes* sp. E				✓			S	S&H
100	*Pericapritermes* sp. F				✓			S	S&H
101	*Pericapritermes* sp.G				✓			S	S&H
102	*Procapritermes prosetiger*[1]		✓			✓	✓	S	S&H
103	*Procapritermes longignathus*[1]		✓			✓		S	S&H
104	*Procapritermes parasilvaticus*[1]		✓					S	S&H
105	*Procapritermes* sp.		✓	✓	✓			S	S&H
106	*Mirocapritermes connectens*[1]						✓	S	S&H
107	*Mirocapritermes prewensis*[1]			✓				S	S&H
108	*Mirocapritermes concaveus*[1]		✓	✓		✓		S	S&H
109	*Mirocapritermes latignathus*[1]		✓				✓	S	S&H
110	*Angulitermes* sp.*			✓				S	S&H
111	*Angulitermes* sp.3*			✓			✓	S	S&H
112	*Homallotermes* sp.*						✓	S	S&H
4.3	**SF. Apicotermitinae (3G, 6SP)**								
113	*Euhamitermes hamatus*[1]				✓		✓	S	S&H
114	*Euhamitermes* sp.				✓			S	S&H
115	*Speculitermes macrodentatus*[1]	✓	✓					S	S&H
116	*Speculitermes rongensis*[2]							S	S&H
117	*Speculitermes* sp.				✓			S	S&H
118	*Indotermes thailandis*[1]		✓					S	S&H
4.4	**SF. Nasutitermitinae (7G, 42SP)**								
119	*Aciculitermes maymyoensis*[1]		✓			✓		S	W

120	*Nasutitermes johoricus*[1]		✓	✓			✓	S	W
121	*Nasutitermes matangensiformis*[1]	✓	✓	✓	✓	✓	✓	A	W
122	*Nasutitermes dimorphus*[1]	✓			✓		✓	S	S&H
123	*Nasutitermes perparvus*[1]	✓	✓					S	S&H
124	*Nasutitermes tungsalangensis*[1]		✓			✓		S	W
125	*Nasutitermes fuscipennis*[1]						✓	S	W
126	*Nasutitermes matangensis*[2]							S	W
127	*Nasutitermes bracynasutus*[2]							S	W
128	*Nasutitermes havilandi*[2]							S	W
129	*Nasutitermes profuscipennis*[3]			✓				S	W
130	*Nasutitermes* sp.1			✓				S	W
131	*Nasutitermes* sp.2			✓			✓	S	W
132	*Nasutitermes* sp.3			✓				S	W
133	*Nasutitermes* sp.4			✓				S	W
Termite species		Distribution						Nest habitat	Food habitat
		C	N	E	W	NE	S		
134	*Nasutitermes* sp.5			✓				S	W
135	*Nasutitermes* sp.6						✓	S	W
136	*Bulbitermes makhamensis*[1]			✓				S	W
137	*Bulbitermes prabhae*[1]		✓	✓		✓	✓	S	W, S&H
138	*Bulbitermes parapusillus*[1]		✓	✓				S	W
139	*Bulbitermes laticephalus*[1]		✓	✓		✓		S	W
140	*Bulbitermes deltocephalus*[2]							S	W
141	*Bulbitermes germanus*[2]							S	W
142	*Bulbitermes* sp.				✓			S	W
143	*Bulbitermes* sp.1			✓				S	W
144	*Bulbitermes* sp.2			✓				S	W
145	*Bulbitermes* sp.3			✓				S	W
146	*Bulbitermes* sp.4				✓			S	W
147	*Hospitalitermes ataramensis*[1]		✓	✓	✓		✓	S	L
148	*Hospitalitermes jepsoni*[1]			✓			✓	S	L
149	*Hospitalitermes birmanicus*[2]						✓	S	L
150	*Hospitalitermes asahinai*[2]							S	L
151	*Hospitalitermes medioflavus*[2]							S	L
152	*Hospitalitermes* sp.1						✓	S	L
153	*Hospitalitermes* sp.2							S	L
154	*Hospitalitermes* sp.3			✓				S	L
155	*Hospitalitermes* sp.4			✓				S	L
156	*Longipeditermes longipes**						✓	S	W
157	*Lacessititermes* sp.1*			✓				S	W
158	*Lacessititermes* sp.2 *			✓				S	W
159	*Havilanditermes proatripennis*[1]						✓	S	W
160	*Havilanditermes* sp.				✓			S	W

* = Newly recorded of Thailand
[1] = List from Ahmad (1965)
[2] = List from Morimoto (1973)
[3] = List from Intanai (1987)

Newly Recorded in Termite Lists of Thailand

From the list of the undetermined species one species of genus *Archotermopsis*, one species of genus *Incisitermes*, two species of genus *Parrhinotermes*, two species of genus *Reticulitermes*,

one species of genus *Prohamitermes*, one species of genus *Synhamitermes*, two species of genus *Angulitermes*, one species of genus *Homallotermes*, one species of genus *Longipeditermes* and two species of genus *Lacessititermes* are considered as new recorded species to Thailand's termite list.

Important wood destroying termite

Somnuwat (1966) reported that only thirteen termite species were found infesting in houses in urban and rural areas. *Coptotermes gestroi*, subterranean termite, is the most important termite species attacking wooden constructions not only in urban area but also in rural areas and widely distributing throughout the country (Table 1). Apart from *C. gestroi*, *C. havilandi* was also found the wood-destroying termite in urban area in my recent survey. *Microcerotermes crassus*, wood feeder, was the main species infested in rural area and now have been found in urban area, this might due to the village extend to their own area. There is another species, *Globitermes sulphureus* was found destroy in rural area as same as *M. crassus*.

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Spectral Sensitivity of the Compound Eyes of the Black-winged Subterranean Termite *Odontotermes formosanus* (Isoptera: Termitidae) Alates

by
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Abstract

Spectral sensitivity of the compound eyes of *Odontotermes formosanus* (Shiraki) alates, a economically important pest of river dikes and reservoir dams, was measured by electroretinogram (ERG) technique. 6 selected wavelength bands between 389 and 940 nm stimulated by light-emitting diodes (LEDs). The form of the ERG was found to be monophasic in nature. The relative spectral sensitivity high significantly different in descending order to violet (wave range, 385-390 nm), blue (465-470 nm), green (520-525 nm), yellow (585-590 nm), infrared (850-940 nm), red (625-630 nm) light. Peaks of electroretinogram were found in the violet and blue. Furthermore, no differences were observed between sexes.

Key words: *Odontotermes formosanus*, termite, spectral sensitivity, electroretinogram

Introduction

Most insects show certain characteristic response to light. In generally, workers and soldiers of termites avoid light, they live in constant darkness, have neither functional compound eyes nor ocelli. However, the alates of *O. formosanus* have functional compound eyes, exhibit a strong tendency to light in particularly, swarming from March to July in southern China annually, This dispersal bring many new colonies to new environment, causing a great many infestations.

Black-winged subterranean termites *Odontotermes formosanus* (Shiraki) are serious pests of the plants, dikes and dams in the south of China, Vietnam, Burma and other Southeast Asia countries. Its nests systems are subsurface defects that can cause infiltration and collapse to the body of dikes and dams when water level is high. Within all dam-termite species, *O. formosanus* responsible for the greatest economic losses of dam damage in China (Li 2004). In southern China, river dike and reservoir dam bodies are made mostly of earth, over 90% of them >15 years old are damaged by *O. formosanus*, moreover, during annual flood season, 20% of dam leaks were cause by termite gallery systems which drill through the dames, and many disaster caused by this termite will always be remembered (Li 1991). An example in China is the collapse of the Dongkaomiao reservoir Dam in Zhejiang province, which washed away villages and fields and claimed the lives of over 180 people in June 1971. There is a long history of combating dike and dam termite in China. Their damage was first described as "a small leak will sink a great ship" in the early 234 BC.

Photosensitivity is well known in Hymenoptera and Lepidoptera (Ding et al. 1974, Eguchi et al. 1982, Wei et al. 2002, Wu and Lin 1990, Yang et al. 1998), especially Hymenoptera possesses a trichromatic visual system (Peitsch et al. 1992), however, literature on Isoptera is very scant (Cabrera and Rust 1996, Cheng and Qing 1963, Chang et al. 2001, 2004, Huang 1993, Jing and Lei 2004, Park and Raina 2005). We present the result of laboratory experiments of the responses of *O. formosanus* alates to light of different spectrum with the same intensities. Hoping to have application in developing management strategies against this economically important species for maintenance and protection of the dike system in South China and neighboring Southeast Asia region.

Materials and Methods

Termite Collection and Maintenance

During the dispersal season, alates of O. formosanus were collected from Longdong reservoir dam (23° 11'N; 113° 23'E), Guangzhou, China. Captured and transferred them into carton box with soil as soon as possible which soil maintained at 55.0%~61.4% humidity and 25±1℃ (Liu et al. 1998, Rao et al. 1987).

Electrophysiological recordings

Spectral sensitivity of the compound eyes of *O. formosanus* was measured using the electroretinogram (ERG) technique, defined by Goldsmith and Bernard (1965) as the retinal action potential reflecting activities of both receptors and higher order neurons (Brown and Anderson 1996, Brown et al. 1998). Alates were partially embedded in a polyethylene glycol 6000 block. Care was taken to ensure that all appendages were secured and that the uppermost compound eye was unobstructed. The positive electrode end consisted of glass microelectrode which tip resistance was about 3-5 MΩ. The indifferent electrode end consisted of silver needle that inserted into the termite abdomen. Potential were recorded by physiological collection system (RM6240BD, Chengyi, China).

Light-emitting diodes (LEDs) over the range 389-940 nm as optical stimulator for the alates' compound eyes. We regulate the voltage and electrified time by physiological collection system to control the illumination intensity and duration. 30 min dark adaptation passed before electroretinogram recording. All illumination at the compound eyes maintained at 100 Lux and flashed duration was 0.1 second once every 5 seconds (Agee 1972,1973; Chang et al. 2004). Mean sensitivity was calculated for males (n=5), females (n=5) and overall for both sexes (n=10). Elaborate operation with micromanipulator was under a microscope (Olympus, Japan) in a stainless steel box to screen environmental static.

Results and Discussion

The spectral sensitivity of the compound eye of a female *O. formosanus* is shown in fig. 1. It was found to be monophasic. The value of electroretinogram-determined spectral sensitivity of the compound eyes was between 0.6 V and -0.05 V. The relative spectral sensitivity high significantly different in descending order to violet (wave range, 385-390 nm), blue (465-470 nm), green (520-525 nm), yellow (585-590 nm), infrared (850-940 nm), red (625-630 nm) light (t-test, $P<0.01$). Two peaks of sensitivity are clearly visible, firstly in the range of violet and secondly in the blue. Alates are not sensitive in the red region, however, alates *O. formosanus* show a little sensitivity in the infrared region, they may perceive infrared red through compound eyes or other organs.

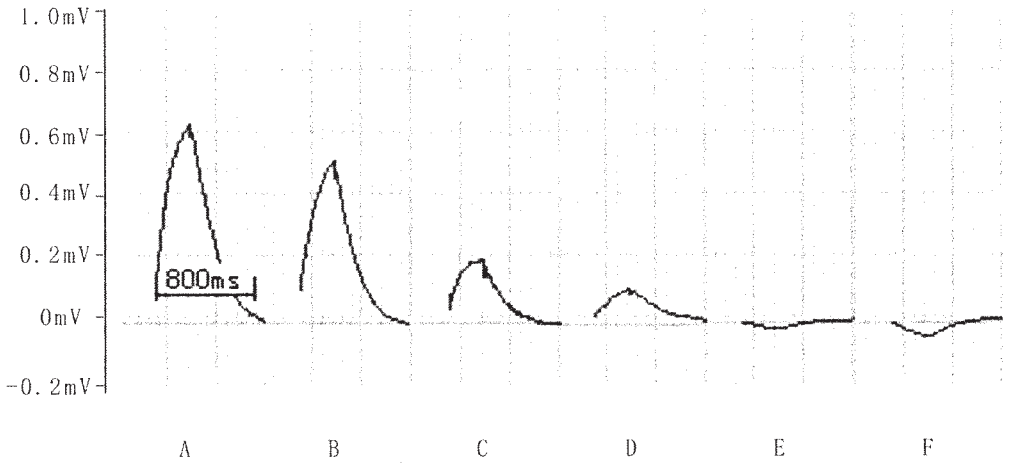

Fig 1. Electroretinogram spectral sensitivity of one female alate *Odontotermes formosanus* stimulated by six kinds of LEDs, shown as the means of 10 replications. A: violet (wave range, 385-390 nm); B: blue (465-470 nm); C: green (520-525 nm); D: yellow (585-590 nm); E: red (625-630 nm); F: infrared (850-940 nm).

Figure 2 shows no significant differences were observed between males and females at any wavelength. Sensitivity peaks were seen in violet region and blue region distinctly for both sexes.

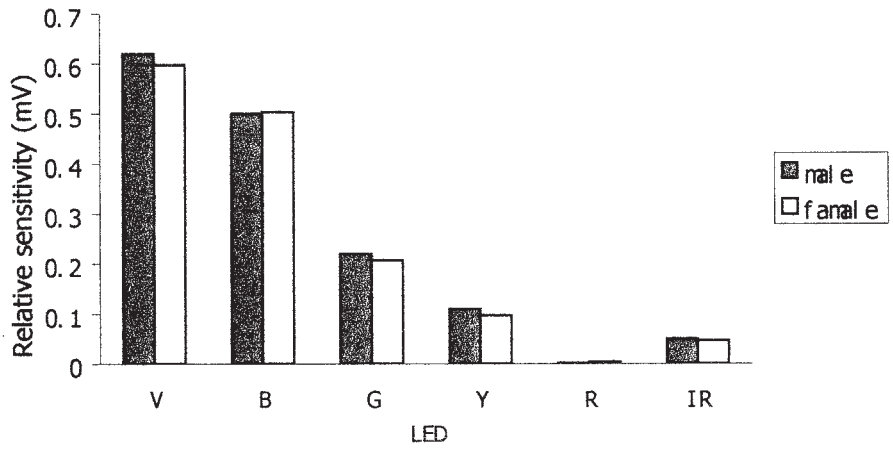

Fig 2. Electroretinogram spectral relative sensitivity of *Odontotermes formosanus* stimulated by six kinds of LEDs, shown as the results of 5 males and 5 females. A: violet (wave range, 385-390 nm); B: blue (465-470 nm); C: green (520-525 nm); D: yellow (585-590 nm); E: red (625-630 nm); F: infrared (850-940 nm).

In contrast with *C. formosanus*(Chang et al. 2004), alates of *O. formosanus* possessed a broadly similar electroretinogram. The major difference is the relatively much greater sensitivity of *C. formosanus*. This may reflect the fact that alates of *C. formosanus* are a much more vigorous, as longer flight and longer chasing activity before paired than alates of *O. formosanus* which appear in the air shortly.

O. formosanus are usually established in the earthwork at a depth of about 2 m and responsible for many collapses of river dikes and reservoir dams. It is well document that most primary productives of *O. formosanus* on dikes and dames swarmed from hills or woods nearby, other than immigrated form dikes themselves (Li and Huang 1991, Wang et al. 2002, Zhong and Liu 2002). So preventing alates swarming towards dikes and dames has great control significance.

Many common lights have been setting for traffic and fishing on dikes and dames, this lights attract considerable alates to land on dams from nearby environment during annual dispersal flight season.

Most of the attention in termite management has focused on foraging workers of mature colonies. Howerer, every year swarming alates have the potential to establish new subterranean nests on river dikes and reservoir dams. This poses great dangerous for infiltration, cavity and even collapse for dikes and dams.

In this study showed that violet and blue wave bands are of high sensitivity, otherwise, red, yellow and green are low sensitive to *O. formosanus*. The high level of relative sensitivity in the violet and blue region suggests that activation of violet or blue receptor by appropriate wavelengths was causing attraction or repulsion to the source of illumination. While low sensitivity may represent indifferent. Our observation found that filament lamps exhibit the least phototaxis, sodium light exhibit less phototaxis than fluorescent lamps for *O. formosanus* alates. Further behavioral experiments need to test the practical spectral lights efficiency.

River dikes and reservoir dames termite is a serious calamity in southern China. Especially in the Pearl River Delta Economic Zone, one of China's leading economic regions that covers an area of 41,700 km^2, has 12,198 km dikes and dams, has a population of 40.8 million people. By 2001 its GDP rose to just over US$100 billion accounted for 8.7 percent of China's. Security of dikes and dams in this zone is of vitally importance to China economy development (State Statistical Bureau 2001).

This study is probably a guide for selection of dame lighting. If apply the repellent or indifferent wave band to light, settle the repellent or indifferent lights at reasonable distance, we may prevent the alates swarming to dikes and dams effectively. Our study provides a novel approach for effective dam termite management in southern China and neighboring Southeast Asia region.

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Clever Chewing: Termites Get Information from their Noisy Nibbling

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Abstract

Termites are notoriously cryptic foragers, and their foraging behaviour is notoriously fickle. How termites choose their food, especially considering that they typically encounter only one small part of it before they decide to attack it, is a mostly unanswered question. We investigated the possibility that worker termites use vibration signals in foraging. Worker termites must be able to hear vibration/acoustic signals, because termite soldiers communicate alarm using such signal, and vibro-acoustic signals could be a clue to explaining their foraging behaviour. Vibration signals were recorded for worker drywood termites of *Cryptotermes domesticus* (an pest species that is an invasive to Australia) feeding on wooden blocks of a range of sizes. The dominant frequencies were dependent on wood block size. The vibration signals from various wooden blocks were played into differently sized blocks to ascertain termite response; these playback experiments demonstrated that the termites used these frequencies to ascertain the size of the wooden block.

Progress of Biological Studies on Primary Reproductives in *Cryptotermes Domesticus* (Haviland) (Isoptera: Kalotermitidae)

by
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Abstract

Cryptotermes domesticus (Haviland) is serious pest causing wood damage. It's one of main important termites species in China. This paper is to summarize the progress of biological studies on primary reproductives' life cycle, ecological factors influence on swarming, new colony development and behavior features.

Key words: *Cryptotermes domesticus*, primary reproductives, biology

Introduction

Cryptotermes domesticus (Haviland) (Isoptera: Kalotermitidae) is a serious species termite to damage wood and wood products in the worldwide (Termite research laboratory of Guangdong entomological institute 1979, Edwardo and Mill 1986, Ping and Xiu 1997). It originated in India and Malaysia (Gay 1969, Huang et al. 1989, Li 2002). Now it has spread into Sri Lanka, Indonesia, Thailand, Singapore, Japan, Finland island, Panama, Samoa, Solomon Islands, New Britain, Society Islands, Fiji, Australia, Guma and southern provinces of China such as Taiwan, Guungdong, Guangxi, Hainan, Yunnan etc by carrying of importing wood and wood products. In Zhanjiang, Guangdong Provinces, P. R. China, its damage was much serious to wood and wood products (Gay 1969, Huang et al. 1989, Zhu et al. 1994, Li 2002).

C. domesticus was easily spread and difficult to control. In order to prohibit the spreading of *C. domesticus*, our group studied the biology of primary reproductives in *C. domesticus* in Guangzhou for many years.

Progress of biological studies on primary reproductives in *C. domesticus*

1 The life cycle of forming primary reproductives in *C. domesticus*.

C. domesticus was reared using *Pinus massoniana lamb*, *Pinus spp.*, *Pseudotsuga menziesis*, *Cyclobalanopsis spp.*, *Schima spp.*. A life cycle from a couple of primary reproductives stablishing a new colony to reproduce the new primary reproductives was investigated (Huang et al. 2005). The results indicate that finishing a life cycle at indoor temperature needed seven years in Guangzhou and six or seven years in Zhanjiang; but at constant temperature of 27℃ and relative humidity of 80%, it can complete a life cycle in two or three years (Huang et al. 2005).

2 The swarm period of primary reproductives in *C. domesticus*.

The swarm period of primary reproductives in *C. domesticus* was longer than other species and occurred yearly from middle of April to late August in Guangzhou based on the three years observation (Huang et al. 2004a). The first day of the swarm period was 15th April and the last day of that was 16th September (n=60). The swarm period was about 55~80 days and the peak occurred from May to July. The swarm time generally at 18:30~19:30, the peak was at 19:00~19:30, in which 66.66% of primary reproductives swarming about in this time (Huang et al. 2004a).

3 Influence of temperature, relative humidity and atmosphere pressure to swarming of primary reproductives in *C. domesticus*.

The relationship between the swarming of primary reproductives in *C. domesticus* and temperature, relative humidity and atmosphere pressure was observed for three years in laboratory (Huang et al. 2004b). The results indicated that the swarming of primary reproductives are suitable for broad-spectrum temperature, relative humidity and atmosphere pressure, the range of temperature, relative humidity and atmosphere pressure are respectively 25~30℃, 70%~90% and 999~1006mPa. At temperature 27~30℃, the swarming times are more, in which the most swarm times are at temperature 28~29℃, about 47.49% total swarming times. At relative humidity 70%~85%, the swarming times are more, in which the most swarm times are at relative humidity 75%~80%, about 34.77% total swarming times. At atmosphere pressure 1003~1005mPa, the swarming times are more, in which the most swarm times are at atmosphere pressure 1004~1005mPa, about 35.58% total swarming times.

4 Formation and development of new colonies in *C. domesticus*.

4.1 At indoor temperature in June & July every year in Guangzhou, the female (n=40) started to produce eggs at 8~17 days, average 11.33±2.32 days; hatching stage of eggs are 46~71 days, average 55.13±6.42 days. The numbers of offspring are 3~8 individuals in one year (n=15), 10~16 individuals respectively in two year old new colony (n=20), 12~35 individuals in three years old colony (n=5), 23~57 individuals offspring and 1~3 individuals soldiers in four year old colony (n=11). Sex mature vacation of a new colony is seven years (n=6), it has 36~115 individuals offspring and 1~4 individuals soldiers in a colony (Qian et al. 2005a).

4.2 At constant temperature 27℃ & relative humidity 80%, the female (n=30) started to produce eggs at 8~18 days, average 11.6±2.66 days; hatching stage of eggs are 50~73 days, average 57.8±5.79 days. The numbers of offspring are 6~10 individuals in a one year old colony (n=15), 16~34 individuals offspring and appearing 1~2 individuals soldiers in a two year old colony (n=16). Sex mature vacation of a new colony is 2~3 years (n=12), it has 18~40 individuals offspring and 1~2 individuals soldiers in a colony (Qian et al. 2005a).

5 Behaviour features of primary reproductives in *C. domesticus*.

5.1 Behaviour features of primary reproductives

Primary reproductives during swarming have phototaxis.

Male & female primary reproductives have no obvious tandem behaviour.

Primary reproductives losing wings like search and go deep into the concealing place.

Primary reproductives after going into holes close the mouths of holes against the disturbing of external factors.

Primary reproductives move, eat, excrete and reproduce offspring in holes.

Primary reproductives push out excrements from holes after forming colony.

Primary reproductives spread easily after forming colony (Huang et al. 2003).

5.2 Methods identifying whether termites *C. domesticus* exist in wood and wood product or not.

∅0.3~1.5 mm, closed or no closed, circular holes appear in the surface of wood and wood products.

Fine sand shape and no easy crushed excretion appear in the outside of holes (Huang et al. 2003).

6 Influences of different species of wood to new colonies of *C. domesticus*.

62 species of woods from Southeast Asia were collected and acted as the food of *C. domesticus*. Alates of *C. domesticus* were transferred to the wood for their pairing in the course of nature. After pairing, new colonies entered wood and developed new colonies inside the wood. Influences of different species of woods on termite's feeding and new colony's developing were investigated (Qian et al. 2005b). The results indicated that *C. domesticus* has broad recipe and can feed on most species of wood which have large difference in taxonomy except *Eusideroxylon zwageri*. All wood tested had no influence on alate's pairing and were caved by new couples. Dissecting the colonies after pairing for 3 months, we found that female and males survived and offspring grew normally in

most species of wood except *Artocarpus sp., Artocarpus sp., Cinnamomun sp., Eusideroxylon zwageri, Kokoona sp., Madhuca sp., Pentace sp., polyalthian sp., Pometia sp., Tectona grandis, terminalia sp.* (Qian et al. 2005b).

Conclusions

We recognized the activity regularity of primary reproductives in *C. domesticus* and relationship between colonies and ecological factors, and searched out the weak stages to control the termites based on our studies on swarming, formation, developing, reproducing, eating and life cycle of primary reproductives in *C. domesticus* for many years. Understanding the knowledge in order to reduce the damage of wood and wood products, offer assistance for effective control this termite reproduce and spread, and facilitate wood termite biology research development.

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Behavioral and Feeding Response of *Coptotermes formosanus* (Isoptera: Rhinotermitidae) to Pine Sawdust Inoculated with Five Fungus Species

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Abstract

The aggregation behavior and feeding preferences of *Coptotermes formosanus* to *Pinus massoniana* sawdust infected with five fungus species, *Ganoderma lucidum*, *Ganoderma sinense*, *Poria cocos*, *Gloeophyllum trabeum* and *Phanerochaete chrysosporium*, were examined. For each fungus species, termites were observed aggregating into sawdust infected with fungus and termites showed a strong feeding preference for the fungus-inoculated sawdust for all fungus species tested in paired choice tests. In multiple-choice tests, the results showed that termites mostly preferred sawdust infected with among *P. cocos*,*G. lucidum* or *G. trabeum* in two nest populations.

Key word: Behavioral and feeding response, fungi, *Coptotermes formosanus*.

Introduction

There is a complicated association between termites and wood decay fungi. Like many other insects, subterranean termites tend to discriminate when choosing among different food. Many studies have examined the aggregation and the feeding preferences of subterranean termite for different species of substrate inoculated with wood decay fungi. Subterranean termites prefer wood decayed by certain species of basidiomycete fungi including brown rot fungi, white rot fungi and litter rot fungi. For example, the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, showed a strong preference for sawdust or wood blocks inoculated with some species of basidiomycete fungi, *Gloeophyllum trabeum*, *Phanerochaete chrysosporium*, and *Marasmiellus troyanus* (Matsuo and Nishimoto 1973, Amburgey 1979, Cornelius et al. 2002, 2003, 2004).

In contrast, several previous studies have also reported that wood decayed by some species of white rot fungi are avoided by termites (Amburgey and Beal 1977), and that wood decayed by the white rot fungus *Ganoderma applanatum* contains compounds that are toxic to termites (Amburgey 1979). However, other studies found that wood decayed by other species of white rot fungi are attractive to termites (French et al.1981, Waller et al.1987).

The medicinal fungus including *Ganoderma lucidum*, *Ganoderma sinense* and *Poria cocos* were widely cultured popularly in China. The pine and other wood were often used as culture medium of medicinal fungi. The pine wood used as culture medium of medicinal fungi including *G. lucidum*, *G. sinense* and *P. cocos*, in most cases, were severely fed and damaged by termites (Yi 1984, Lin 1988, Jia 1991). The Objective of this study was to determine that Masson pine, *Pinus massoniana*, sawdust inoculated with three medicinal fungus, *G. lucidum*, *G. sinense* and *P. cocos*, influenced the feeding preferences of *C. formosanus*. The behavioral and feeding response of *C. formosanus* to Masson pine sawdust inoculated with two species of fungus, *G. trabeum* and *P. chrysosporium*, were also conducted the same observations for comparison.

Materials and methods

Termite Collections and Maintenance

Formosan subterranean termites, *C. formosanus*, were collected from field colonies in Heshan County, Guangdong Province of China, using underground bucket traps (Su and Scheffrahn 1986) baited with blocks of Masson pine wood *(P. massoniana)*. Termites were kept in the laboratory in 37-liter covered plastic boxes containing moist sand and blocks of pine wood until they were used in experiments. The nests of the Formosan subterranean termites that were reared by using blocks of Masson pine wood as food in 250-L covered glass boxes (100 by 50 by 50 cm) in laboratory were also used in this study.

Fungus Cultures

One isolate of the brown rot fungus, *G. trabeum*, was obtained from Research Institute of Tropical Forestry of Chinese Academy of Forestry (Guangzhou, China). One isolate of *P. chrysosporium, G. lucidum, G. sinense* and *P. cocos* were obtained from Guangdong Institute of Microbiology (Guangzhou, China).

Inoculation of Pine Sawdust with Different Fungus

Experiments were conducted using the following five different fungi: *G. trabeum, P. chrysosporium, G. lucidum, G. sinense* and *P. cocos*. The pine sawdust of 400 g and 800 ml water was mixed and placed in an autoclavable polypropylene bag (39 by 25.5 cm) (Southern Packaging (Nanhai) Limited Company) with cotton plug vent. The bag was sealed and autoclaved in 121℃ for 40 min. Potato dextrose agar (PDA) plates were inoculated with the five different fungi and placed in incubators set at 27-30℃ with a darkness condition for 3-5 d. After 3-5 d, plugs of the five different fungi were removed from the PDA plate and used to inoculate 100 ml of sterile Sabouraud dextrose broth in shake flasks of 500 ml, respectively. The broth in shake flasks was inoculated at ambient temperatures in an orbital shaker at 150 rpm for 2-3 d. After 2-3 d, 100 ml of sterile Sabouraud dextrose broth with the five different fungi was added to each autoclaved bag of 400g pine sawdust after cooling to room temperature. The vent bags were placed in ambient conditions in the laboratory for 1-3 mo during which 10-17.5% weight loss caused by the five different fungi were recorded. Bags of sawdust without fungus were prepared using the same procedure (400 g of sawdust in 800 ml of water) to serve as controls.

Paired Choice Tests with Fungus-Inoculated Sawdust Versus Sawdust without Fungus

Test 1 Bioassays Evaluating Aggregation Behavior in Sawdust

Bioassays were conducted using GLAD® ware containers (15.5 by 15.5 by 5 cm) (Clorox China (Guangzhou) Limited) with lids. Each container contained 100 g of vermiculite moistened with 300 ml of water. In each container, two 15-ml (4.8 cm in height by 2.4 cm in diameter) PET plastic bottles (Southern Packaging (Nanhai) Limited Company) were sloped embedded in vermiculite on two opposite sides at 2 cm from the container corner, respectively. Direction of the mouth of both bottles in each container was sloped up and towards a corner of container. Termites were able to move freely between the container and the both of bottles. For paired choice tests of fungus-inoculated sawdust versus control sawdust of each fungus species, there were one treatment bottle and one control bottle placed to each container. Each bottle filled with sawdust up to 2 g, and 2 ml of water was added to the sawdust in each bottle to provide moisture. Treatment bottle was filled with sawdust infected with the five fungus species and control bottle was filled with sawdust without fungus. Groups of termites of 1g (226.4±1.3 termites/g) were placed in the center of each

container. These tests were conducted in ambient conditions in the laboratory. There were three replicates each from five fungus species. These experiments lasted for 6 d. The number of termites in treatment bottle and control bottle were counted and compared using a *t*-test for matched pairs.

Test 2 Bioassays Evaluating Aggregation Behavior in Sawdust and Termite Consumption of Sawdust

The method was same as test 1 described. But, groups of termites of 2g (226.4±1.3 termites/g) were placed in the center of each container. There were six replicates each from five fungus species for the paired choice tests. These experiments lasted for 15 d. The number of termites in treatment bottle and control bottle were counted and compared using a *t*-test for matched pairs. At the same time, sawdust in the bottles were oven-dried and weighed to determine termite consumption (including sawdust fed and removed by termites). Final weights of sawdust were compared using a *t*-test for matched pairs.

Multiple-Choice Tests with Sawdust inoculated with the Five Fungus Species

Test 3 Bioassays Evaluating Termite Consumption of Sawdust in Nest of C. formosanus

Bioassays were conducted using two nests of the Formosan subterranean termites that have been reared by using blocks of Masson pine wood as food in laboratory in 250-L covered glass boxes (100 by 50 by 50 cm) for 13 years. Thirty-six 50-ml PET plastic bottles (6.6 cm in height by 3.6 cm in diameter) was placed on blocks of pine wood in the nest of the Formosan subterranean termites. The positions of the bottles in the nest were placed arbitrarily and the mouth of each bottle contacted the blocks of pine wood in the nest. Termites were able to move freely between the thirty-six bottles. These tests were conducted using the five fungus-inoculated sawdust and sawdust without fungus. Each bottles was filled with sawdust infected with the five different fungus species or sawdust without fungus up to the 5g. And 3 ml of water was added to the sawdust in each bottle to provide moisture. These tests were conducted in ambient conditions in the laboratory. There were 12 replicates, with six replicates each from two termite nests. For these multiple-choice tests, the experiments lasted for 3 d. Sawdust in the bottles were oven-dried and weighed to determine termite consumption (including sawdust fed and removed by termites). Final weights of sawdust were compared using a one-way analysis of variance (ANOVA) to determine if there were significant differences.

Results

Bioassays Evaluating Aggregation Behavior in Sawdust

Paired Choice Tests with Sawdust versus Sawdust without Fungus

In tests where pine sawdust were infected by the five fungus species, numbers of *C. formosanus* in bottles with fungus-infected material were significantly greater than numbers in control bottles after 6 and 15 days exposure (Table 1 and Table 2). When termites encountered the pine sawdust were infected by the five fungus species, they immediately clustered into bottles with fungus-inoculated sawdust. Termites were observed aggregating into the bottles with fungus-inoculated sawdust throughout the experiment.

Bioassays Evaluating Feeding Preferences in Sawdust

Paired Choice Tests with Fungus-Inoculated Sawdust versus Sawdust without Fungus

For each fungus species, termite consumption of fungus-inoculated sawdust was significantly greater than consumption of control sawdust after 15 days exposure (Table 2). Because termites

aggregated into the bottles containing sawdust infected with fungus, the aggregation effect of the sawdust infected with fungus resulted in increased consumption of the sawdust.

Multiple-Choice Tests with Sawdust Inoculated with the five Fungus species in termite nests

After 3-d exposure, consumption of sawdust infected with the five different fungus species was significantly greater than consumption of control sawdust (Fig. 1). Consumption of sawdust infected with *P. cocos* was significant greater than consumption of sawdust infected with either *G. sinense* or *P. chrysosporium*, but not significant greater than consumption of sawdust infected with either *G. trabeum* or *G. lucidum*. Consumption rates showed that termites preferred sawdust infected with the following three fungus species, *P. cocos,G. lucidum* and *G. trabeum*. There were no significant differences in the responses of termite populations of two *C. formosanus* nests to sawdust infected with fungi for different species except for *P. chrysosporium*.

Table 1. Aggregation behavior of termites in paired choice tests after 6-d exposure

Fungus species	Number of termites/bottle	
	Fungus-inoculated	No fungus
G. lucidum	206.7 ± 9.7	5.7 ± 5.7*
G. sinense	134.3 ± 30.0	0*
G. trabeum	125.3 ± 46.1	0*
P. cocos	118.3 ± 22.4	0.7 ± 0.7*
P. chrysosporium	153.7 ± 42.5	0.7 ± 0.7*

*P<0.05, *t*-test.

Table 2. Aggregation behavior and feeding preferences of termites in paired choice tests after 15-d exposure

Fungus species	Number of termites/bottle		Weight loss of sawdust/bottle (g)	
	Fungus-inoculated	No fungus	Fungus-inoculated	No fungus
G. lucidum	156.5 ± 15.8	32.3 ± 18.2*	1.13 ± 0.09	0.18 ± 0.03*
G. sinense	179.7 ± 21.8	50.0 ± 15.8*	1.33 ± 0.08	0.29 ± 0.09*
G. trabeum	164.8 ± 30.0	82.8 ± 27.8*	1.29 ± 0.08	0.45 ± 0.05*
P. cocos	222.0 ± 17.1	31.5 ± 14.5*	0.92 ± 0.06	0.49 ± 0.08*
P. chrysosporium	165.5 ± 32.7	54.0 ± 33.9*	1.43 ± 0.12	0.18 ± 0.05*

*P<0.05, *t*-test.

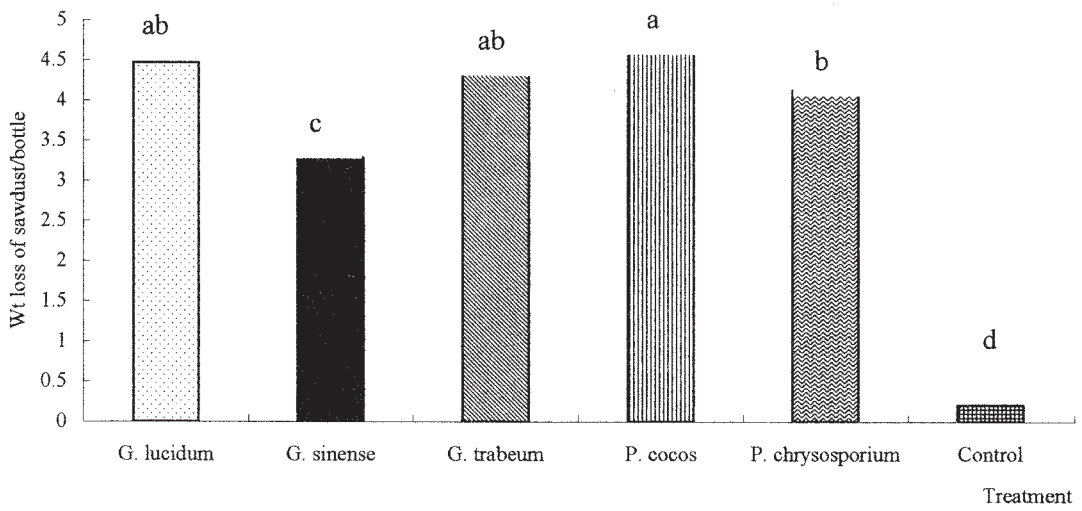

Fig. 1. Feeding preferences of termites in multiple-choice tests in termite nests after 3-d exposur

Discussion

In conclusion, the sawdust inoculated with among fungi tested clearly affected termite aggregation and feeding behavior. *C. formosanus* showed a strong aggregation and preference for the fungus-inoculated sawdust for all fungus species tested. Our results confirmed several previous studies that *C. formosanus* prefer wood decayed either G. *trabeum* or *P. chrysosporium* (Amburgey 1979, Cornelius et al. 2002, 2003). Moreover, this study is the first report of termite aggregation and preferences to pine sawdust inoculated with among *G. lucidum*, *G. sinense* or *P. cocos* that were cultured widely in China. We are also the first determination of termite preferences to pine sawdust inoculated with wood decay fungus in multiple-choice tests in termite nest. Further studies are necessary to determine the behavioral response of termites to various weight loss of sawdust infected with different fungus.

There is significance to understand mechanism of preferences of termites to wood decay fungi for better control populations of termite pests. There are other species of wood rot fungi that may produce chemicals that act as cues to foraging termites (Esenther and Beal 1979). These chemicals could potentially be used to direct termite foraging toward bait stations in the field. Wood decay fungi have the potential to develop more effective bait for controlling the Formosan subterranean termite. Moreover, some baits formulation using material infected with wood decay fungi have been used for control of termite, including *C. formosanus*, *Odontotermes formosanus* (Shiraki) and *Macrotermes barneyi* Light, and obtained better control of termite populations in China (Gao 1985, Luo et al. 1988, He et al.1997, Li et al. 2001). It is worthwhile to developing bait system containing material inoculated either *G. lucidum* or *P. cocos* for management of termites.

Wood decayed by the brown-rot fungus, *G. trabeum*, elicits trail-following and aggregation behavior in *Reticulitermes* spp. and *Coptotermes formosanus* (Esenther and Beal 1979, Tokoro et al.1992, Rust et al.1996). However, bioassays of active fractions of *Serpula lacrymans* indicated that trail-following activity of termites to extracts of wood decayed by *S. lacrymans* was not due to the compound isolated from wood decayed by *G. trabeum* (Ohmura et al.1995). Su (2005) considered that the attractants produced by decayed wood that may locate food source by termites were not isolated and identified, but they are most likely water-soluble. Therefore, the chemicals produced by

wood decay fungi that may elicit trail-following activity in termites should be diverse. Many studies are necessary to isolate and identify the chemicals causing the behavioral response of termites.

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Identification of Two Subterranean Termite Species using the Loop-Mediated Isothermal Amplification (LAMP) Method with Sequences of Endo-β-1,4-glucanase Genes of Hindgut Protozoa

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Abstract

To discriminate *Coptotermes formosanus* Shiraki and *Reticulitermes speratus* (Kolbe), forward- and backward-inner primers and outer primers were designed for loop-mediated isothermal amplification (LAMP) based on partial sequences of the DNA that encodes endo-ß-1,4-glucanase *of Holomastigotoides hartmanni*, a symbiotic protozon of *C. formosanus*, and EG homologue of a hindgut symbiont of *R. speratus*. Using these primers, the LAMP method successfully amplified genomic DNA extracted from whole gut in which symbiotic protozoa are harbored, but failed in amplifying genomic DNA extracted from galleries constructed by the termites.

Key words: loop-mediated isothermal amplification, endo-ß-1,4-glucanase, symbiotic protozoa, *Coptotermes formosanus*, *Reticulitermes speratus*

Introduction

Coptotermes formosanus Shiraki and *Reticulitermes speratus* (Kolbe) are the most widespread termite pests on the Japanese mainland (Yoshimura, 1996). Features of the soldier castes of these termites, such as head shape and length, are commonly used as criteria for the identification based on morphological characters (Morimoto, 1994). Worker caste termites of these species lack some defining features, so it is difficult to identify them based only on the morphological features of worker castes. Polymerase chain reaction (PCR) successfully discriminates these termites using nucleotide sequences encode endo-ß-1,4-glucanase (EG) of termite origin (Itakura *et al.*, 2006b). However, PCR uses heat denaturation of double-stranded DNA products to promote the next round of DNA synthesis and requires a precise and costly thermal cycler for amplification. Loop-mediated isothermal amplification (LAMP) is a simple and easy-to-perform method that requires only four primers, a pre-mixed solution containing buffer and dNTP, a DNA polymerase, and a water bath or heat block for the reaction to occur. LAMP has the advantage of enabling DNA amplification to be carried out with high efficiency under isothermal conditions and is highly specific for the target DNA (Notomi *et al.*, 2000).

Using the primers designed for discriminating the single nucleotide polymorphisms on the genomic DNA that encodes EG of termites, the LAMP method had been examined previously and successfully amplified genomic DNA that was extracted from the heads of *C. formosanus* and *R. speratus*, but failed in amplifying genomic DNA extracted from galleries of these termites (Itakura *et al.*, 2006a). The identification of termite species by the LAMP method using genomic DNA of symbiotic protozoa has not been reported. Galleries are built of particles of soil and wood and fecal material (rectal content) deposited via the anus (Noirot and Darlington, 2000). In case symbionts are excluded as a part of feces used for building material of galleries, it could be possible to distinguish termite species by LAMP using nucleotide sequences encode EG and EG homologue of the symbiotic protozoa. The precise identification of termite species is essential for appropriate pest control operations. Correct identification of termite species from galleries would be valuable for obtaining data on the history of termite infection to house.

Materials and methods

Insects: *C. formosanus* individuals were collected from a nest that had been maintained with blocks of *Pinus densiflora* as the food source at 26°C for 6 years in our laboratory. *R. speratus* individuals were collected from a wild colony located in infested wood in the Wakayama Prefecture, Japan, and maintained with their nest materials and filter paper at 26°C for a few months in our laboratory. Worker-caste individuals were used for all experiments.

Preparation of genomic DNA from termites and their galleries: Genomic DNA was extracted from

the head and whole gut of 20 worker-caste individuals of *C. formosanus* and *R. speratus* using a DNeasy tissue kit (Quiagen), according to the manufacturer's recommended conditions. To identify termite species using genomic DNA that was remaining in the termite's galleries, genomic DNA was extracted from the galleries that were constructed by *C. formosanus* and *R. speratus*. Approximately 0.5 g of wet gallery was collected from the container maintaining *C. formosanus* or *R. speratus*; this gallery was then used for genomic extraction using an ISOIL (Nippon gene). The amount of extracted DNA was determined by spectrophotometric assay (Sambrook *et al.*, 1989a).

Oligonucleotide primers: To amplify 150 or 154 bp fragments (between F2 and B2 in Fig. 1) from genomic DNA that encodes EG or EG homologue by the LAMP method, the forward inner primer (FIP), the backward inner primer (BIP) and the two outer primers (called B3 and F3) were designed (Table 1) using the web-based Primer Explorer (http://primerexplorer.jp/). Primer FIP consisted of the complementary sequence of F1c and F2, whereas primer BIP consisted of B1c and the complementary sequence of B2. The two outer primers of F3 and B3 were F3 sequence and the complementary sequence of B3, respectively (Table 1).

LAMP of genomic DNA: LAMP was performed in a Cool thermo unit CTU-N (Taitec) with the primers FIP (Cf), BIP (Cf), F3 (Cf) and B3 (Cf), or with the primers FIP (Rs), BIP (Rs), F3 (Rs) and B3 (Rs). Each reaction (25 μl) contained 0.8 μM of each FIP and BIP, 0.2 μM of each F3 and B3, 8 units of *Bst* DNA polymerase, 12.5 μl of pre-mixed solution (Eiken chemical, 1.4 mM of each dNTP, 0.8 M betaine, 20 mM Tris-HCl (pH8.8), 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 8 mM $MgSO_4$, 0.1% Tween 20, in final concentration) and 50 ng of template DNA. In the LAMP with the primers for symbiont of *R. speratus*, the concentration of betaine was raised from 0.8 M to 1.05 M to prevent unspecific LAMP-amplification of DNA. The template DNA was preheated at 95°C for 5 min and then chilled on ice for 3 min, before addition to the mixture. The mixture was incubated at 63°C for 1 h followed by heating at 80°C for 5 min to terminate the reaction.

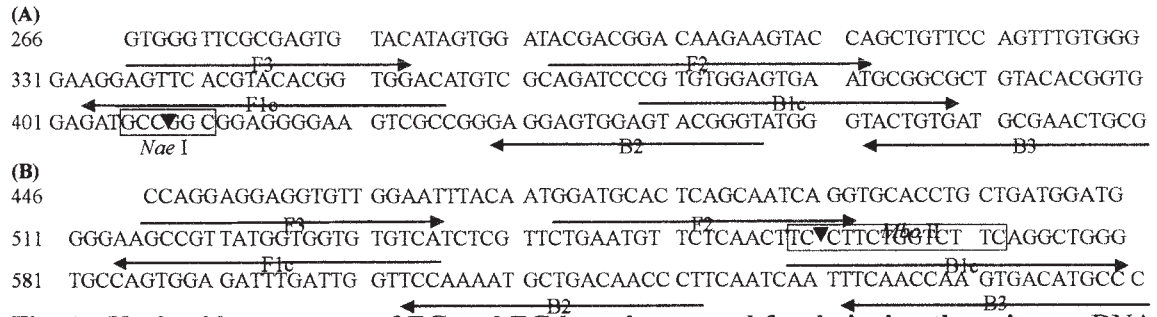

Fig. 1 Nucleotide sequences of EG and EG homologue used for designing the primers. DNA sequences used for primer design are shown by arrows. The restriction sites for *Mbo* II and *Nae* I are indicated by boxes. Numbers at the left end of sequences correspond to the position in (A) EG of *H. hartmanni* (*H. mirabile*, GenBank accession no. AB 071011) and (B) EG homologue of *R. speratus* hindgut symbiont (GenBank accession no. AB045179).

Table 1 Oligonucleotide primers for amplification of EG and EG homologue of symbionts.

Primers	Sequence (5'→3')
EG of *H. hartmanni* (*H. mirabile*)	
FIP (Cf)	GTCCACCGTGTACGTGAACTCC-ACGACGGACAAGAAGTACCA
BIP (Cf)	CGTGTGGAGTGAATGCGGCG-ACCCGTACTCCACTCCTC
F3 (Cf)	GTGGGTTCGCGAGTGTAC
B3 (Cf)	CGCAGTTCGCATCACAGTA
EG homologue of hindgut symbiont of *R. speratus*	
FIP (Rs)	TGACACACCACCATAACGGCTT-GGATGCACTCAGCAATCAGG
BIP (Rs)	TCCTTCTGGTCTTCAGGCTGGG-AGGGTTGTCAGCATTTTGGA
F3 (Rs)	CCAGGAGGAGGTGTTGGAAT
B3 (Rs)	GGGCATGTCACTTGGTTGAA

Analysis of LAMP-amplified product: Aliquots of 10 μl of LAMP-amplified products were electrophoresed in 1% agarose gels with 0.5 × TBE, followed by staining with ethidium bromide. For digestion by restriction enzymes, 25 μl of LAMP-amplified products were concentrated by ethanol precipitation (Sambrook *et al.*, 1989b). The pellet that was recovered by ethanol

precipitation was dissolved in 10 μl of water followed by addition of restriction enzyme and L buffer (10 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 1 mM dithiothreitol). Aliquots (3 μl) of dissolved DNA that was LAMP-amplified with the primers for *H. hartmanni* was incubated with 10 units of *Nae* I (Takara-bio), whereas aliquots (3 μl) of dissolved DNA that was LAMP-amplified with the primers for symbiont of *R. speratus* was incubated with 7 units of *Mbo* II (Toyobo) at 37°C for 3 h, respectively. Aliquots of 10 μl of the *Nae* I and *Mbo* II digested products were electrophoresed as mentioned previously.

Results and discussion

The LAMP reaction produced many bands of different sizes over ~150 bp (Fig. 2, lanes 2 and 9). The specific primers for *H. hartmanni*, FIP (Cf), BIP (Cf), F3 (Cf) and B3 (Cf), successfully produced bands from the genomic DNA of symbionts of *C. formosanus*, but failed to produce bands from the genomic DNA of symbionts of *R. speratus* as well as from the genomic DNA of both termites. On the contrary, the specific primers for hindgut symbiont of *R. speratus*, FIP (Rs), BIP (Rs), F3 (Rs) and B3 (Rs), formed the bands not from the genomic DNA of both termites and symbionts of *C. formosanus* but from the genomic DNA of symbionts of *R. speratus*.

Amplified products by LAMP are observed as many bands on the agarose-gel electrophoresis due to the mechanism of LAMP. In the LAMP method (Notomi *et al.*, 2000), the inner primer FIP hybridizes to F2c in its complementary strand and initiates sense-strand synthesis. The outer primer F3 slowly hybridizes to F3c in the complementary strand and initiates DNA synthesis to displace the strand that was synthesized with FIP, which forms a looped-out structure at one end by hybridization between the complementary sequence of F1c on the FIP and the sense-strand sequence of F1 on the strand synthesized with FIP. This single-strand DNA with the loop structure serves as the template for BIP-initiated DNA synthesis and subsequent B3-primed DNA synthesis to displace the strand synthesized with BIP, which forms stem-loop DNA by self-primed DNA synthesis. FIP hybridizes with F2c (the complementary sequence of F2), then the single-strand DNA in the loop of the stem-loop DNA structure primes DNA synthesis and displacement of the double-strand DNA. As a result, another stem-loop DNA with B2c (a sense-strand sequence) in the loop of the stem-loop DNA structure is produced. BIP hybridizes with B2c in the loop of the stem-loop DNA structure to prime DNA synthesis and displacement of the double-strand DNA. The final products are a mixture of a stem-loop DNA with various stem lengths. The anticipated structures of LAMP-amplified products are schematically represented using 6 repeating-units of F+ ; 5'-F1c-F2-(flanking sequence)-F1-3', F- ; 5'-F1c-(flanking sequence)-F2c-F1-3', B+ ; 5'-B1c-(flanking sequence)-B2c-B1-3', B- ; 5'-B1c-B2-(flanking sequence)-B1-3', + ; sense strand between F1 and B1c, and - ; antisense strand between B1 and F1c, as shown in Fig. 4.

To confirm the structure, the LAMP-amplified products were digested with the restriction endonucleases and analyzed using electrophoresis. The LAMP-amplified products of *H. hartmanni* were expected to be fragmented to 191 and 227 bp fragments, whereas those of symbiont of *R. speratus* were expected to be fragmented to 82 and 135 bp fragments (Fig. 4). As shown in Fig. 3, the products of *H. hartmanni* were digested to approximately 180-190 and 220-230 bp fragments, whereas the products of symbiont of *R. speratus* were digested to 140-150 bp fragments. Although the band of ~82 bp was veiled with the bands of primers on the agarose gel (Fig. 3, lane 4), these patterns of fragmentation were almost consistent with the expected sizes.

If the DNA remaining in the galleries could be used as a DNA template for LAMP, it would be possible to build up a history of termite invasion into house, and the invading termite species would be easily detected. Such information would help to diagnose termite attack to house. As shown in Fig. 5, LAMP amplification both with the specific primers for *H. hartmanni* and hindgut symbiont of *R. speratus* failed in the amplification of genomic DNA of symbionts of *C. formosanus* and *R. speratus* that was extracted from the galleries made by termites. The ratios of absorbance at 260-280 nm of extracted DNA from the galleries of *C. formosanus* and *R. speratus* were 1.85 and 1.77, respectively. These values were very similar to the ratio of pure DNA (approximately 1.8). This implies that substances as phenolic compounds, which inhibit elongation of nucleotide strand by DNA polymerase (Suhara *et al.*, 2005), would not contaminate the extracted DNA. The DNA extracted from the galleries could come from bacteria inhabit the galleries, this should cause the fault for amplification of the genomic DNA of symbionts.

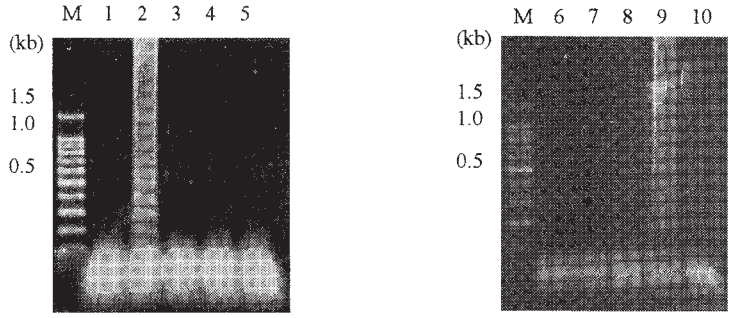

Fig. 2 Elctrophoretic analysis of the LAMP-amplified products. Genomic DNA from head and whole gut of *C. formosanus* and *R. speratus* were amplified by LAMP method with the specific primers shown in Table 1. Lane M, 100-bp ladder marker; lanes 1-10, LAMP amplification with the primers for *H. hartmanni* (lanes 1-5) and for symbiont of *R. speratus* (lanes 6-10); genomic DNA from *C. formosanus* (lanes 1 and 6), from *R. speratus* (lanes 3 and 8), from whole gut of *C. formosanus* (lanes 2 and 7) and from whole gut of *R. speratus* (lanes 4 and 9) was amplified, respectively; lanes 5 and 10, negative controls (LAMP without DNA).

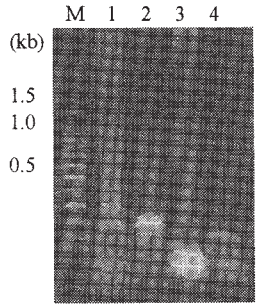

Fig. 3 Digestion of the LAMP-amplified products by restriction enzymes. Lane M, 100-bp ladder marker; lanes 1 and 2, LAMP-amplified genomic DNA from whole gut of *C. formosanus* with the primers for *H. hartmanni*; lanes 3 and 4, LAMP-amplified genomic DNA from whole gut of *R. speratus* with the primers for symbiont of *R. speratus*; the LAMP-amplified products were digested with *Nae* I (lane 2) and *Mbo* II (lane 4).

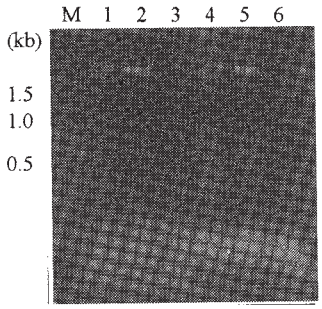

Fig. 5 LAMP of genomic DNA extracted from galleries of the termites. Genomic DNA extracted from the galleries made by *C. formosanus* and *R. speratus* was LAMP-amplified. Lane M, 100-bp ladder marker; lanes 1-6, LAMP with the primers for *H. hartmanni* (lanes 1-3) and for symbiont of *R. speratus* (lanes 4-6); genomic DNA from galleries of *C. formosanus* (lanes 1 and 4) and *R. speratus* (lanes 2 and 5) were amplified; lanes 3 and 6, negative controls (LAMP without DNA).

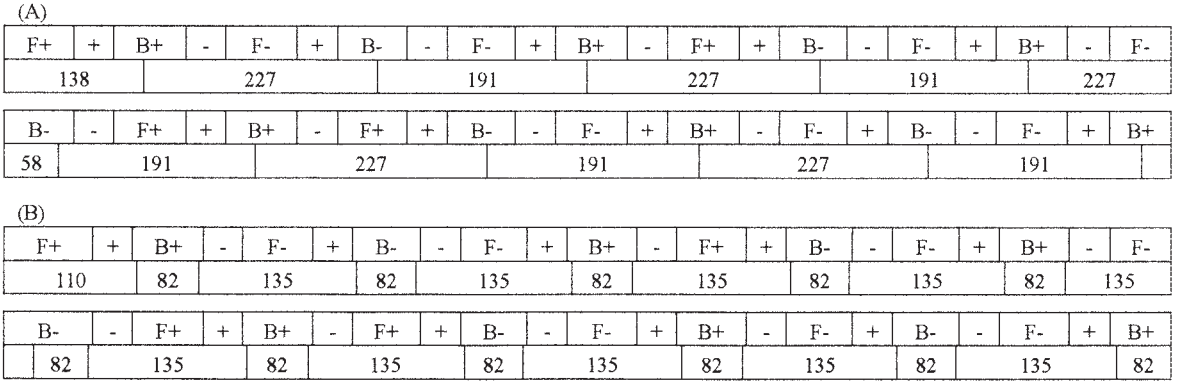

Fig. 4 Schematic representation of the anticipated structure of LAMP-amplified products and the sizes of the restriction fragments. F+ , 5'-F1c-F2-(flanking sequence)-F1-3'; F- , 5'-F1c-(flanking sequence)-F2c-F1-3'; B+ , 5'-B1c-(flanking sequence)-B2c-B1-3'; B- , 5'-B1c-B2-(flanking sequence)-B1-3'; + , sense strand between F1 and B1c ; - , antisense strand

between B1 and F1c. The restriction sites for *Nae* I and *Mbo* II are shown as vertical lines and the sizes of the restriction fragments are in the boxes; (A) LAMP-amplified genomic DNA from whole gut of *C. formosanus* with the primers for *H. hartmanni*; (B) LAMP-amplified genomic DNA from whole gut of *R. speratus* with the primers for symbiont of *R. speratus*.

Conclusions

The specific LAMP primers for *H. hartmanni* and for hindgut symbiont of *R. speratus* successfully discriminated the termite species by amplifying genomic DNA extracted from whole gut of host insects. However, the primers failed in amplification of genomic DNA extracted from termite's galleries, so a history of termite invasion into house was not demonstrated by LAMP using DNA from galleries and the primers in this study.

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Phylogenetic relationship of the Asian subterranean termite, *Coptotermes gestroi* (Wasmann) and Philippine milk termite, *Coptotermes vastator* Light (Isoptera: Rhinotermitidae) as inferred from 16S mitochondrial DNA

by

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Abstract

The Asian subterranean termite, *Coptotermes gestroi* (Wasmann) and the Philippine milk termite, *Coptotermes vastator* Light were compared using molecular phylogenetic technique. Partial sequence of ribosomal RNA large subunit 16S was obtained from 7 colonies of *C. gestroi* and 4 colonies of *C. vastator*. In addition, 4 colonies of *C. formosanus* Shiraki with *Globitermes sulphureus* (Haviland) were used as the outgroups. DNA sequencing of the 16S ribosomal DNA amplicon revealed an average size of 428 bp. Consensus sequences were obtained and aligned using the BioEdit v7.0.5 software. *C. vastator* and *C. gestroi* were likely synonymous based on the DNA sequence with differences detected at only 3 base pairs across the partial 16S gene. On the basis of partial 16S rDNA sequences determined, phylogenetic trees were constructed using maximum parsimony, likelihood, and distance methods. The results revealed 2 minor subclades of *C. gestroi* and *C. vastator* within a major clade. The interspecific pairwise sequence divergence, base on uncorrected "p" distance between *C. gestroi* and *C. vastator*, varied up to only 0.79%. Other findings that further support the synonymity are discussed.

Keywords: *Coptotermes gestroi*, *Coptotermes vastator*, 16-S ribsomal DNA, molecular phylogenetic, synonymous.

Introduction

Amongst the termite genera within Rhinotermitidae, the genus *Coptotermes* is probably regarded as one of the most important genera. Several species of *Coptotermes* including the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, and the Asian subterranean termite, *Coptotermes gestroi* (Wasmann) have been known for their destructive nature to buildings and structures in the subtropical and tropical regions, respectively. Globally, *C. formosanus* accounted a substantial amount of the USD 22 billion of termite damage yearly (Su 2002). In Malaysia, Thailand and Singapore, *C. gestroi* contributed more than 85% of the total termite damage in buildings and structures in the urban area (Lee 2002; Lee *et al.* 2003). Despite being located in the tropics, *Coptotermes vastator* Light is the primary subterranean termite species of urban environment in the Philippines, and not *C. gestroi*. *C. vastator* is a serious structural pest that accounted for >90% of the termite damages to timber and wooden structures. These damages cost nearly USD 1 million to residential and commercial properties of the Mariana Islands and between USD 8 and 10 million for the damages in and around Manila (Yudin 2002). Both *C. gestroi* and *C. vastator* are very similar based on analysis of morphological characteristics, and it was long suspected that *C. vastator* is a junior synonym of *C. gestroi* (Kirton & Brown 2003; Kirton 2005). However, no attempt has been executed so far to address this issue from molecular phylogenetic perspective. Molecular phylogenetic analyses are able to reveal the relationship among the populations and differentiate species regardless of the termite caste (Szalanski *et al.* 2003). In this study, we used 16S mitochondrial gene to elucidate the relationship between *C. gestroi* and *C. vastator*.

Materials and methods

Morphology: Samples were collected or obtained as shown in Table 1. They were preserved in absolute ethanol (Table 1). Micrographs pictures were made of the heads, mandibles, and whole bodies for each species. Morphometric measurements of the length of mandible, maximum width of head, and length of head were measured for 10 soldier termites for all 7 colonies of *C. gestroi*, 4 colonies of *C. vastator*, and 4 colonies of *C. formosanus*.

Table 1. Summary of the samples and information used in this study.

Sample code/ Gene bank accession no.	Species	Collection site	Collector
CG001MY	*C. gestroi*	Malaysia, Penang, USM.	B.K. Yeap
CG004MY	*C. gestroi*	Malaysia, Kuala Lumpur, Bangsar.	K.T. Koay
*CG005MY	*C. gestroi*	Malaysia, Muar.	C.Y. Lee
CG001SG	*C. gestroi*	Singapore, Serenity Terr.	SPMA
CG002SG	*C. gestroi*	Singapore, Serangoon.	SPMA
CG001TH	*C. gestroi*	Bangkok, Thailand1.	V. Charunee
CG002TH	*C. gestroi*	Bangkok, Thailand1.	V. Charunee
CF001JP	*C. formosanus*	Japan, Wakayama.	T. Yoshimura
CF002JP	*C. formosanus*	Japan, Wakayama.	T. Yoshimura
CF003JP	*C. formosanus*	Japan, Okayama.	T. Yoshimura
CF001HW	*C.formosanus*	USA, Hawaii, Oahu.	J. Yates III
CV001HW	*C. vastator*	USA, Hawaii, Oahu.	J. Yates III
CV001PHI	*C. vastator*	Los Banos, Laguna Philippines, colony1	C. Garcia
CV002PHI	*C. vastator*	Los Banos, Laguna Philippines, colony2	C. Garcia
CV003PHI	*C. vastator*	Los Banos, Laguna Philippines, colony3	C. Garcia
GS001MY	*G. sulphurues*	Malaysia, Penang, USM.	B.K. Yeap
AY302709	*C. gestroi*	Thailand, Bangkok.	
AY558907	*C. gestroi*	USA: Miami, Florida	
AY558906	*C. gestroi*	Turks and Caicos Islands, Grand Turk.	
AY558905	*C. gestroi*	Antigua and Barbuda	
AY302713	*C. vastator*	Philippines: Wedgewood	
AY302712	*C. vastator*	Philippines: Manila	
AY302711	*C. vastator*	USA: Honolulu, Hawaii	

*Dried specimens.

DNA Extraction: The specimen preserved in absolute ethanol was washed with distilled water and dried on a filter paper. Total genomic DNA was extracted from single termite using Dneasy tissue kit manufactured by QIAGEN (Valencia, CA). Extracted genomic DNA from each sample was used as polymerase chain reaction (PCR) template. PCR amplification was performed in a standard 25- µl reaction volume with 2µl of total genomic DNA, 1 pmol of each primer, 1.5 mM $MgCl_2$, 2 mM dNTPs, and 5U/µl Taq DNA polymerase. Amplification was accomplished in a MJ Research PTC-200, Peltier Thermol Cycle, with a profile consisting of a precycle denaturation at 94°C for 2 min, a postcycle extension at 72°C for 10 min, and 35 cycles of a standard three-step PCR (53.1°C annealing). Reaction conditions were optimized with respect to $MgCl_2$ concentration and annealing temperature. Primer set of LR-J-13007 (TTA CGC TGT TAT CCC TAA) and LR-N-13398 (CGC CTG TTT ATC AAA AAC AT) was used to amplify mtDNA 16S gene. Two µl of each PCR product was visualized by UV transillumination on a 1.2% agarose gel containing 0.5 mg/ml ethidium bromide. Double-stranded PCR products were purified using SpinClean Gel Extraction Kit (column) and subjected for direct sequencing.

Data Analysis: BioEdit v7.0.5 software was used to edit individual electropherograms and to form contigs. Multiple consensus sequences were aligned using CLUSTAL X. The alignment results were adjusted manually for obvious alignment errors. The data were imported into PAUP4.0 (Swofford 2000) and analyzed to generate maximum likelihood, neighbour-joining (NJ), and parsimony bootstrapped trees, based on nucleotide data. A bootstrap test was used to test the reliability of trees (Felsenstein 1985). Using the heuristic search option, 1000 replicates were performed and 50% majority rule consensus trees were generated. Gaps were treated as missing data.

Results and Discussion

Morphology: The soldier termites were used for morphological studies. *C. formosanus* was readily distinguished from *C. vastator* and *C. gestroi* with two pairs of setae projecting dorse-laterally from the base of the fontanelle, compared with only a pair of setae in the latter two species. It is difficult to distinguish *C. vastator* from *C. gestroi* by the size, shape of its postmentum and head, as they are highly parallel. As reported in Kirton and Brown (2003), there is a continuous variation in size and shape of a single species. The morphology of termites can be influenced by the age and state of the colony, or the environment of the habitat. Furthermore, additional variability in coloration may be attributed to sample age and storage condition (Scheffrahn *et al.* 2005).

Nucleotide analyses: Average amplicon size of 16S gene resulting from DNA sequencing was approximately 428 basepairs (bp). Thirty five bp from the 5' end of the amplicon was excluded in the analysis to facilitate genetic comparisons with existing GenBank DNA sequences. The average nucleotide compositions among *Coptotermes* species (excluding GenBank sequences) for 16S gene for A, C, G and T are 42.82%, 24.61%, 10.57%, and 22.00%, respectively. The multiple sequences alignment for 16S gene, including the outgroup taxon resulted in a data matrix with 385 characters, of which 326 are constant and 20 parsimony-informative. The interspecific pairwise sequence divergence based on uncorrected "p" distance between *C. gestroi* and *C. vastator* ranges from 0 to 0.79% across the entire 16S gene. On the other hand, the divergence values between the *Coptotermes* species and the outgroup vary from 12.45 to 13.75% in 16S gene. From the aligned data matrix, there were only 3 characters (at base -39, -103, and -135) in the 16S of *C. vastator* which were different from those of *C. gestroi*. The divergence values and the high similarity in the sequences suggested that *C. gestroi* and *C. vastator* are conspecific.

Phylogenetic relationships inferred from 16S gene: Phylogenies derived using maximum parsimony, neighbour-joining and maximum likelihood methods showed the same tree topology. Only the tree from maximum parsimony analysis is shown here (Fig.1). The parsimony analysis using the heuristic search algorithm of the aligned sequences yielded a single maximally parsimonious tree with 68 evolutionary steps, index of consistency (CI) of 0.971 and 0.976 retention index (RI). The robustness of the trees was tested by bootstrapping with 1000 replicates. Nodes with less than 50% support were collapsed. The strict consensus tree (Figure 1) consisted of two clades with strong bootstrap support of 99% and 100%. The first clade comprises *C. gestroi* and *C. vastator* from various populations. There is only one or two changes along the branches between *C. gestroi* and *C. vastator* which falls within the same clade. The second clade is composed of *C. formosanus* from Japan and Hawaii. *C. vastator* has been previously suggested to be closely related to the *C. gestroi* based on morphological characters under the *C. gestroi* complex (Kirton and Brown 2003; Kirton 2005). The results of the analysis of mitochondrial gene sequences in this study further support these earlier reports.

Geographical distribution: C. gestroi is a native species of Asia. Zoogeographical regions of this species include Nearctic, Neotropical, and Oriental. In the New World tropics, *C. gestroi* was first reported in Brazil in 1923 and in Barbados in 1937. Later, the distribution of this species is endemic to Antigua and Barbuda, Grand Cayman, Grand Turk, Jamaica (Montego Bay and Port Antonio), Little Cayman, Montserrat, Nevis, Providenciales, Peurto Rico (San Juan), St. Kits, and United States. It has also been collected in southern Mexico (Scheffrahn and Su 2000). There is limited information on the distribution of *C. vastator*. Thus far, it is a notably pest in Guam and the Philippines as well as Hawaii.

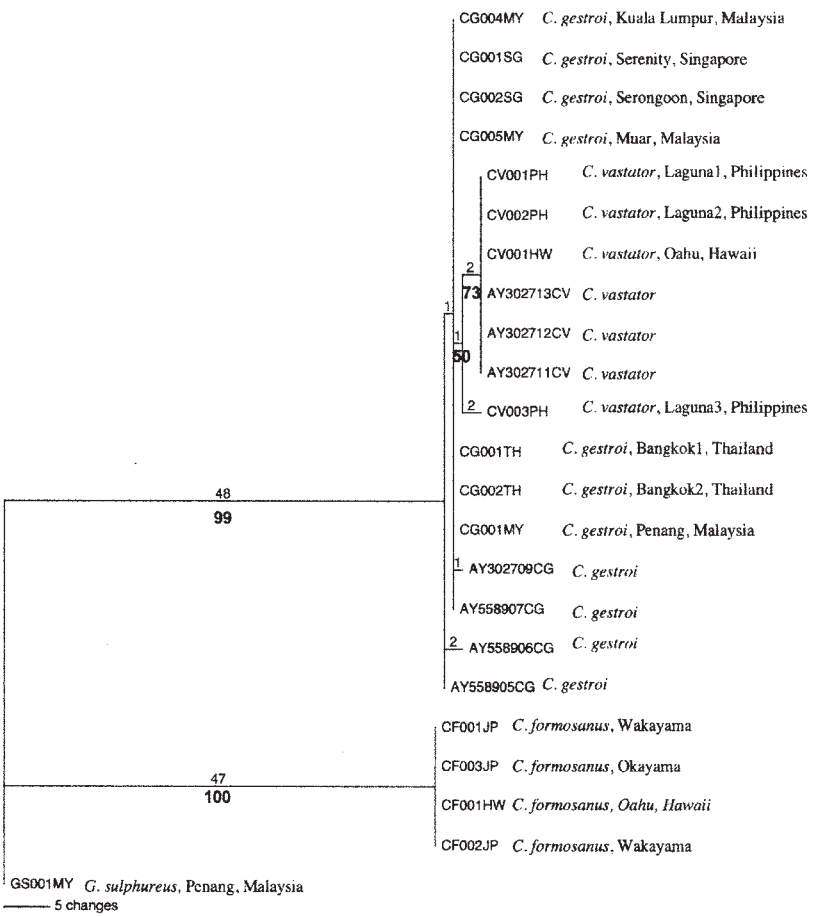

Fig. 1. Single most parsimonious tree obtained from 16S gene sequences. Branch lengths are drawn proportional to the number of changes per branch. Numbers of changes are shown above the branches while bootstrap values (1000 replicates) are mapped under the branches.

Summary

Based on the outcome of this study, it is suggested that *C. vastator* is a junior synonym of *C. gestroi*. More studies are currently being undertaken to further substantiate current findings.

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Termite Nests in Dam Site Foundation before Construction

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Abstract

Among termite species already found so far in Vietnam dams, 51 species under Termitidae family are found locating their nests in ground at various diameters and depths. At dam site foundation, the taking way of the top layer by 0.3~0.5 m during the construction process has helped to remove most of shallow- and small-nested species except Macrotermes and Odontotermes genera which are mostly still existent under the dam site foundation. This trouble has been existing for a long period without given due concern as compared with such other troubles as geology, hydrogeology, soil mechanic, rock mechanic. In order to assure the stability to the dam, the author proposed a standard for the investigation and control of termite nests in dam site foundation right before the construction, including the investigation and elimination of all termite colonies of Odontotermes and Macrotermes in the foundation and adjacent area, filling up by the way of paste injection of all the holes caused by termites in the foundation.

Key words: Macrotermes, Odontotermes, dam site foundation, treatment, standard 14-TCN-88-93.

Introduction

Nowadays, before construction of dams, various surveys are conducted like topography, geomorphology, geology, hydrogeology... together with various laboratory and site experiments, with a view to detect and remove harmful elements to the dams in terms of stabilisation and infiltration. During surveying process, such interstice of even several millimeters wide will be studied thoroughly to investigate their base infiltration possibility, however, much bigger and/or deeper termite nests are out of the norms to be investigated and treated before construction of dams. In 1982, during the ground-breaking for construction of Dau Tieng dam in South Vietnam, constructors found hundreds of termite nests locating several meters beneath the base, thus embarrassed technical managers because technical norms by that time contained no article like this, while construction standards only allowed the removal of 0.3~0.5 m of the top layer of the dam site foundation. This meant that deep and /or big holes caused by termites still existent under the ground base. This report will make a clarification about this issue.

I - Termitidae-family termite species in dams in Vietnam

In the list of 64 species of 4 families already found in dikes and dams of Vietnam, only Termitidae (51 species) dig their nests in the earth at different sizes and depths. Basing on the depth from the natural earth ground to the main chamber, we divide 51 species in this family into different groups:

Group 1 - including Odontotermes, Hypotermes and hypogenous nests of Macrotermes. In this group, main mature chambers have diameter of 0.4~0.6 m up and 0.7~3 m depth. Above and around the main chamber of Odontotermes species, normally there are many small auxiliary ones. A typical example of this Group is Odontotermes hainanensis.

Group 2 - including Macrotermes species having epigenous nests, and Odontotermes hainanensis. The species under this Group build their nest as epigenous earth mound above the ground, and a mature nest may have diameter of approximately 1 m up to 4~5 m. The earth made up the mound is taken by termites from the deep ground, therefore the bigger the nest is, the emptier the earth ground will be. In addition, their tunnel network will have large section and may go deep down

to even more than 10m, and far to even 100m. A typical example of this Group is Macrotermes gilvus.

Group 3 - including remaining genus under Termitidae family like Microtermes, Microcerotermes, Termes, Globitermes, Bulbitermes, Nasutitermes, Procapritermes, Pseudocapritermes, Pericapritermes, Microcapritermes, Discuspiditermes... Nests of most genus in the Group are all small, or just tiny and superficial interstices locating 0.2~0.3 m down from the earth surface, or up on the trees. A typical example for this Group is Microtermes dimorphus.

As an exceptional case, Globitermes sulphureus builds their special type epigenous nest, but not from earth material, thus not emptying the earth base very much.

II - Effects of the excavation of the top layer of the foundation during dam construction process according to current norms in Vietnam

According to current norms of Vietnam, the top layer of dam site foundation will be removed about 0.3 or 0.5 m depending on each case. What kind of efficiency that such removal brings to the elimination of empty holes created by termite nests in the natural foundation?

- For termite nest belonging to Group 1: though the excavated layer is 0.3 or 0.5 m, only subsurface auxiliary chambers of Odontotermes species will be taken away, while main chambers are untouched, i.e the termite colony and most important hollow chamber they create will continue to exist below the foundation afterwards.

- For Group 2: the nest mound and the main chamber locating above excavation limit will be removed, while chambers underneath and tunnel system remain exist and cause damage to the dam foundation afterwards, though the extent of hazards depends on the thickness of the excavated layer and size of the termite nests.

- For Group 3: almost all hollow chambers created by termites will be eliminated.

It is therefore easy to see that current Norm applied in Vietnam in the excavation of the top layer of 0.3~0.5 m is fundamentally having no effects to the elimination of most dangerous termite species to the dam base. This is quite understandable because they are set up not to solve termite trouble.

III-Termite hazard at different geomorphological units of dam foundation

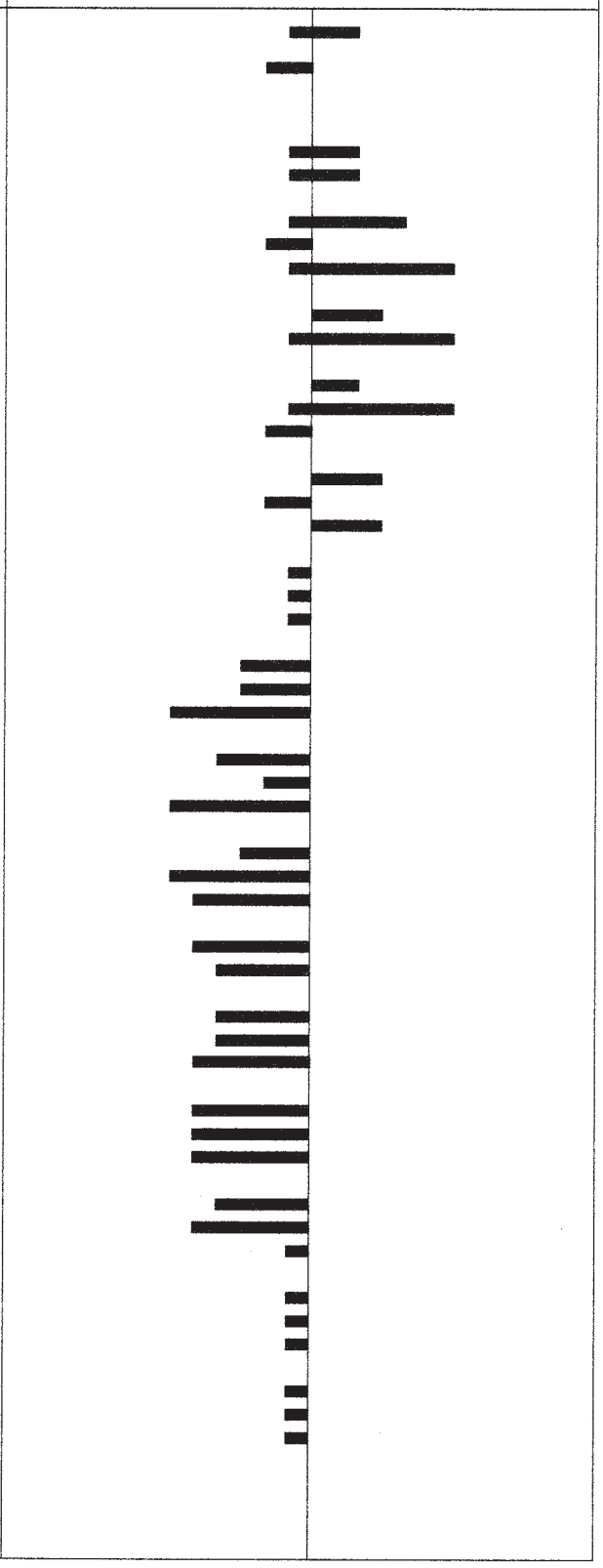
Usually, dams are constructed across the rivers to establish water reservoirs, their foundations were based on different geomorphological units with different distribution status of termite hazard.

III-1- Hill slope (slope of site dam): According to our observations, termite hazard in the weathering eluvial horizons depends on the following factors:

- Petrographic compositions of source rocks: If source rocks are conglomerates, gravelites, silstones, quartz sandstones.., their weathering products are poor in clay compositions. As a consequence, the grass-covered grounds are scattered, termite nests also are very rare. In contrast, if the source rocks are shales, claystones, marlstones, magmatic rocks of mafic and ultramafic compositions ect.., the weathering eluvial horizons are riched in clay compositions, botanical covers are therefore developed, then termite nests are also strongly distributed.

- The thickness of eluvial deposits: At the slopes with great pitch angles, the erosion is too strong so the eluvial covers are thin in comparison with small pitch angles. As a result, the number of termite nests at slopes with great pitch angles is smaller than those in small-angled slopes.

Depth and height of different species

Species	depth m -4 -3 -2 -1 0 1 2 3 4 m height
Globitermes sulphureus Hav	
Microcerotermes bugnioni Holm	
M.crassus Snyder	
M.dammermani	
Termes comis Hav	
T.majoriae Hav	
Macrotermes annandalei Silv	
M.barneyi Light	
M.givus Hag	
M.chaiglomi Ahmad	
M.carbonarius Silv	
M.tuyeni Vuong	
M.malaccensis Havilandi	
M.maesodensis Ahmad	
M.serrulatus	
M.latignathus Thapa	
M.menglongensis Han	
Microtermes dimorphus Tsai&Chen	
M.pakistanicus Ahmad	
M. obesi Holm	
Hypotermes makhamensis Ahmad	
H. xenotermitis Wasmann.	
O. ynnanensis Tsai et Chen	
O. horni Wasm	
O. feae Wasmann	
O. angustignathusTsai& Chen	
O. djampeensisWasm.	
O. hainanensis Light	
O. formosanus shir.	
O. proformosanus Ahmad	
O. pahamensis Duc kham	
O. obscuriceps Wasm	
O. sumatrensis Holmgren	
O. malaccensis Holmgren	
O. cornignathus	
O. latignathus	
O. ceylonicus	
O. profeae	
O. giriensis Roonwall	
Procapritermes nitobei Shir	
P. sowerbyi Light	
P. albipenis Tsai et Chen	
Pseudocapritermes sp	
Pericapritermes semarangi Holm	
Microcapritermes sp	
Nasutitermes gardneri Snyder	
N. deltocephalus Tsai et chen	
Bulbitewrmes prabhae Krisna	
Discuspiditermes orientalis Harris	

III-2. The alluvial terraces. The river terraces of steps 1, 2, 3. have different mechanical sedimentary compositions and different thickness, soil-pH, humidity, organic compositions, leading to different termite nest distribution. For example, alongside the Cam river of Huu Lung district, Lang son province, there are 3 terraces. Among them, the 3^{th} terrace is composed of yellow clay with black ion oxide points, hence, there is a great number of termite nests of Macrotermes annandelei. Meanwhile, the 2^{th} terrace composed of gray soil and the 1^{th} terrace composed of soil and sand have almost no termite nests. Similarly, the 3^{th} terrace of the small river across Ranchi city in India also has strongly developed termite nests of Odontotermes walloensis, meanwhile in other terraces no termite nests of this species are observed.

IV - Consequences of the non-treatment of termites in dam foundation before construction

IV - 1 - The empty hole in the dam foundation causes difficult-to-cure consequences. Take the Dau Tieng dam (Tay Ninh province, South Vietnam) as an example. This dam is 27km long. According to our survey in 1991, the pre-constructed dam site foundation contained 1,113 big termite nests belong of Macrotermes, Odontotermes, Globitermes, among them:

- Macrotermes species contained 656 nests, most were epigenous with average height of 1.72m and bottom diameter of 1.92m. If these nests are considered to be cone-shaped, then each nest would have a volume of 1.659m^3, and total volume of all Macrotermes nest mounds would be 1,088.3m^3. All researches of scientists proved that earth made up the nests were taken by termites from the deeper ground, i.e Macrotermes nest mounds alone had created empty holes in dam base of up to 1,088.3m^3.

- Odontotermes contained 71 nests at 2m average depth and average volume of 0.150m^3/nest(based on the volume of mortar injected into the nests), thus these species alone made up empty holes of 10.65m^3 in dam base.

Together, Macrotermes and Odontotermes nests made up empty holes of approximately 1,100m^3 totally, making up an incredibly big figure to dam designers. As the big holes could not be removed if only 0.3 to 0.5m-thick top layer were excavated, the Dau Tieng dam met with a lot of occurrences, mainly water leakage the treatment of which was very costly with low efficiency.

IV-2 - Termite colonies inside dam-foundation may still be alive and continue existent: Observations in non-treated pre-constructed base showed that the colonies lelft in the base may still alive after the construction of dam. The proof for that is through observations of parts of dams where termite nests underneath were not controlled, then termites appear very quickly after the dam was dammed up, for example Tri An dam in Dong Nai province... Truly, after the water reservoir was put into use, the water level raised slowly, then the dam base was gradually soaked. At that time, the colony left in the base would move upwards to the upper new area that is not disturbed by the water. That's why on the whole dam, the nearer to the old base a place is, the sooner and more termites appears.

IV-3 - On an operative dam body where termites are already available, the non-treatment of termites before elevation leads to the same consequences as the non-treatment of termites in the foundation before construction.

Termite nests inside dam body are normally lying above the line of ambibition of the dam. Once the dam is elevated, a new line of ambibition will appear at a higher position than the old line. That's why when the reservoir water level goes up, the termite nest in the old dam body will step by step be soaked then flooded, the termite colony has to move upwards to the newly elevated body part.

A typical example of this case is the Vinh Trinh dam in Quang Nam province. This dam which was put in use for years had so many termite nests inside. By 1989, it was elevated by 1m and just about 1 year after that, there appeared on the newly elevated surface a series of swarming holes of Odontotermes and a number of Macrotermes' epiginous mound.

V - Solution

To avoid such consequences as demonstrated in Article IV above, in 1993, we proposed the Ministry of Irrigation of Vietnam to allow the issuance of a Standard on treatment and control of termites in dam foundation prior to the ground-breaking. This Standard would include the following items:

- Objects to be controlled: base of dam from 6m high up;
- Termite species to be controlled: Odontotermes, Macrotermes;
- Requirements:

+ For termite nests inside dam base (or inside an operative dam body): to eradicate the colony, then fill the empty hole created by termites with sprayed mortar;
+ For termite nests located in the surrounding area 50m~100m away from the dam foot: only eradication of termites colony is required.

This Standard has been approved by the Ministry of Irrigation of Vietnam (now the Ministry of Agriculture and Rural Development), encoded 14-TCN-88-93 and named "Composition and quantity of surveillance and control of termites dangerous to wholly earth-made or partly earth-made dam". This Standard is compulsorily enforceable in the whole country since 1st January, 1994.

Conclusion

After 11 years, this Standard has applied efficiently in the construction of hundreds of big and small dams in Vietnam, contributing a great importance to the safety of these dams. The control of termites shall then be carried out just once before the damming up. However, as the main author of this Standard, we recognize some shortcomings of the Standard to be remedied, for example the application of new technology to restrain the environmental pollution, and some other issues.

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Baits of Molybdenum and Tungsten Salts for Termite Control Part-3. Field Efficacy Tests at Dams and Dikes in China

by
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Abstract

Bait formulations containing molybdenum and tungsten salts show a slow-acting but high termiticidal efficacy against termites. Based on the facts that molybdenum and tungsten are trace elements in the soil and their salts are low toxic for mammals and pose almost no impact to the environment, we have tried to apply molybdenum and tungsten salts as bait formulations for the control of termites including *Macrotermes* and *Odontotermes* which are known to cause serious damages to dams and dikes in China.

During the initial stages of development works, sodium salts of molybdenum and tungsten were employed, proving the fear of their leaching out in the field conditions such as rainfall in spite of high termite control efficacy. Later formulation studies led us to prepare a bait formulation in which sodium salts of molybdenum and tungsten were converted to water-insoluble barium salts. This barium salt formulation showed a high termiticidal efficacy almost equal to that of sodium salt bait, and its active ingredients were found not to leach out in the water and in the soil. By applying particle-board techniques as well, we developed bait formulations which were easy to use and applicable to mass production. Since July of 2005, field tests of these barium bait formulations have been conducted at 2 dams near Wuhan and one dam near Chongqing in China.

At the 2nd conference of TRG held in Bangkok, we reported the outlines of fundamental test results and the achievements of field tests of this type of baits at Rubber Experiment Station of Phuket in Thailand. This time the progress of field tests of molybdenum and tungsten baits conducted at dams and dikes in China will be reported.

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

Introduction

1. Bait formulations containing molybdenum and tungsten salts

Salts of molybdenum and tungsten compounds show a slow-acting but high efficacy against termites. From the obtained test results on mode of action, the primary cause for the termiticidal efficacy of molybdenum and tungsten salts is considered to be the influence on the symbiotic intestinal flagellates, or the physiological depression resulted from the accumulation of their oxides in the body, especially in the fat body (Yoshimura *et al.* 1989).

Based on the facts that molybdenum and tungsten are trace elements in the soil and their salts are low toxic for mammals and pose almost no impact to the environment, we have tried to apply molybdenum and tungsten salts as bait formulations for the control of termites including *Macrotermes* and *Odontotermes* which are known to cause serious damages to dams and dikes in China (Nakayama *et al.* 1998).

During the initial stages of development works, sodium salts of molybdenum and tungsten were employed at 2 sites of Baifen and Huinu dams, proving the fear of their leaching out in the field conditions such as rainfall in spite of high termite control efficacy. Later formulation studies led us to prepare a bait formulation in which sodium salts of molybdenum and tungsten were converted to water-insoluble barium salts. This barium salt formulation showed a high termiticidal efficacy almost equal to that of sodium salt bait, and its active ingredients were found not to leach out in the water and in the soil (Kanzaki *et al.* 1994). By applying particle-board techniques as well, we developed bait formulations which were easy to use and applicable to mass production. Since July of 2005, field tests of these barium bait formulations have been conducted at 2 dams near Wuhan and

one dam near Chongqing in China.

To confirm the practicability of this type of baits, we have also carried out field tests successfully at Rubber Experiment Station of Phuket in Thailand where *Macrotermes* and *Odontotermes* cause awful damages to natural rubber production by attacking roots of young rubber trees (Charunee *et al.* 2005).

At the 2nd conference of TRG held in Bangkok, we reported the outlines of fundamental test results (Katsuda & Nakayama 2005) and the achievements of field tests in Thailand (Vongkaluang *et al.* 2005). This time the progress of field tests of molybdenum and tungsten baits conducted at dams and dikes in China will be reported.

2. Damages of dams and dikes caused by termites in China

In China, damages by termites that eat woods or destroy dams and dikes on the large scale become social concern. In particular, the damage of dams and dikes by *Odontotermes formosanus*, *Odontotermes hainanensis*, *Globitermes*, or *Macrotermes* is serious.

These kinds of termites make big nests with holes and large numbers of mud tubes under the surface of dams and dikes instead of making termite mounds above the ground. When the water level of dams or rivers goes up due to the flood at the rainy season, the water sinks into the mud tubes and cause a threat of destroying dams and dikes.

In China, there are about 80,000 large and small dams. In Hubei Province having the largest number of about 7,000 dams, as many as about 70% of them have been found to be nested by termites. Decrease in the strength of dams causes serious concern. Moreover, almost all dikes of numberless rivers and canals in China are made of the soil and also have suffered from termite damages. In 1998 when the water level of Yangtze River went up, dikes were immediately before collapse and got into dangerous situations.

The sphere of a termite colony reaches the radius of about 50 m from its main nest and termites make lots of satellite nests by extending mud tubes. In case that a colony is aged 10 years or so, the nest is often found about 3 m in depth under the ground. A more aged colony tends to have a nest deeper in the ground. The size of a nest is often 1 m or more in diameter and sometimes reaches as big as that of a car.

To preserve the dams and dikes, eradication of termite colonies is indispensable, and conventionally bait formulations such as mirex were employed for this purpose. However, the use of organic chlorine compounds was banned because of residual toxicity and environmental pollution, and the development of new alternatives has been desired.

Materials and Methods

1. Test materials

According to the previously-mentioned process (Vongkaluang *et al.* 2005), we prepared bait formulations containing salts of molybdenum and tungsten. The test materials served for field tests are shown in Table 1. Using particle-board manufacturing techniques applicable to mass production, wood chips impregnated with salts of molybdenum and tungsten were solidified with resin adhesive. These bait formulations were cut into the shape likes stakes.

Table 1. Bait formulation for field tests

Materials	Contents (%)	
	Barium salt bait	Sodium salt bait
$Na_2MoO_4 \cdot 2H_2O$ (MW:242)	13.0 (5.2% as Mo)	13.0
$Na_2WO_4 \cdot 2H_2O$ (MW:330)	5.0 (2.8% as W)	5.0
$BaCl_2 \cdot 2H_2O$ (MW:244)	15	–
Wood chip	57-61	70-72
Resin adhesive	6-10	10-12
Total	100%	100%

2. Test methods

We selected some test sites at dams or dikes where termite activities were found vigorous. Test bait stakes were hit into the ground at intervals of about 1-2 meters. To monitor the termite activities, several stakes of pine tree were also hit into the ground. Then the level of termite activities was observed periodically.

Results and Discussion

1. Sodium salt bait formulation

1) Baifen Dam, Guangdong Province (1997.7-)

At Baifen Dam in the suburb of north of Guangdong Province, where colonies of *Odontotermes formosanus* and *Reticulitermes speratus* were active, 70 stakes of sodium salt bait and 20 stakes of pine tree for monitoring were hit into the ground at a square area of 10 m x 15 m. Three months after installation, the baits started to receive termite attacks. And after 10 months, 29 out of 30 bait stakes were heavily damaged by termites. On the other hand, the stakes of pine tree received only slight damage. At the final observation of 16 months after installation, no termite activities were observed in many emasculated satellite nests and mud tubes.

2) Huinu Dam, Fujian Province (1999.8-)

At the orchard and the slope nearby Huinu Dam in Fujian Province, where colonies of *Odontotermes formosanus* and *Macrotermes barnyi* were found, 30-65 stakes of sodium salt bait were applied to each of 6 test sites. Some baits were eaten after 2 months of post-installation. At the observation time of 24 months after installation, no termite colonies were found at 5 out of 6 sites. At another one test site where termite activities were observed, baits received considerably less termite damages.

From the above field test results, it was found that sodium salt baits of molybdenum and tungsten compounds generally show high termiticidal efficacy against termites but there are some cases where good results have not been obtained in some of test sites. The cause for this problem is considered to be the bait attractancy or the leaching out of water-soluble sodium salts of molybdenum and tungsten as active ingredients. As these results suggested that molybdenum and tungsten baits are promising as a control measure of termite colonies at dams and dikes in China, we have stepped forward to practical field tests of barium salt baits of molybdenum and tungsten which are water-insoluble.

2. Barium salt bait formulations

1) Suburb of Wuhan City, Hubei Province (2005.7-)

At a little dam in Xianning City, Hubei Province, where colonies of *Macrotermes barnyi* were found, 40 stakes of barium salt bait and 9 stakes of pine tree were installed in the ground of the slope of the dam.

Furthermore at another dam in the suburb of Wuhan City, which was a habitat of colonies of *Odontotermes formosanus*, 25 stakes of barium salt bait and 6 stakes of pine tree were installed similarly, especially in sites where termite activities were vigorous.

2) Dazu County, Chongqing Municipality (2005.7-)

At a little dam in Dazu County, Chongqing Municipality, where colonies of *Odontotermes formosanus* were found, 50 stakes of barium salt bait and 7 stakes of pine tree were installed in the ground of the slope of the dam.

These field tests of barium salt baits are still in continuation. We are scheduled to observe these test sites at the end of February of 2006 and make a presentation on the interim report at the 3rd conference of TRG.

Considering good achievements obtained from field efficacy tests of barium salt bait form ulations at Rubber Plantations in Thailand, high control efficacy against termites can be expected for field tests in China as well.

Conclusion

Molybdenum and tungsten compounds are metal elements distributed on the earth, and show a slow-acting but high termiticidal efficacy against termites without causing problems of residual toxicity as organic chlorine-based termiticides, proving promising as a new termiticide. Especially barium salt bait formulations are useful for the control of termites, which cause severe damages to dams and dikes on the large scale in China.

In the future there will be room for improvement as to physical properties of baits and the selection of base materials suitable for the districts where baits are applied.

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Evaluation on Sentricon Termite Elimination System against *Coptotermes formosanus* Shiraki

by
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Abstract

Evaluations of territory and group size of *Coptotermes formosanus* Shiraki with the 2/20/2006-recapture method on 15 wild colonies and the effectiveness of Sentricon Termite Elimination System to control 23 colonies of *Coptotermes formosanus* Shiraki in Guangzhou were conducted. The results indicates that the longest distance of foraging territory of is 40 meters and the shortest is 8 meters among the 15 *Coptotermes formosanus* Shiraki colonies, and the largest group has about 2,140,000 workers and soldiers and the smallest has only 160,000. The results of using Sentricon Termite Elimination System to eliminate 23 termite colonies shows: the shortest time of eliminating 8 *Coptotermes formosanus* Shiraki colonies in different kinds of buildings with the bait containing 0.5% Hexaflumuron is 32 days, and longest is 75 days, and average is 51.75±22.76 days; the shortest time of eliminating 6 *Coptotermes formosanus* Shiraki colonies in different kinds of buildings with the bait containing 0.25% noviflumuron is 31 days, and longest is 45 days, and average is 38.5±5.4 days; the shortest time of eliminating 9 *Coptotermes formosanus* Shiraki groups in different kinds of buildings with the bait containing 0.50% noviflumuron is 31 days, and longest is 45 days, and average is 44.11±13 days.

Key words: *Coptotermes formosanus* Shiraki, territory, group size, hexaflumuron, noviflumuron

Introduction

Coptotermes formosanus Shiraki is from South China originally, and named by Japanese expert SHUMUDEYI according to samples he got from Taipei. At present, *Coptotermes formosanus* Shiraki is distributed in all provinces in south of Yangtse river in China, and spread aboard to Japan, America, South Africa, Ceylon, India, Pakistan, and Sri Lanka (Li Gui Xiang 1989), The number of termite in *Coptotermes formosanus* Shiraki colony could be over 10 million, and the diameter of its foraging range could be over 200 meters (Tamashiro,1990). *Coptotermes formosanus* Shiraki is one of the most destructive termite species in the world. *Coptotermes formosanus* Shiraki usually damages buildings and architectures, communication line, electrical wires and cables, traffic facilities, city trees, and crops. The provinces at south of Yangtse river in China had good geography and climate conditions for *Coptotermes formosanus* Shiraki's propagating and spreading. The damage of *Coptotermes formosanus* Shiraki is very popular in Guangdong, Guangxi, and Hainan, the damage rate of buildings is over 70---90%（Huang Fu Sheng 2000）.

In China, Arsenic trioxide (also named as arsenious acid, arsenic acid, or arsenic) and mirex (a kind of organic chlorine pesticide) are mainly used for *Coptotermes formosanus* Shiraki control. In 1950's, Mr Si Tu Yao, Si Tu Qiao and Li Shi Mei from Guangdong opened the prescription of arsenic termiticide. Now, the use of termiticide powder made of Arsenic trioxide is widest in China. Mirex was introduced in China at 1968 and is mainly used for elimination of termite and ants. Arsenic trioxide is a virulent pesticide, and could quickly kill animals or human beings without apparent symptom (Zhu 1989). Arsenic trioxide chemicals are banned in Finland, Swiss, Hungary, Russia and other countries. Mirex is a kind of steady organic chlorine pesticide, and its half life in soil could last for more than 10 years. Mirex also can accumulate in fat of human or animal's body, so it is prohibited to use in most countries in the world. Form 1990's, the "green conception" is cried up in many developed countries, people started to look for termiticides and methods harmless to human health and ecological equilibrium such as insect growth regulator, biological, physical measures. Many experiments proved that the termiticides which contain hexaflumuron and noviflumuron as its active ingredients can significantly kill many kinds of termite colonies (Su 1994,

1996; Lee 2001).

It's known that *Coptotermes Formosanus* Shiraki causes damage and losses to national economy. But in China, high toxicity and high remaining pesticides are still used in *Coptotermes Formosanus* Shiraki prevention and control, and they are absolutely inconsistent with the "green conceptions" and "sustainable development strategy". Till now, only few Africa counties and China still use mirex and arsenic, so, it's very necessary and urgent to find new termiticides which have effectiveness and low toxicity. From May 2001, we did experiment on *Coptotermes formosanus* Shiraki colonies by Sentricon Termite Elimination System from Dow AgroSciences, and now we list details of our experiment as well as results below.

Experimental materials

1. Red or blue filter papers, tissue paper

Dye the filter papers, tissue paper red and blue with 0.1% central-red and SUDAN blue. The filter papers, tissue paper are used for evaluating group size and territory of *Coptotermes Formosanus* Shiraki colonies (Lai,1983).

2. *Pinus massoniana* Lamb board, bar

Specification of *Pinus massoniana* Lamb board: 1.8cmX20cmX25cm, baking at 50C° for 72 hours, then weight up it. Specification of bar: 4cm X 4cm X 30 cm, nib an end.

3. Trapping case

Take a 25cm X 25cm X 30 cm plastic pail with cover, and then saw its bottom.

4. Sentricon Termite Elimination System

Sentricon Termite Elimination System from Dow AgroSciences. Baits contains the active ingredient noviflumuron at 0.25% (w/w), 0.50% (w/w) and hexaflumuron at 0.5% (w/w), respectively.

5. The termite colonies for experiment

The termite colony for experiment is *Coptotermes formosanus* Shiraki which causes most damages in buildings and architectures in South China. 23 Coptotermes formosanus Shiraki groups in different types of buildings are chosen for the experiment.

Experimental method

The experiment includes three main steps which are: monitoring/checking (evaluating colony's size and territory), poisoning, and re-monitoring.

1. Evaluation on territory and size of *Coptotermes formosanus* Shiraki colonies

Set termite trapping box at galleries or affected parts, or strike 4cmx4cmx30cm deal bars into soil around buildings at 2-3 meters distance to observe termite activity. When termites were found on bar, the termite trapping box can be set close to damaged position. Separate the *Coptotermes formosanus* Shiraki from the box in the lab, then feed them with blue or red filter paper, weight up and take account of them when all termite were colored then release them (at least 2000 workers) to the trapping box again. Then check trapping box, galleries and affected parts every 7 days to ascertain the territory and group size of the *Coptotermes formosanus* Shiraki colony.

2. Installation of Sentricon Termite Elimination System

(1). Indoor installation

When termites were found in galleries and damaged places, remove dusts and water on the surface by clean cloth (paper), but not to ruin the galleries for disturb termites to leave. Use a 1 mm-thick double-surface adhesive fabric to fix AG Station on the damaged position, then put wet toilet paper roll baits in AG Station, and the baits should be close to places with termites' trail, at last, seal the baiting box by opacity fabric. 2 or more AG Station are installed for each experimental termite colony.

(2). Outdoor installation

Strike 4cm x 4cm x 30cm deal bars into soil around buildings at 2-3 meters distance to observe termite damage. Replace the deal bars with IG stations when termites were found, and leave some monitoring stations for comparison.

3. Observation

Termites' activities and death, baits consumption, termiticides consumption should be checked and recorded regularly (at least once two weeks) after Ag or Ig Stations and monitoring stations are installed. Add or replace baits when they were consumed over 1/3－1/2. Baits consumption should be recorded. Add more trapping stations when new termites are found.

4. Identification about death of *Coptotermes Formosanus* Shiraki colonies

When termites are fading in trapping stations and monitoring stations (compared to original situation) in buildings and the percentage of soldiers increases sharply (usually 70%), and no live termite was found, Elimination of *Coptotermes formosanus* Shiraki colonies could be ascertained. At this time, reclaim all the baits and replace trapping stations with monitoring stations for observation.

Results and discussion

This experiment investigated territory and size of 15 *Coptotermes formosanus* Shiraki colonies in Guangzhou territory (see results in Table 1.). Results indicates that the longest distance of *Coptotermes formosanus* Shiraki colonies' territory is 30 meters and the shortest distance is only 8 meters, and average distance is 12.93±7.93 meters. The largest colony has 2,140,000 workers and soldiers. The smallest group has only 160,000 workers and soldiers. The average number of each colony is 727,300±567,800. The results shows: territory of *Coptotermes formosanus* Shiraki group usually has a distance within 20 meters in Guangzhou, but *Coptotermes formosanus* Shiraki groups are dense in Guangzhou.

The *Coptotermes formosanus* Shiraki colony in Guangzhou is of limited territory, small size and high density. The reasons of the above characteristics lie on the ecological conditions, such as climate and food. Favorite climate makes a new *Coptotermes formosanus* Shiraki group easily survive and abundant food such as underground abandoned wood in buildings save the group members from long distance journey for food. Therefore, the group can maintain itself without a large scale of members.

Table 2 indicates the results about using Sentricon Termite Elimination System to eliminate *Coptotermes formosanus* Shiraki colonies. The results indicates: the shortest time for eliminating 8 *Coptotermes formosanus* Shiraki colonies in different buildings by baits containing 0.5% Hexaflumuron is 32 days, and longest time is 75 days, and average time is 51.75±22.76 days, and the least baits consumption is 36.92g and the most is 279.73g, and average is 134.03±76.80g, and there is 0.67±0.384g active ingredient in the baits; the shortest time for eliminating 6 *Coptotermes formosanus* Shiraki groups in different buildings by baits containing 0.25% Noviflumuron is 31 days, and longest time is 45 days, and average time is 38.5±5.4 days, and the least baits consumption for each *Coptotermes formosanus* Shiraki nest is 16.56g and the most is 94.59g, and average is 42.37± 28.97 g, and there is 0.106 g noviflumuron as active ingredient in the baits; the shortest time for eliminating 9 *Coptotermes formosanus* Shiraki colonies in different buildings by baits containing 0.5% noviflumuron is 26 days, and longest time is 65 days, and average time is 44.11±13 days, and the least baits consumption is 20.42 and the most is 142.94 g, and average is 65.87±44.40 g, and there is 0.332 g noviflumuron as active ingredient in the baits.

Sentricon Termite Elimination System completely eliminated 23 *Coptotermes formosanus* Shiraki colonies at different kinds of buildings in the experiment.

The two kinds of noviflumuron baits at different consistence hasn't apparent difference on time to eliminate *Coptotermes formosanus* Shiraki group ($P>0.05$), but there's significant difference on consumption of active ingredient ($0.05>P>0.01$), in other words, the baits in 0.25% noviflumuron for eliminating a *Coptotermes formosanus* Shiraki colony is only 32% of baits containing 0.5% noviflumuron.

Compared with noviflumuron baits and hexaflumuron baits, we can see that there is significant difference on time to eliminate *Coptotermes formosanus* Shiraki colony between them ($P<0.01$). Noviflumuron baits could save rolls 50% and shorten time 32-34% (about 20 days) compared with Hexaflumuron baits. Besides, there's significant difference between them on the consumption of active ingredient ($0.05>P>0.01$,. $P<0.01$). Compared with hexaflumuron baiting

system, noviflumuron can reduce 50-84% active ingredient consumption for eliminating each *Coptotermes formosanus* Shiraki colony. So, noviflumuron baits are more superexcellent products to eliminate or control *Coptotermes formosanus* Shiraki in different kinds of buildings.

The same as Hexaflumuron baiting system, noviflumuron baiting system also utilized termite's biological characteristic to spread termiticide around inside a whole colony, at last, the whole *Coptotermes formosanus* Shiraki colony is killed. According to our research, after a *Coptotermes formosanus* Shiraki colony started to eat noviflumuron baits around 25 days, the workers which take responsibility of nesting and foraging as main body of the colony, gradually became stagnant, and the soldier percentage increased from 10% to 70% in the group, therefore, their destructive power to architectures is lost or severely weakened even if the *Coptotermes formosanus* Shiraki group wasn't completely eliminated yet at that time.

Only 0.106g—0.332 g of noviflumuron in baits as active ingredient could kill a group. Noviflumuron is sorted as IGR (insect growth regulator), and its mechanism is to inhibit the synthesis of chitin. The mammal body contains no chitin, so it's harmless and innoxious for mammalian. The dosage of pesticides could be significantly reduced in application of termite-baiting elimination method, and remaining material could be recycled and reused after every termite eliminating action, therefore, pollution from pesticides could reduce a lot. Besides, in the application of termite-baiting elimination method, termite monitoring stations and termite-damage alarm system are established, and these facilities also can prevent termites and their damage, so termite-baiting elimination method can prevent and eliminate termite at the same time. On the other hand, mirex is on the list of persistent organic pollutants (POPs) by United Nations Environment Programme and to be completely forbidden in near future（UNEP 2001）. The new generation termiticides (like hexaflumuron, noviflumuron, and so on) which are very safe for animals, human and environment will substitute mirex, therefore, termite control operators could be much safer and feel secure than ever before. So, development of termite-baiting elimination technology has a bright future in China.

Table 1. Foraging territory and colony size of 15 *Coptotermes formosanus* Shiraki colonies in Guangzhou

Colony	Foraging territory	Colony size
1	25	142
2	30	214
3	8	56
4	8	160
5	15	63
6	10	35
7	15	45
8	10	38
9	8	16
10	11	90
11	20	41
12	13	18
13	8	73
14	13	35
15	20	65
	12.93±7.93	72.73 ±56.78

Table 2. Results of Sentricon Termite Elimination System against 23 *Coptotermes formosanus* Shiraki colonies

Colony Site	%AI	Date Monitors Baited	Date of Cessation of Activity	Days to cessation	AI Consumed (g)
Floor 1	0.5% H*	2001/6/26	2001/9/9	75	0.35
Floor 1	0.5% H	2001/7/3	2001/8/28	56	0.609
Floor 1	0.5% H	2001/7/3	2001/8/28	56	0.185
Floor 2	0.5% H	2001/7/18	2001/9/19	63	0.367
Floor 1	0.5% H	2001/8/9	2001/10/19	71	1.399
Floor 5	0.5% H	2001/9/7	2001/11/7	61	0.777
Floor 6	0.5% H	2001/8/16	2001/10/5	50	0.847
Floor 1	0.5% H	2001/8/16	2001/9/17	32	0.828
Floor 1	0.25% N*	2002/8/5	2002/9/11	37	0.236
Floor 1	0.25% N	2002/7/14	2002/8/26	43	0.085
Floor 1	0.25% N	2002/8/15	2002/9/15	31	0.103
Floor 1	0.25% N	2002/8/22	2002/9/25	34	0.041
Outdoor Ground	0.25% N	2002/7/22	2002/9/5	45	0.041
Floor 20	0.5% N	2002/8/15	2002/9/25	41	0.128
Floor 1	0.5% N	2002/5/17	2002/6/26	40	0.745
Floor 1	0.5% N	2002/7/14	2002/8/26	43	0.584
Floor 1	0.5% N	2002/8/5	2002/10/4	60	0.479
Floor 1	0.5% N	2002/7/22	2002/8/22	31	0.102
Floor 1	0.5% N	2002/7/22	2002/9/5	45	0.380
Floor 1	0.5% N	2002/7/22	2002/9/25	65	0.248
Floor 1	0.5% N	2002/10/18	2002/12/9	52	0.191
Floor 16,17	0.5% N	2002/8/30	2002/10/4	35	0.116
Outdoor Ground	0.5% N	2002/9/4	2002/9/30	26	0.147

- H= hexaflumuron, N= noviflumuron

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A New Bait Toxicant - Bistrifluron

by
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Abstract

Bistrifluron is one of the benzoylphenylurea (BPU) compounds which are less toxic to mammals. Laboratory no-choice feeding test demonstrated that bistrifluron was higher in efficacy against *Coptotermes formosanus* Shiraki than hexaflumuron and diflubenzuron and that the efficacy against *C. formosanus* did not vary among colonies. Optimum formulations and application should be further studied to use bistrifluron as a bait toxicant in addition to a wide range of field tests.

Key words: bistrifluron, benzoylphenylurea, *Coptotermes formosanus*, bait toxicant

Introduction

Benzoylphenylurea (BPU) compounds such as hexaflumuron were proved to be effective as a bait toxicant for eliminating colonies of subterranean termites like *Coptotermes* and *Reticulitermes* in the mid of 1990's (Su 1994, Su *et al.* 1995). Since then, baiting products made of slow-acting insecticides have widely launched in the market, especially in the United States (Jones 2003). Bait systems with BPUs including hexaflumuron have been getting popularity from the public due to the good performance in the management of subterranean termites in Australia and Asian countries like Japan and Malaysia (Tsunoda *et al.* 1998, Lee 2002, Peters & Fitzgerald 2003).

Bistrifluron (Fig. 1) is the BPU compound (Kim *et al.* 2000), and has been developed as a commercial termite bait toxicant by industries. The characteristics of bistrifluron and its efficacy against *Coptotermes formosanus* Shiraki as a bait toxicant are reported here.

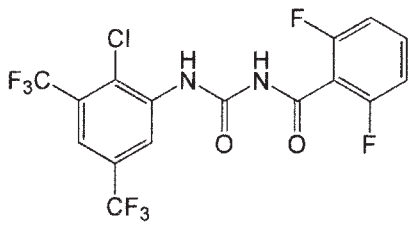

Fig. 1. Chemical structure of bistrifluron

Toxicological Information and Chemical and Physical Properties of Bistrifluron

Since bistrifluron is the IGR insecticide that inhibits insects' molting as other BPU, its toxicity to mammals is low (Table 1). However, it shows certain toxicity to crustaceans that have similar molting mechanisms as insects. Low solubility of bistrifluron in various solvents including water (Table 2) and type of usage as a termite bait seem to suggest that the chemical would not do dame to the environment. However, bait products containing bistrifluron should be securely discarded. All the data shown in table 1 & 2 are cited from Kim *et al.* 2000.

Table 1. Toxicological information of bistrifluron

Items	Results
Acute oral (rat)	LD_{50}(mg/kg): >5,000
Acute dermal (rat)	LD_{50}(mg/kg): >2,000
Ames test	Negative
Eco-toxicity to Carp (*Cryprinus carpio*)	LC_{50} (mg/l, 48 hr): >0.5

Table 2. Chemical and physical property of bistrifluron

Items	Outline of property
Appearance	Odorless white powder
Vapor pressure	$2.7x10^{-11}$ Pa
Melting point	172 -175℃
PH	6.8 – 6.9 at 25℃
Solubility in water	<0.03 mg/l at 25℃
Stability	Stable at room temperature and pH 5 - 9

Efficacy against *C. formosanus* in Laboratory

Materials and Methods

1) Termites

Undifferentiated larvae (workers) of *C. formosanus* were obtained from three different colonies that have been cultivated for more than one year in the laboratory at the Agricultural Chemicals Research Laboratory of Sumitomo Chemical Co., Ltd (SCC-ACRL), Hyogo Pref.,, Japan).

2) Chemicals

Three BPUs, bistrifluron [technical grade (96.5% a. i., Dongbu Hannong Chemical Co., Ltd., Seoul, Korea)], hexaflumuron [technical grade (>95.0% a. i., synthesized by SCC-ACRL)] and diflubenzuron [standard (>98.0% a. i., Wako Pure Chemicals Industry Ltd., Osaka, Japan)] were used.

3) No-Choice Feeding Test

3-1. Comparison between bistrifluron and conventional BPUs

A sheet of milk sediment disks (No.1026, ϕ 33 mm, ca. 0.2 g) (Toyo Roshi Kaisha, Ltd., Tokyo, Japan) was treated with 1.0 ml of a 1.0 g/l acetone solution of bistrifluron, hexaflumuron or diflubenzuron by pipetting to obtain filter papers with a chemical loading of 5,000 ppm (m/m) of each BPU. Both sides of the disk were pipetted evenly. After treatment, the disks were dried at ca. 25℃ for several hours until the acetone completely evaporated. Untreated (solvent) control disks were also prepared by treating them with 1.0 ml of acetone.

Each disk was placed in a small plastic cup (ca. 14 ml) with small entry holes for termites. This container was then put in a larger plastic container (ca. 200 ml) together with 100 termite workers. The bottom of the larger container had several small holes and was covered with 2-3 mm of plaster. These assembled units were placed on a damp cotton pad in an incubation chamber so that termites could uptake water through the plaster. Five units were prepared for each BPU treatment, including untreated controls. The termites from single colony were used. The units were maintained at ca. 25℃ for 12 wk. Termites were removed from the test containers and the number of live termites was counted at 2, 4, 6, 8 and 12 wk to determine the change in mortality with time. Live termites were put back into the same containers from which they were originally removed. Sluggish termites were counted as surviving insects as long as they could stand and walk by themselves. Termite mortality at given inspecting times were compared among BPUs and control by the Tukey-Kramer test ($p < 0.05$). Percentage mortality was transformed into the arc sine of the square root for statistical analysis (Yamamura 2002).

3-2. Efficacy of bistrifluron against termites from different colonies

The same no-choice test as above was conducted to evaluate efficacy of bistrfluron against termites from the other two *C. formosanus* colony than that used in the above-mentioned test. The filter-paper baits treated with 5,000 ppm bistrifluron and control baits were used. For one colony, four replications were made for bistifluron treatment and control and the number of live termites was counted at 2, 4, 6 and 8 wk to determine mortality. For the other colony, five replications were made and mortality was determined at 2, 4, 6 and 9 wk. For each colony, mortality of termites exposed to bistrfluron bait at given inspecting times were compared to that for control by Student's t test ($p < 0.05$) after determining equality of variance between mortality values for bistrifluron and control by F test ($p < 0.05$). Percentage mortality was transformed into the arc sine of the square root for

statistical analysis (Yamamura 2002).

Results and Discussions

Mortality of termite exposed to bistrifluron exceeded significantly that for control at 4 wk and later, while mortality for hexaflumuron was slightly greater than that for control only at 12 wk and mortality for diflubenzuron was not significantly different from that for control at any inspecting time ($p < 0.05$, Tukey-Kramer test, table 3). Therefore bistrifluron appeared to have better efficacy than hexaflumuron and diflubenzuron. Mortality of termites from each of three different colony exposed to bistrifluron exceeded significantly that for control at 4 – 6 wk ($p < 0.05$, Tukey-Kramer test and Student's *t* test, tables 3 & 4). From these results, baits containing bistrifluron might be effective for *C. formosanus* control and its efficacy might be better than conventional baits with regards to speed of colony elimination.

Table 3. Mortality of the termites exposed to three BPUs at given inspecting times in the no-choice feeding test

Bait*	Inspecting time – Mortality (%) [mean ± SE]**				
	2 wk	4 wk	6 wk	8 wk	12 wk
Bistrifluron	1.2±0.49a	27.0±8.47a	90.6±5.56a	100.0±0.00a	100.0±0.00a
Hexaflumuron	7.8±1.24b	10.2±1.39ab	11.8±1.53b	13.2±1.98b	26.2±2.87b
Diflubenzuron	3.0±1.41ab	5.0±1.67b	7.0±1.87b	10.0±1.26b	22.4±1.81c
Control	5.2±1.16ab	6.2±1.83b	8.8±2.03b	11.6±2.60b	17.2±2.42c

* Concentration of each BPU was 5,000 ppm.
** Values in the same column with different letters are significantly different by the Tukey-Kramer test ($p < 0.05$)

Table 4. Mortality of the termites from two different colonies exposed to bistrifluron at given inspecting times in the no-choice feeding test

Colony & Bait*	Inspecting time – Mortality (%) [mean ± SE]				
	2 wk	4 wk	6 wk	8 wk	9 wk
Colony-1					
Bistrifluron	3.8±2.46	56.5±24.54	100.0±0.00#	100.0±0.00#	Not inspected
Control	6.0±4.24	5.8±4.52	13.3±3.35	24.3±6.22	Not inspected
Colony-2					
Bistrifluron	3.0±1.05	15.2±1.39	90.4±5.01#	Not inspected	100.0±0.00#
Control	4.4±0.68	9.4±0.81	15.8±2.03	Not inspected	56.4±11.51

* Concentration of bistrifluron was 5,000 ppm.
# Values are significantly different compared to the mortality values of control at same inspecting times ($p < 0.05$; Student's *t* test).

Compounds of BPU have different efficacy/palatability from each other against termites: It is indicated by laboratory evaluation that hexaflumuron has better efficacy than diflubenzuron against *C. formosanus* and *Reticulitermes flavipes* (Su & Scheffrahn 1993). However, baits containing hexaflumuron and that containing diflubenzuron took apparently similar periods to eliminate laboratory-culturing *C. formosanus* colonies (Rojas & Morales-Ramos 2001). Practical efficacy of BPUs to eradicate termite colonies will be influenced by activity and magnitude of them (Grace *et al.* 1996). Although efficacy shown in laboratory tests will not always be reflected directly on time required for eradicating termite colonies in field, materials and application methods of baits containing bistrifluron are desired to be examined for achievement of intensive consumption by termites and consequently quicker elimination of colonies by the potency of bistrifluron.

Conclusion

Bistrifluron was proved to be promising as a bait toxicant to eradicate *C. formosanus* colonies in the laboratory, although a wide range of field tests should be conducted to draw conclusions on the efficacy under simulated practical conditions. The potential to bistrifluron might attract researchers and industrial technicians to develop new termite management technologies in the future.

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Laboratory Efficacy Test Methods of Termiticides in Thailand and Evaluation of Silafluofen Products by the Methods

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Abstract

According to standard laboratory efficacy test methods of termiticides in Thailand, silafluofen products were evaluated in Thailand and Japan. As test termites, *Coptotermes gestroi* Wasmann was used in Thailand and *Coptotermes formosanus* Shiraki was in Japan, respectively. Silafluofen EC (emulsifiable concentrate) was diluted with water to concentrations of 0.10% and 0.15% as active ingredient for efficacy tests.

As a result, mortality of termites at silafluofen plots treated with each concentration reached 100% within 5 days to 2 weeks with no or slightly wood weight loss of wood pieces in tests of both countries, whereas the control plots received considerable termite damage after 2 months. This indicated that Thai standard laboratory efficacy test methods of termiticides can be applied to tests in Japan. And silafluofen was confirmed to be effective as termiticidal ingredient also in Thailand when used at concentrations of 0.10% and 0.15% .

Key words: laboratory efficacy test methods, silafluofen, *Coptotermes gestroi* Wasmann, *Coptotermes formosanus* Shiraki

Introduction

Termites cause damages to wooden constructions and others all over the world and a lot of termiticides have been widely used for the control of these harmful termites. To assess the effectiveness of termiticides, laboratory efficacy test methods have generally established in accordance with circumstances of each nation such as living termite species, environments and structures of constructions.

For example, laboratory efficacy test method of termiticides for soil treatment in Japan has been determined as JWPA (Japan Wood Preserving Association) Standard No. 13, Tunneling Test. In the test, a glass tube stuffed with termiticide-treated soil is connected to two glass cylinders at the both ends. Wood flakes are put into one of the two cylinders and termites are placed on the soil in another cylinder. After the test duration, the distance of boring of the formation layer caused by the termites is measured. On the other hand, Thai standard laboratory efficacy test methods stipulate that one container has untreated sand with termites, termiticide-treated sand and untreated sand with a wood piece in 3 layers and that wood weight loss of the wood piece by termite attacks is measured after the test duration.

Silafluofen (Katsuda *et al.* 1986, 2005, Minamite *et al.* 1990, Nakayama *et al.* 1998), endowed with low fish toxicity and chemical stabilities under sunlight, in the soil and under alkaline environments in addition to high termiticidal activities and low mammalian toxicity, has been widely used as termiticides such as EC formulations for soil treatment and oil formulations for timber treatment since 1991 in Japan.

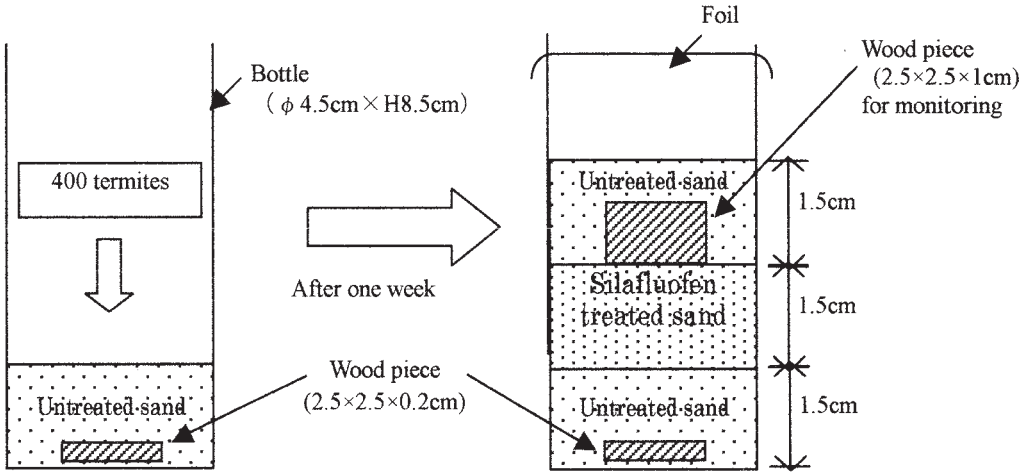
This time laboratory efficacy tests of silafluofen EC on the market in Japan were conducted in Thailand and Japan according to Thai standard laboratory efficacy test methods. Comparative test results obtained will be reported.

Materials and Methods

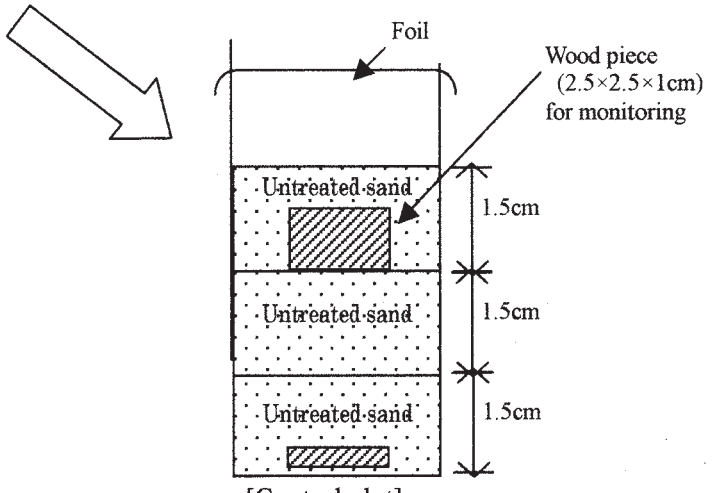
1. Standard laboratory efficacy test methods of termiticides in Thailand

(1) Test procedures

1) Into a glass bottle (Φ;4.5cm, H; 8.5cm), put a wood piece of rubber tree (2.5×2.5×0.2cm) and then a mixture of the sterilized sand and distilled water at a rate of 3:1. Make the height of the sand at 1.5cm from the bottom of the bottle and flat the surface of the sand.
2) Put 400 termites of *Coptotermes gestroi* Wasmann into the bottle and acclimatize for one week.
3) Prepare a dilution of test termiticide formulation.
4) Mix the sterilized sand with the dilution of test termiticide formulation at a rate of 3:1 and leave the mixture as it is for 2 weeks.
5) Put the mixture of 4) into the bottle so that the height gets at 1.5cm from the original sand surface. Place a wood piece of rubber tree (2.5×2.5×1cm) on the sand surface. And then put the sterilized sand mixtured with distilled water at a rate of 3:1, making the height of the sand at 1.5cm from the new sand surface (4.5cm from the bottom in total). The wood piece should be placed in the last-poured sand layer. Close the bottle with foil.
6) For a control plot, use the sterilized sand mixtured with distilled water at a rate of 3:1 in stead of the mixture of 4).
7) Observe and record the activities of test termites in test plots in comparison with a control for 2 months.



[Silafluofen-treated plot]



[Control plot]

Fig.1 Scheme of test methods

(2) Evaluation of effectiveness

A. No termite damage to the wood piece (2.5×2.5×1cm) ; Excellent effectiveness
B. Slightly damage (under 10%) ; Good
C. Moderate damage (10-40%) ; Moderate
D. Considerable damage (40-80%) ; Not good
E. Severe damage (above 80%) ; Can not be used.

(3) Evaluation criteria

Performance requirements should be the above A or B.

2. Laboratory efficacy test methods in Japan

Tests were conducted according to procedures described in the above "Standard laboratory efficacy test methods of termiticides in Thailand" excepting that *Coptotermes formosanus* Shiraki was used in stead of *Coptotermes gestroi* Wasmann as test termites and a wood piece of pine tree in stead of a wood piece of rubber tree, respectively.

Results and Discussion

1. Test results in Thailand

- Test duration: April, 2005～June, 2005
- Test place : Royal Forest Department
- Test results : Table 1

Table 1 Test results of dilutions of silafluofen termiticide

Test termiticide	Mortality of termites days after treatment (%)							
	1 d	3 d	5 d	7 d	10 d	14 d	30 d	60 d
Silafluofen 0.10%	0	5	10	50	60	100	100	100
Silafluofen 0.15%	5	20	40	100	100	100	100	100
Control	0	0	0	10	10	20	50	80

- At silafluofen plots treated with concentrations of 0.10% and 0.15% as active ingredient, mortality of termites reached 100% within 2 weeks with no or slightly wood weight loss of wood piece, whereas the control plots received considerable termite damage after 2 months.

2. Test results in Japan

- Test duration: April, 2005～June, 2005
- Test place : Research & Development Laboratory of Dainihon Jochugiku Co., Ltd. (room temp.; 25±2℃)
- Test results : Table 2

Table 2 Test results of dilutions of silafluofen termiticide

Test termiticide	Wood weight loss of wood piece (%) and termite activities			
	2-3 d	5 d	1 M	2 M
Silafluofen 0.10%	Movements of test termites got slow.	Existence of alive termites was not confirmed visually.	0 Alive termites were not found.	—
Silafluofen 0.15%			0 Alive termites were not found.	—
Control	—	—	9.3	16.3 Termite activities were vigorous.

- At silafluofen plots treated with concentrations of 0.10% and 0.15% as active ingredient, existence of alive termites was not confirmed visually 5 days after treatment and no termite damage was observed on wood pieces at the observation time of one month after treatment.

3. Discussion

These test results indicated that Thai standard laboratory efficacy test methods of termiticides can be applied to tests in Japan. And silafluofen was confirmed to be effective as termiticidal ingredient also in Thailand when used at concentrations of 0.10% and 0.15%.

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Tunneling and Survival of Japanese Subterranean Termites in Soil Treated with a Nonrepellent Termiticide, Fipronil

by
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Abstract

Tunneling tests were conducted with fipronil-treated soil rich in available phosphate using two species of Japanese subterranean termites, *Coptotermes formosanus* Shiraki and *Reticulitermes speratus* (Kolbe) in the laboratory. Termites (100 workers and 10 soldiers) were exposed to the treated soil in a glass tube (13 mm ø), and tunneling distance was measured regularly for 7 days together with the examination of termite survival. Both termite species could penetrate through the 50 mm long treated zone within a single day, and they were active 7 days after the test was initiated. Workers of *C. formosanus* could penetrate approximately 30 mm and died after 5 days at 5 ppm (m/m), while they showed reluctance to penetrate into the treated soil at $\geq$ 50 ppm and the mean penetration distance did not exceeded 10 mm with 100% mortality in 5 days. Similar results were produced by *R. speratus*, provided that the termite mortality reached 100% in 3-5 days at all concentrations of fipronil. The current results clearly indicated that fipronil is nonrepellent.

Keywords: tunneling, nonrepellent termiticide, fipronil *Coptotermes formosanus* Shiraki, *Reticulitermes speratus* (Kolbe)

Introduction

Treatment with soil poisoning liquid termiticides has been widely applied to termite prevention and control since chlordane, a chlorinated hydrocarbon appeared on the market in the 1950's (Ingle 1965), although the use of chlordane was banned in the 1980's in some countries including Japan (Tsunoda & Nishimoto 1985, Tsunoda 1991). Chlorinated hydrocarbons were replaced by organophosphates and synthetic pyrethroids. These soil termiticides were thought to repel termites from access points by the continuous layer of treated soil along outside and inside structures. However, these repellent termiticides cannot diminish termite population from treated zones (Su & Scheffrahn 1990, Su *et al.* 1993). Alternative termiticides such as imidacloprid, bifenthrin, fenobucarb, and chlorfenapyr have recently appeared in the market, and others are currently used in Japan (Tsunoda & Yoshimura 2004).

Although physical barriers and other options are available, chemical treatment is still ranked first as a termite management measure (Fushiki 1998, Tsunoda 2003). Among commercially available termiticides, non repellent termiticides such as fipronil and imidacloprid have recently been attracting researchers (*e.g.*Thorne & Breisch 2001, Osbrink *et al.* 2001, Shelton & Grace 2003, Ibrahim et al. 2003, Remmen & Su 2005b) since the application of these termiticides definitely contribute to the reduction of environmental impact due to the less amount of termiticides used (Potter & Hillery 2002). Since there is only a limited number of articles concerning termite response to soil treated with (Remmen & Su 2005a), a laboratory tunneling test was conducted to demonstrate the nonrepellency of fipronil using treated soil in the present study.

Materials and methods

Two Japanese subterranean termites were tested for their response to the soil treated with fipronil.

Termites: Sound undifferentiated workers and soldiers were obtained from a laboratory colony of *Coptotermes formosanus* Shiraki. Workers older than 5th instar and soldiers of *Reticulitermes speratus* (Kolbe) were collected from a field colony in Uji Campus of Kyoto University

Preparation of treated soil: Treatment solutions were evenly mixed with air-dried soil rich in available phosphate to prepare 5 concentrations of fipronil: 5, 50, 100, 150, and 200 ppm (mass [AI]/mass [air-dried soil]). Mixing ratio was 2g of treatment solution/10g of air-dried soil. Soil samples were kept under ambient conditions for at least three weeks prior to the tunneling test.

Test apparatus: A glass tube (13 mm ø and 150 mm long) was used for termite tunneling test (Grace *et al.* 1993). Treated soil (*ca* 2 g + 2 ml distilled water, 50 mm long) was sandwiched between tow plugs (20 mm long each) of 7% agar in the tube (Fig. 1). A few pieces of corrugated cardboard were placed at either end of the tube as food.

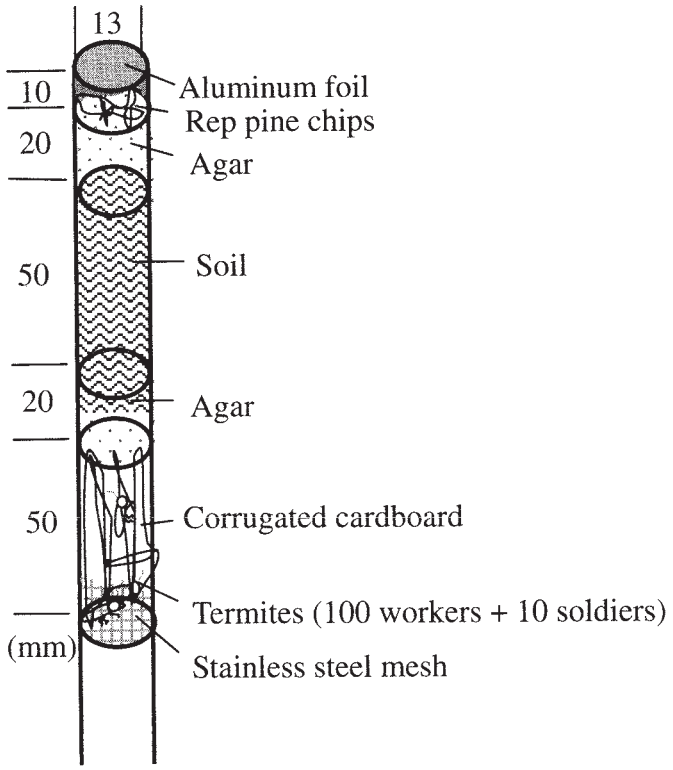

Fig. 1 Test apparatus for tunneling test

Tunneling test: Termites of a single species (100 workers + 10 soldiers) were introduced into the bottom space of the tube. The bottom end was sealed with stainless steel mesh, and the top was capped with aluminum foil. Penetration distance was recorded regularly for 7 days or until all termites died. The tunneling test was conducted at 28±°C in the dark using three replicates for each test concentration and termite species.

Results and discussion

Since the tolerance of workers of *C. formosanus* to insecticides varied with contents of available phosphate in soil (unpublished data), the soil rich in available phosphate was used as substrate in the current experiment.

When workers of *C. formosanus* were forced to contact fipronil-treated sandy loam, periods required to reach 100% mortality ranged from 4 to 48 hr at ≤ 5 ppm (unpublished data). Accordingly, 5 ppm was selected as the lowest concentration tested this time.

Termites penetrated through the full length (50 mm) of untreated soil within a single day after the initiation of the test. Although the degree of penetration decreased with increase in treatment concentrations, both termite species penetrated into the treated soil zone to some extent. At ≥ 50 ppm *C. formosanus* succeeded in penetrating longer than 10 mm of the treated soil only in a single case,

while there was no such record with *R. speratus*. As expected, the results well supported the nonrepellency of fipronil.

Workers of *C. formosanus* were active in moving and penetrating in the untreated soil after 7 days, while all termites were dead after 7 days at 5 ppm and 5 days at ≥ 50 ppm. *R. speratus* was less tolerant to fipronil-treated soil than *C. formosanus*. Workers of *R. speratus* were as active as *C. formosanus* in the untreated soil after 5 days, and all termites unexceptionally died after 3-5 days in the treated soil.

As already demonstrated by other researchers, soil types are important in determining persistence of soil treatment termiticides (Grace *et al.* 1993, Gold *et al.* 1996). In addition, the remarkable effect of substrate and colonies on the tolerance of termites to termiticides is seen with *C. formosanus* (Osbrink & Lax 2002). Therefore, it is worthy to note that results in this study must be interpreted with caution.

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Evaluation of several novel and conventional termiticide formulations against the Asian subterranean termite, *Coptotermes gestroi* (Wasmann) (Isoptera: Rhinotermitidae)

by
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Abstract

Five novel and conventional termiticide formulations, i.e. DPX-E2Y45 18.5% SC, indoxacarb 14.5% SC, WellTech 0980 24% SC, Termidor® (fipronil) 2.5% EC and Biflex® (bifenthrin) 24% SC were tested against the Asian subterranean termites, *Coptotermes gestroi* in the laboratory to determine their efficacies and their repellent properties. Two methods were used in this study: modified glass tube method, and petri-dish method. Four concentrations (1, 10, 50 and 100 ppm w/w) were evaluated for all termiticides in the glass tube method, while only recommended concentration was tested in the petri-dish method. Results indicated that with exception to bifenthrin, all termiticides demonstrated non-repellent properties. At extremely low concentration (1 ppm), bifenthrin did not show repellency effect against termites. The efficacies of each termiticide based on tunneling and wood consumption activities of the termites are discussed.

Keyword: subterranean termites, termiticides, non-repellent, tunneling, wood consumption.

Introduction

The Asian subterranean termite, *Coptotermes gestroi* (Wasmann) is an economically important subterranean termite species in South East Asia (Kirton and Brown 2003). Management of this species has been relying heavily on the use of chemical methods such as soil termiticide, baiting and dusting (Lee et al. 2003). Soil termiticide treatment, via creating a chemical barrier for exclusion of subterranean termites from buildings and structures, has been a popular mode of termite control for the last 50 years (Ibrahim et al. 2003; Su et al. 1997; Miller 2002; Jones 2003). In the past, repellent termiticides have been the principle approach to exclude termites from structures. Insecticides especially those from the pyrethroid group are key candidates of repellent termiticides. However, over the last several years, there has been increasing popularity in using termiticides with non-repellent properties. This characteristic is crucial because termites will not be able to detect the presence of the treated termiticide in soil, and thus will continue to forage through until the demise or suppression of the whole colony (Spence 1998; Shelton & Grace 2003). These compounds also have a delayed mode of action that continues to allow termites to forage freely in and out of the treated zone, thus promising a greater impact to the whole colony (Shelton & Grace 2003). In this study, we examined the laboratory performance of several novel and conventional termiticide formulations against the Asian subterranean termite, *Coptotermes gestroi* using two evaluation methods.

Materials and methods

The Asian subterranean, *C. gestroi* was used in this study. They were freshly collected from the underground monitoring stations which were established earlier in the Universiti Sains Malaysia Minden campus. They were brought back to the laboratory and separated from debris using the method described by Tamashiro *et al.* (1973). Five termiticide formulations, i.e. DPX-E2Y45 18.5% SC [DuPont Professional Products], indoxacarb 14.5% SC [DuPont Professional Products], WellTech 0980 24% SC [WellTech Healthcare Co. Ltd, Thailand], Termidor® (fipronil) 2.5% EC [Bayer Environmental Science] and Biflex® (bifenthrin) 24% SC [FMC] used in the evaluation.

Two evaluation methods had been adopted: Glass-tube method and Petri-dish method. The glass tube method, modified after Su and Scheffrahn (1990) (Figure 1) is a 30-cm glass tube (1.4 cm diam.) that contained a 21-cm long moistened sand and 2-cm termiticide-treated sand sandwiched between 2-cm sawdust and 2-cm 10% agar. Two pieces of moistened filter paper were placed into the 3-cm void adjacent to the agar layer. Two hundred termite workers and 10 soldiers were introduced into the void and allowed to tunnel freely. The cumulative tunneling distance was measured daily up to 7 days post-treatment. The number of termite survivors was recorded at the end of the experiment. Four different concentrations [1, 10, 50 and 100 ppm (w/w)] were tested and each concentration was replicated 3 times.

For the petri-dish method, a polyethylene container measuring 16 cm diam. x 6 cm height) was separated into 2 sections with a piece of glass. One section was filled with 140 g of untreated sand, while the other was filled with equal amount of sand that was treated with the termiticide. Two pieces of rubber wood (*Hevea brasiliensis*) measuring 2 x 1 x 1 cm were placed in each section. Four hundred workers and 20 soldiers were then introduced into the untreated section and allowed to acclimatize for 48 hours. After that period, the glass pieces was removed and termites were allowed to forage freely. Termite survivorship and total wood consumption were calculated after one week post-treatment. Tunneling activities in both treated and untreated section were qualitiatively ranked. Each experiment was replicated 5 times.

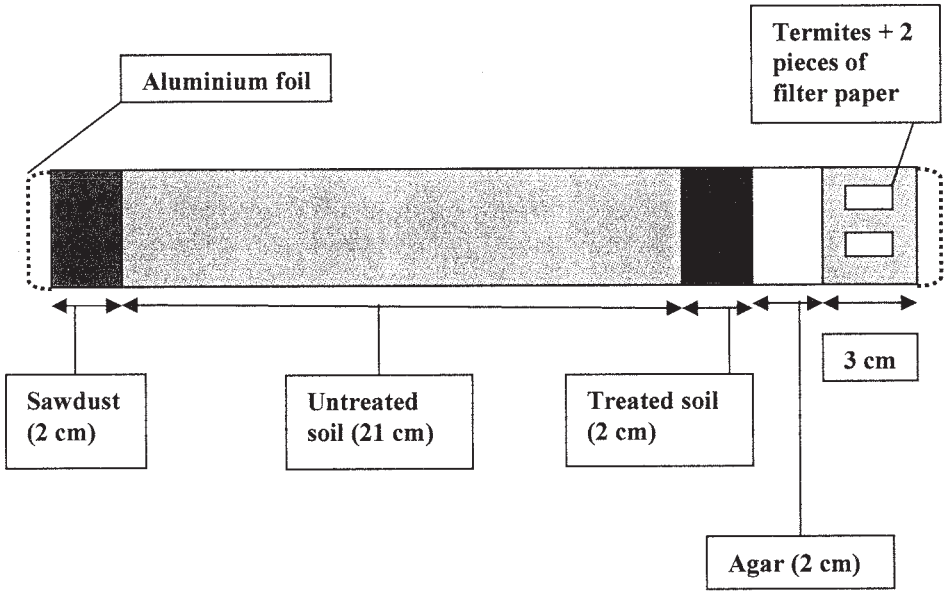

Figure 1: Experimental setup of the tube method for evaluation of termiticide performance.

Results and Discussion

Results indicated that all termiticide formulations (with exception to bifenthrin) evaluated show non-repellent characteristics (Table 1). Under glass-tube method, both DPX-E2Y45 and indoxacarb showed excellent performance even at 10 ppm w/w. Termites continued to tunnel forward without detecting the presence of the compounds, thus resulting complete mortality of the test insects after 7-day post-treatment. As for fipronil and WellTech 0980, similar efficacies were recorded at 1 ppm w/w. At higher concentrations, most formulations showed a faster killing action, as evident from the shorter tunneling distance of the termites. In this experiment, there was minimal mortality of termites in the control replicates and the maximum tunneling distance was achieved within 48 hours post-treatment.

Table 1: Summary of the performance of the termiticides evaluated using the modified glass tube method.

Termiticide	Conc. (ppm)	Evaluated parameters		Characteristic
		Tunneling distance[1]	Mortality of termites[2]	
Control (water)	-	Long	Low	-
DPX-E2Y45	1	Long	Low	Non-repellent
	10	Long	High	
	50	Short	High	
	100	Short	High	
Indoxacarb	1	Long	Low	Non-repellent
	10	Long	High	
	50	Long	High	
	100	Short	High	
WellTech 0980	1	Long	High	Non-repellent
	10	Long	High	
	50	Long	High	
	100	Short	High	
Fipronil	1	Long	High	Non-repellent
	10	Short	High	
	50	Short	High	
	100	Long	High	
Bifenthrin	1	Long	Low	Repellent
	10	Short	Low	
	50	Short	High	
	100	Short	High	

[1]Tunneling distance: 0 – 30% total distance = short; 31 – 60% total distance = moderate; 61 – 100% total distance = Long.
[2]Termite mortality: 0 – 25% = Low; 25 – 70% = moderate; >75% = High.

Evaluation using the petri-dish method confirmed the characteristics of the compounds evaluated in the earlier experiment. WellTech 0980 was found to be an excellent non-repellent termiticide candidate. Termites was found to forage freely between the two sections (treated and untreated) and complete mortality of termites was recorded after several days (Table 2). Despite the foraging activity of termites in the treated zone, the amount of wood consumption was still relatively low. Both DPX-E2Y45 and indoxacarb also demonstrated good non-repellent insecticide properties with moderate tunneling activities in treated zone and minimal wood damages. Fipronil, however, showed a quicker killing action when compared to the former three candidates. On the other hand, bifenthrin was clearly a repellent insecticide with high termite tunneling activity in untreated section and no activity in the treated zone.

To achieve greater colony suppression or even elimination, it is crucial that the termiticide used is slow-acting and non-repellent. This is because termites demonstrate necrophobic behavior where quick mortality of the poisoned termites may result in abandon or sealing of tunnels that leads to the treated zone by healthy colony members (Su et al. 1982). Once the decomposed corpses accummulated near and in treated zones, the healthy termites will no longer come in contact with the treated zone and thus survived the treatment (Su 2005).

Table 2: Summary of the performance of the termiticides evaluated using the petri-dish method.

Termiticide	Conc. (ppm w/w)	Tunneling activity (wood consumption)		Termite mortality
		Untreated section	Treated section	
Control (water)	-	High (high)	High (high)	Low
DPX-E2Y45	100	High (moderate)	Moderate (low)	High
Indoxacarb	100	High (moderate)	Moderate (low)	High
WellTech 0980	30	High (moderate)	High (low)	High
Fipronil	11	Moderate (low)	Low (low)	High
Bifenthrin	30	High (high)	No (no)	Low

In this study, two methods were used to evaluate the termiticides. The glass tube provides a reliable quantitative method that permit measurement of tunneling distance of test insects under the presence of a treated barrier (layer). On the other hand, the petri-dish method provides a simple qualitative approach to study the response of termites between the treated and untreated zones. This method also enables accurate determination of the wood damage in both treated and untreated zones. It is believed that when all parameters obtained from the two methods were taken in account, it can provide a relatively reliable interpretation on the actual performance of termiticide formulation.

Acknowledgments

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Comparative Study on Seven Different Entomopathogenic Fungi on the Mortality of *Coptotermes* sp.

by
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Abstract

Termite cause a serious problem world wide destroying many buildings and furnitures. Chemical insecticides is widely used to prevent the lost from termites attack. The use of chemical insecticides, however, do not recomended as it may cause risk to human health, environment, and may also harm to the non-target organisms and lead to the development of pest resistance.. These situations encourage many scientists to develop and evaluate various prospective biological controls of termites.

Fungal has a considerable potential for controlling insects including termites. There are a large number of fungi as an insects parasite (entomopathogenic fungi). Utilization of entomopathogenic fungi has being developed in many countries, but not in Indonesia. For this purpose, the use of biological agents to control termite should be initiated to minimize the damages caused by chemical insecticide.

In this research, seven different species of fungi namely *Metarhizium* sp., *Beauveria* sp., *Acremonium*, *X-1* (not identified yet) and three *Fusarium* sp. (*Fusarium* A, B and C) were evaluated. The fungi are grown on rice culture media (RCM). The production of conidial powder (bio-insecticide) and its entomopathogenic activity were observed. Number of conidia of seven fungi species obtained from a 10 days cultures were evaluated. The total number of conidia of *Metarhizium* found to be the highest followed by *Acremonium, Fusarium* B, *Beauveria, X-1, Fusarium* A and *Fusarium* C respectively. Our observation shows that the number of fungal conidia do not directly correspond to the mortality rate of termites tested. *Beauveria* caused 100% mortality of termite after 10 days observation. But the total number of conidia of this fungus lower compared to that of *Metarhizium, Acremonium* and *Fusarium* B.

Key words: biological control, entomopathogenic fungi, subterranean termite.

Introduction

Termite cause a serious problem world wide as they have a strong destructions on buildings and furnitures. The Formosan subterranean termite, *Coptotermes* sp., is one of a major economic important subterranean species contributing to this problem (Culliney & Grace 2000). To prevent the lost, chemical pesticides was widely and successfully used to control termite populations. The use of chemical pesticides, however, do no longer being supported by scientist community, policymakers and public as it may cause human health risks, environmental pollution and may harm on non-target organisms and could lead to the development of pest resistance.

These situations encourage many scientists to develop an alternative solution to the conventional pesticide. In recent years, evaluation on various prospective biological controls of subterranean termites e.g *Coptotermes*, continued. Butt (2000) noted that fungal has a considerable potential for controlling insects, diseases as well as weed.

According to Burges (1998) and Butt *et al.* (1999) fungi (*Verticillium lecanii, Metarhizium anisopliae, M. flavoviride, Beauveria bassiana, B. brongniartii, Paecilomyces fumosoroseus* and *Lagenidium giganteum*) could be developed as biological control e.g pesticides. *M. Anisopliae* is being developed as biological control in Japan and USA against termites.

According to Moore-Landecker (1996) there are a large number of fungi living as insects parasite. He noted that there are more than 700 species of insect parasitic fungi include some chytrids,

almost all Entomophthorales groups, a few of yeasts, numerous members of Ascomycota, Deuteromycota, and Uredinella (a member of the Septobasidiales). Those fungi which is called an entomopathogenic fungi, mostly invade the host insect through the exoskeleton or the cuticle and may involve complex biochemical interactions between the host and the fungus before germination, penetration, growth and reproduction of the fungus (Lacey *et al.* 2001). The spores of fungus is commonly inisiated an infection on the arthropods. Typically, the spore binds nonspecifically to the host exoskeleton and germinate (Moore-Landecker 1996).

Today, biological control of insect using entomopathogenic fungi has developed in many country, and the report included the mass production technology for fungal conidia, *Metarhizium* in particular (Jenkins *et al.* 1998). Unfortunately, in Indonesia this kind of research has not been developed yet. Termite control practice in Indonesia is still virtually adopted a conventional techniques applying chemical pesticides. In order that, the use of biological agents to control termite should be initiated to minimize human health risks, environmental pollutions, elimination of non-target organisms and to prevent the development of pest resistance. For this purposes, seven different genus of fungi namely *Metarhizium* sp., *Beauveria* sp., *Acremonium*, *X-1* (not identified yet) and three *Fusarium* sp. (*Fusarium* A, B and C) were evaluated. This initial work is dedicated to developed a simple and yet economic system for the production of spore as an alternative pesticide to control termite in the country.

Materials and methods

Microorganism. Seven different fungi namely *Metarhizium* sp., *Beauveria* sp., *Acremonium*, *X-1* and three *Fusarium* sp. (*Fusarium* A, B and C) were used. *Metarhizium* sp was obtained from LIPI Microbial Culture collection. While other fungal isolates were isolated from infected termites and soil around Science Center LIPI, Cibinong, West Java. Those cultures were grown on Potato Dextrose Agar Slant at room temperature for 7 days to sporulate and the sporulated cultures were stored in refrigerators with the temperature around 10^0 C.

Rice Culture Media (RCM). Local variety of rice commonly available in the market was used. To the amount of 10 g of rice placed in a 250 ml erlenmeyer flask, a 6 ml of distilled water was added. Covered the flask by a non absorbance cotton and let it stood for 2 hours. It was then sterilized using autoclave for 15 min at 121°C.

Production of fungal conidia. Sterile, 10 ml aquadest was added to a pure sporulated culture of fungi grown on Potato Dextrose Agar Slant. The spores were scraped off the agar with an inoculating wire aseptically. One ml of spore suspension was drop using sterilized pippet on a sterilized RCM. This preparation was incubated for 10 days at room temperature to produce conidia.

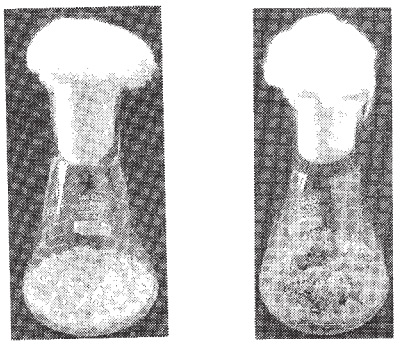
Counting of fungal conidia. Sterile, 50 ml aquadest was added to fermented RCM, and then it were mixed using magnetic stirrer to homogenize suspension. About 1 ml of conidial suspension was moved to 9 ml aquadest. Then conidias were observed microscopically and counted in a hemacytometre chamber.

Production of Biopesticide. Amount of 20 g of sago powder was placed on. Alumunium foil or Petri Dish. The powder was then dry sterilized for 6 hours at 106°C using Germany made oven (Memmert, 2000 W). Sterilized sago powder were then transferred aseptically to the 10 days fungal cultures. It was then blended and mixed with the colonized RCM. The mixture was then kept in sterilized bottle, dried for 4 days at 40°C according to the methods of Jenkins *et al.* (1998). The preparation was then blended to form a powder. This powder will be called a biopesticide.

Entomo-Pathogenic Activity (Anti-Termite Test). Anti termite test was carried out by using contact method. Fifty workers of *Coptotermes* sp. were sprayed with about 0,5 g biopesticide (dust spraying). The untreated termites were used as a control. Each treatment carried out in three-replicates. Eeach test specimen of *Coptotermes* sp. was placed on Petri Dish and kept at 28^0C under high humidity in the dark for 14 days. Anti termite potentials were determined from the mortality rate of termites during 14 observation days.

Results and Discussion

There is wide range of solid substrates which could be used in the production of fungal biomass as biological control. Solid particles, such as rice, wheat, maize, sorghum, potato and others cereals are suitable for the production of aerial conidia. They do support a good sporulation of many fungi. The most commonly selected substrate for production of fungal conidia is white rice. Meanwhile, most fungi could also grow readily in liquid media, but only mycelium or a hyphal body is developed (Jenkins *et al.* 1998). Cooked rice was used by Rusmin and Ko (1974) to produce conidia and spore of *Rhizopus oligosporus* to be used as an inoculum for the manufacturing of tempe. On cooked rice, spores of *Rhizopus oligosporus* are germinated and the subsequent growth covered the rice lumps with a white mycelial layer. On the second day the color turned to light gray due to the formation of sporangia. Our observation on the growth of seven fungi tested, all of the fungi successfully colonized the whole part of rice after 8-10 days of incubation (figure 1). Visual characters of each individual culture could distinguish as a result of distinct fungal characters in the way of sporulation behaviour. According to Jenkins *et al.* (1998), rice may be used for the production of *Metarhizium flavoviride* aerial conidia. When it is incubated at 25±2°C, it required an incubation period of 10-12 days before production of conidial powder.



a b

Figure 1. Visual character of fungi after 14 observation days (a. untreated RCM, b. fungal colony of inoculated RCM represented by *Metarhizium*)

Abundant conidia were produced by seven fungi tested after 10 days incubation period. The following table presents the difference of sporulation behaviour of seven fungi.

Table 1. Growth behaviour of 7 different fungi on RCM observed after 10 days

Fungi	Number of conidias (per gram substrate)
Metarhizium	$9.\ 10^8$
Beauveria	$1{,}7.\ 10^8$
Acremonium	$7.\ 10^8$
X-1	$7{,}5.\ 10^7$
Fusarium A	$4{,}5.\ 10^7$
Fusarium B	$2{,}3.\ 10^8$
Fusarium C	Not found

Table 1 shows that *Metarhizium* produced abundant conidia in highest number followed by *Acremonium*, *Fusarium* B, *Beauveria*, *X-1*, *Fusarium* A and *Fusarium* C respectively. Conidial production of each individual culture could distinguish as a result of distinct fungal characters in the way of sporulation

behaviour. In 1996, Moore-Landecker stated that sporulation behaviour was controlled by genetic factors as well as by hormonal, nutritional and environmental factors.

Our observation, shows that finely conidial powder (bio-pesticide, shown by Figure 2) produced is found to be effective against termites. The termite mortality of biopesticide containing conidial powder of all fungi tested is higher than control. The effect of fungal conidia of *Metarhizium* and *Beauveria* could caused 100 % of termite mortality after 14 days. Meanwhile, fungal conidia of *X-1*, *Acremonium*, *Fusarium* A, B and C caused 95,8 %; 81,7 %; 91,7 %; 79,2 % and 80,8 % respectively after 14 days (Figure 3). *Metarhizium* and *Beauveria* are already identified as an effective entomopathogenic Hyphomycetes for commercial production and use against a broad range of insect pests, including termites (Lacey *et al.* 2001). *M. anisopliae* is developed for microbial insecticide of termite, while *Beauveria bassiana* and *Fusarium oxysporum* are developed for another pests control, such as stem borers and nematodes (Langenwald & Cherry 2000). In the other hand, there is no much information about *Acremonium* using as biological control.

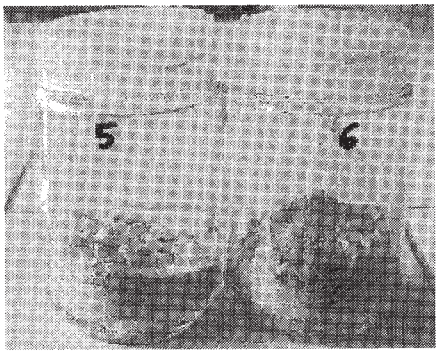


Figure 2. Performance of conidial powder kept in sterilized bottles

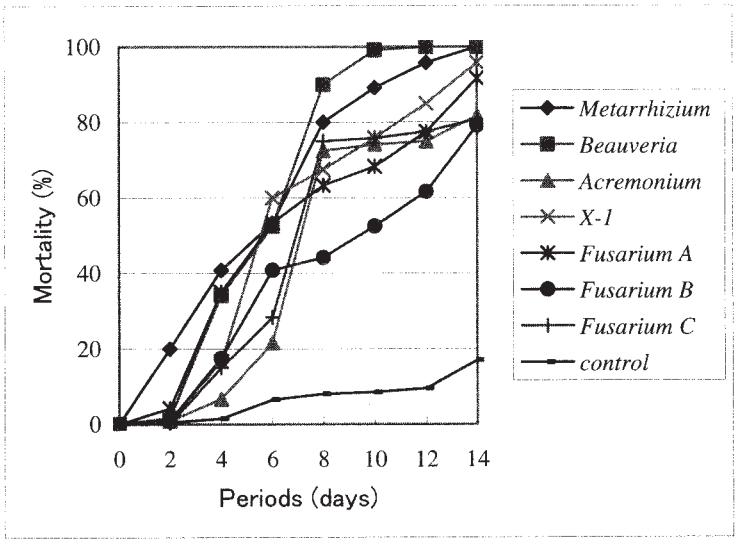


Figure 3. Mortality of termite after fungal infection during 14 observation days

The number of fungal conidia does not always correspond with infection activity. According to Lacey (1997) Siegel thought that infection may occurs as living agents cause a disruption to the host

either by multiplication in tissue because of spores germination, toxin production, or both. Therefore, we can say that spore or conidia is not primary factor in termite mortality, it could be caused by fungal toxic substances. In this research, we can see that *Beauveria* caused 100% mortality of termite after 10 days observation days, faster than other fungi, although it has less conidia compared with *Metarhizium, Acremonium* and *Fusarium* B. In 1980, Domsch *et al.* noted that *B. bassiana* produced toxic substances such as chitinase, jointly with other enzymes in decomposing insect integument. He also reported, production of toxic substances act as a contact insecticide, known to be a proteinase complex consisting of two fractions with different molecular weights. As well as *Fusarium* C, this fungus caused termite mortality which was not different significantly with *Acremonium* and *Fusarium* B effect, although there was no conidia of *Fusarium* C found in RCM. For *X-1*, it would be prospective for suppressing termite population. This fungus caused termite mortality almost 100 % (95,8 %) in 14 days of observation. Even though it just has a few number of conidia, much less than *Metarhizium* and *Beauveria*. Hopefully it might caused higher mortality of termite if this fungus has same number of conidia with *Metarhizium* or *Beauveria*. Based on this study, it considerably recommends *Beauveria* and *X-1* beside *Metarhizium* for being developed in further biological control research.

Conclusion

RCM grown fungi produced abundant conidia after 10 observation days. *Metarhizium* produced abundant conidia in highest number followed by *Acremonium, Fusarium* B, *Beauveria, X-1, Fusarium* A and *Fusarium* C respectively. However, spore or conidia are not primary factor in termite mortality, it could be caused by fungal toxic substances, or both. Based on entomopathogenic activity test, *Beauveria, X-1*, as well as *Metarhizium* are prospective for being developed as biological control agents of termites.

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Laboratory Evaluation of the Termite Resistance of Plastic Tubes

by
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Abstract

Plastic tubes were tested for their resistance to the subterranean termite *Coptotermes formosanus* Shiraki under the forced feeding condition in the laboratory. A single plastic specimen was exposed to 300 workers and 30 soldiers in a test container at 28±2℃ for three weeks in the dark. After exposure to termites, test specimens were recovered and cleaned off surface debris with tap water to visually examine the extent of termite attack. There was no visible termite attack on any plastic tube specimen of the aged and surface-scratched group. The results seemed to suggest that the experimental design was not suitable for the relative comparison of termite resistance among tubes tested.

Key words: termite resistance, *Coptotermes formosanus*, plastic tubes, polyamide

Introduction

The termite resistance of plastic bars and films of polyamide was previously evaluated in the laboratory according to existing standard methods. Since termite did not succeed in attacking any test material, polyamide was proved to be resistant against voracious workers of *Coptotermes formosanus* Shiraki (Rosenblat *et al.* 2005). However, field evaluations are needed to actually determine the termite resistance of plastic materials because previous field evaluation that termite could penetrate into other kind of plastic materials such as polyvinyl chloride and polyethylene plastics (Beal & Bultman 1978).

In the current investigation, plastic tubes prepared by Arkema K. K. were tested for their resistance to termite attack under the forced feeding condition in the laboratory using undifferentiated workers of *C. formosanus*. The results are expected to be available for comparing relative termite resistance of test materials.

Materials and methods

Laboratory bioassay was conducted under the forced feeding condition (nothing but a test specimen as food) according to the newly designed experimental procedure (Fig.1). A single tube specimen (40 mm long) was buried in the center of bottom half of an acrylic cylindrical test container together with 300 workers and 30 soldiers of *C. formosanus*. The test unit was placed on water-moistened cotton pads in a larger container and incubated at 28±2℃ for three weeks in the dark. Following three weeks' exposure, test specimens were recovered and cleaned off surface debris with tap water for the subsequent visual inspection.

Six kinds of plastic tubes were tested in the current experiment. Those were:

1) Low Density Polyethylene (LDPE) 8 mm in outer diameter, 40 mm long with wall thickness of 1mm
2) Arnitel: Thermoplastic Copolyester based elastomer (TPE-E) 13 mm in outer diameter, 40 mm long with wall thickness of 1mm
3) Orgalloy LE 60 THM (Polyolefin and polyamide alloy) 8 mm in outer diameter, 40 mm long with wall thickness of 1mm
4) Rilsan BECV Blue T8L (polyamide 11) 8 mm in outer diameter, 40 mm long with wall thick-

ness of 1mm

5) Rilsan AECV black T8L (polyamide 12) 8 mm in outer diameter, 40 mm long with wall thickness of 1mm
6) Cristamid MS 1700 (amorphous polyamide) 8 mm in outer diameter, 40 mm long with wall thickness of 1 mm

Twenty specimens of each plastic tube kind were prepared, and a half of them were aged (soaking in hot water at 90°C for 21 days). Five each of aged and not aged sets were scratched so that plastic tubes were sorted into 4 conditioned groups, each consisting of 5 replicates: a) not aged/not scratched, b) not aged/scratched, c) aged/not scratched and d) aged/ scratched.

Since it was thought that the group of aged and scratched tubes [group d)] was most vulnerable to termites, laboratory evaluation was conducted with one each of plastic tubes of the group d).

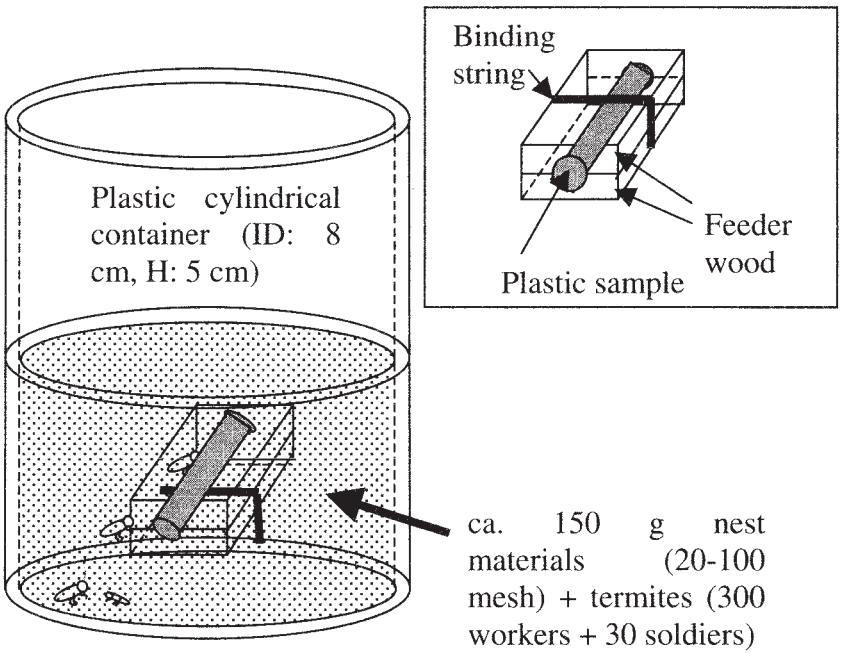

Fig. 1 Experimental design for evaluating the resistance of plastic tubes against termites in the laboratory

Results and discussion

On the basis of hardness of the test tubes, at least aged and scratched low-density polyethylene (LDPE) was thought to sustain some termite nibbling and/or biting (Beal & Bultman 1978). However, there was no visually noticeable termite attack on any plastic tube even when feeder wood attracted termite to gain access to the test tubes. The results were thoroughly contrary to our expectation. This might be due to low termite pressure and short test duration in the current trial. It, therefore, was concluded that the experimental design was not suitable for the present experiment objective. A modified method that can give more termite pressure to test samples should be tried next. For instance, placement of test plastic tubes around a laboratory nest of *C. formosanus* would be worthy of testing.

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International Comparison of Three Field Methods for Assessing the In-ground Resistance of Preservative-treated and Untreated Wood to Termites and Fungal Decay – Summary of Observations after Five Years

by

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Abstract

Results are presented from a five-year study conducted in five locations in Australia, Thailand and the USA. Three methods of exposure were assessed (below-ground, graveyard and ground contact) for evaluating the in-ground termite and decay resistance of *Pinus radiata* D. Don sapwood stakes that had been vacuum pressure impregnated with CCA (Type C) and ACQ (Type D) each at two nominal retentions (2 and 4kg/m^3), and of outer heartwood stakes from five trees of the durable North American baldcypress, *Taxodium distichum* (L.) Rich.

The termite attack and fungal decay ratings, based on the loss of cross section of the stakes, declined more rapidly at the three tropical sites in southern Thailand (Phuket) and in northern Australia (Darwin) compared to the two more temperate sites in southern USA (Gulfport, Mississippi) and southern Australia (Griffith, New South Wales). The mean ratings could differ between the three exposure methods, but the differences were not consistent between locations. For example, the lowest mean ratings for specimens treated with 4kg/m^3 of CCA were found for the below ground exposure method in tropical Australia, but for the graveyard method in Thailand. The pattern of attack on specimens at each location was dependent on the foraging behaviour of the local termite fauna, in particular the depth in the soil at which termites search for food.

Average [n = 15] termite attack ratings (**1st value Australian**, 2nd value ASTM rating) after 5 years in the tropics (T) and non-tropics (NT) were: CCA 2kg/m^3 T = **4.3**/6, NT = **6.7**/8; CCA 4kg/m^3 T =**5.5**/7, NT = **7.4**/9; ACQ 2kg/m^3 T = **4.4**/6 , NT = **6.9**/9; ACQ 4kg/m^3 T = **5.4**/7, NT = **7.1**/9. Baldcypress was more durable in the USA and Thailand (slight attack) and least durable against *Mastotermes darwiniensis* Froggatt in Australia (moderate attack). Fungus decay ratings (**1st value Australian**, 2nd value ASTM rating) after 5 years were: CCA 2kg/ m^3 T = **5.1**/7, NT = **7.9**/9; CCA 4kg/m^3 T = **6.1**/8. NT = **7.8**/9; ACQ 2kg/m^3 T = **5.2**/7, NT = **6.7**/9; ACQ 4kg/m^3 T = **5.6**/8, NT = **7.7**/9. Baldcypress results varied with source tree and test method.

Keywords: subterranean termites, Isoptera; below-ground exposure method, graveyard method, ground contact method, CCA and ACQ wood preservatives; baldcypress