USE OF A SHORT SPAN FIELD TEST TO EVALUATE TERMITE RESISTANCE OF *EUCALYPTUS GRANDIS* AND *BOBGUNNIA MADAGASCARIENSIS* IN A TROPICAL ENVIRONMENT

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Despite being treated with preservatives, the service life of transmission poles and fence posts in the Zambian Copperbelt province is close to fifteen years. However, the service life is only two years for untreated timber, mainly due to termite damage. This short service life is exerting more pressure on an already over-burdened timber resource base. This study used an accelerated field test investigation to facilitate initial assessment of several lesser known indigenous timbers for their termite resistance properties. To determine the service life and natural durability of *Eucalyptus grandis* and *Bobgunnia madagascariensis* to termite attack, samples of each wood species were field exposed to an aggressive species of subterranean termites for 32 days. Morphological and genetic analyses confirmed that the aggressive species of termites in this study was *Coptotermes formosanus,* commonly referred to as Formosan subterranean termites. Results indicated that *E. grandis* can be labeled as susceptible (*S*) following the standard natural durability rating procedure on the basis of service life projected from short term field exposure weight loss determination. Using short duration exposure weight loss and visual designation, similar to the Gulfport scale, *B. madagascariensis* was designated as very durable (*D*). These results showed that natural durability of timber against termites can be estimated after a short duration field exposure to Formosan subterranean termites. This method offers a fast field test for screening of promising lesser known tropical timbers.

Keywords: Formosan subterranean termites; Natural durability; Weight loss/Gulfport scale; Genetic analysis; Morphological analysis; Eucalyptus grandis; Bobgunnia madagascariensis

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INTRODUCTION

Zambia is endowed with 49,468 million hectares of natural forest, of which 300,000 hectares is deforested annually (Global Forest Resource Assessment 2010) due to increased demand for agricultural land and wood fuel. Forest degradation due to high demand for non-durable construction is exacerbated by more than a decade-long lapse (*i.e.* 1990 to 2005) in the replanting of plantation forests (Zambia Forest Information and Data 2010). Additionally, most of the creosote-treated wood poles in Zambia and in other tropical areas are usually replaced every 15 years due to extensive heartwood degradation by termites. Consequently, treated poles replacement increases the demand of timber harvested without being replaced by re-growth.

A program for pole remediation can possibly increase the service life of poles, but freshly treated poles have a high propensity toward leaching, and regular replacement of treated poles would cause pollution (Stilwell *et al.* 2003; Becker *et al.* 2001; Lebow 1996). Although leaching can be controlled by post-treatment conditioning of treated poles (Yu *et al.* 2010), this is not practiced at the pole treatment plants in Zambia. Leaching of polycyclic aromatic hydrocarbons and heterocycles from creosote preservative treated wood during contact with water in soils poses a threat for the surrounding environment and humans (Becker *et al.* 2001). The use of creosote-containing preservatives for protection of wood products in Zambia is of environmental concern, particularly in Copperbelt, Central, Northern, and Luapula provinces. These provinces are characterized by highly weathered, acidic, and easily leachable soils having pH values in the 4 to 4.5 range (Anon 2010). A strategy to reduce leaching of components is necessary for the growth of wood products utilization. Alternative solutions including using steel, plastics, or other materials to substitute wooden poles may pose bigger problems, such as disposal, waste management at the end of the service life, and high water and energy demand for manufacturing.

Strategies that promote the use of biodegradable-renewable-carbon materials, such as wood, require species with considerable natural durability for decay and insects or the use of environmentally benign preservatives (Eriksson 2003). The use and availability of the durable commercial tropical timbers are restricted by certification schemes for sustainable management of timber resources.

Lesser-known timbers are important (Carey and Hendry 1998) as potential commercial timber alternatives. However, if the impact of lesser-known timbers on the world market has to be appreciated, their properties such as natural durability should be specified (Carey and Hendry 1998). Naturally durable timbers usually have longer service life and require replacement less frequently, which consequently reduces the frequency of associated harvesting including commercial tropical timbers. Additionally, naturally durable timbers provide an opportunity for developing environmentally benign wood preservatives from their natural toxic compounds (Zabel and Morrell 1992; Suttie and Orsler 1996).

The assessment of resistance of timber against termites involves initial screening by accelerated laboratory tests of 4 to 16 weeks followed by field tests (AWPA E1-07 2010; AWPA E1-06 2008; ASTM D3345-74 1998). The simulation of termites' natural environment in laboratory tests subjects the insects to undue stress, possibly altering their feeding habits. The other problem is that the special equipment and infrastructure required for raising termites in captivity might not be available in some research institutions. In this study, a short span field test was undertaken to evaluate the natural durability and to determine the hazard class of a lesser-known timber, namely *Swartzia madagascariensis* Desvaux now renamed *Bobgunnia madagascariensis* (Kirkbride and Wiersema 1997). *Eucalyptus grandis* W. Hill ex Maiden was used as a reference because it is highly susceptible to termite attack (Ssemaganda *et al.* 2011). *S. madagascariensis* and *S. fistuloides* are endemic in Africa (Angyalossy-Alfonso and Miller 2002).

Approximately 14.5 million m^3 of merchantable volume of *B. madagascariensis* is available in Zambia (ILUA 2008).

The objective was to explore the possibility of estimating the service life of these timbers using short span field tests and then using this approach as a screening method for several lesser-known tropical timbers in sub-Saharan Africa.

EXPERIMENTAL

Materials

Bobgunnia madagascariensis logs measuring 22 cm diameter by 2.6 m long obtained from Mpata hills woodland outside Luanshya, Zambia were plain sawn into 38 mm thick boards followed by kiln drying to 18 % moisture content at Rainlands Timber, Kitwe, Zambia. The kiln-dried timber was edged to 30 mm width, and trimmed to 300 mm length heartwood boards. The boards were planed to 25 mm width by 25 mm thick samples. *E. grandis* logs of 27 cm diameter by 3 m long were provided by the Copperbelt Forestry Company and prepared using the same procedure described above.

Methods

Identification of subterranean termites

Two groups of termites collected from Mwekera National Forest were identified using morphological characteristics, while one group was further identified by DNA sequence comparison. Specimens were identified to species level using soldier or alate morphology (colour and size of heads of soldiers and/or colour of alates and characteristics of their wings). Three worker termites were selected and used for DNA extraction. DNA was extracted and purified using the Cetyl Trimethyl Ammonium Bromide (CTAB) method (Moller et al. 1992). The nucleic acids were pelleted by centrifugation and washed with 70 % ethanol. The resultant nucleic acid pellets were suspended in sterilised distilled water. Two microliters of RNaseA (10 µg/µL) were added and incubated at 35 ^{o}C for 2 hours to digest any residual RNA as described by Chungu *et al.* (2010). The DNA concentrations were determined using a Nanodrop ND-1000 Spectrophotometer v. 3.6 (Thermo Fisher Scientific, Wilmington, USA).

DNA extracted from termites collected in this study was used as templates for amplification with the polymerase chain reaction (PCR). The Cytochrome c oxidase subunit II (COII) region mitochondrial DNA was amplified using primers A-tLEU 5'-ATGGCAGATTAGTGCAATGG-3' and B-tLYS 5'-GTTTAAGAGACCAGTACTTG-3'primers (Simon *et al.*, 1994). PCR reactions were prepared in a total volume of 25 µL including 1 µL of genomic DNA (100 ng/µL), 1 µL of 1.25 U Taq DNA polymerase (Roche Diagnostics, South Africa), 2.5 µL of 10 x PCR buffer (10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, pH 8.3), 0.5 µL of 10 µM of forward and reverse primers (Inqaba Biotech, South Africa), 2.5 µL of 0.8 mM dNTPs (Roche Diagnostics, South Africa), 4 µL of 2.0 mM $MgCl_2$, and 13.5 µL of double distilled water. The PCR reactions consisted of an initial denaturation step of 96 ^{o}C for 1 min, followed by 35 cycles of template denaturation at 94 ^{o}C for 30 s, primer annealing at 52 ^{o}C for 60 s, followed by extension at 72 ^{o}C for 90 s. A final elongation step of 10 min at 72 ^{o}C completed the program.

PCR products were visualised on 2 % agarose gel containing ethidium bromide under ultraviolet light. Sizes of PCR amplicons were estimated by comparison against a 100 bp molecular weight marker (O' RangeRuler^{TM} 100 bp DNA ladder; Fermentas Life Sciences, USA). Prior to DNA sequencing, PCR products were purified using Centri-sep spin columns (Princeton Separations, Adelphina, NJ) containing Sephadex G-50 (Sigma Aldrich, Amersham Biosciences Limited, Sweden) as outlined by the manufacturer.

Purified PCR products were used as template DNA for cycle sequencing reactions using the ABI Prism Big Dye Terminator Cycle sequencing reaction kit v. 3.1 (Applied Biosystems, Foster City, CA, USA) following the manufacturer's protocol. The same primers as those used for the PCR reactions were used for sequencing reactions. Sequence reactions were run on polyacrylamide gel electrophoresis (PAGE) using an ABI PRISM^{TM} 377 Autosequencer (Applied Biosystems, Foster City, CA, USA). Sequence electropherograms were analysed using Sequence Navigator version 1.0.1 (Applied Biosystems, Foster City, CA) and both the forward and reverse sequences for each isolate were obtained.

Forward and reverse sequences were matched and analyzed using Sequence Navigator version 1.0.1 (Applied Biosystems, Foster City, CA, USA). Additional sequences for use in the analyses were obtained from Genbank (www.ncbi.nlm.nih.gov). Sequence alignments were done using the online interface (http://align.bmr.kyushu-u.ac.jp/mafft/software/) of MAFFT v. 5.667 (Katoh *et al.* 2005) incorporating the G-INS-i alignment algorithm for the purpose of this study. We checked the alignments manually, and sequences not aligned accurately by MAFFT were appropriately adjusted.

The phylogeny of the aligned sequences were determined using PAUP* 4.0 Phylogenetic Analysis Using Parsimony *and other methods) (Swofford 2002). All gaps were coded as missing data and characters were assigned equal weight. The heuristic search option with random stepwise addition and tree bisection reconnection (TBR) was used as the swapping algorithm. The Mulpar option was in effect and branches collapsed if they equalled zero. Confidence levels of the branching points were determined using 1000 bootstrap replicates and distribution of 1000 trees.

For parsimony analyses, the tree length (TL), retention index (RI), consistency index (CI), rescaled consistency index (RC), and homoplasy index (HI) were determined. In the phylogenetic analyses, *R. speratus* and *R. lucifugus* were used as the outgroup taxa and treated as paraphyletic sister groups to the in-groups.

Field exposure of wood samples to subterranean termites

Two sets of 24 *E. grandis* ($E_{1...24}$) and *B. madagascariensis* ($B_{a...x}$) specimens consisting mainly of heartwood, free from growth defects and biotic infestation were randomly selected and labelled appropriately. Sections measuring 20 mm axially were cut from the middle of four representative samples per species $E_{1...4}$ for *E. grandis* and $B_{a...d}$ for *B. madagascariensis*, and were used to determine the initial moisture content according to BS EN 350-1 (1994). The weight and moisture content of each sample were calculated based on the average moisture content and the initial weight of conditioned samples. Twenty samples of each species were buried 150 mm into the ground close to an active termite hill for 32 days during the dry months of October and September 2010.

After 32 days, the specimens were removed from the ground, thoroughly cleaned, and weighed to determine their oven dry weight.

Data analysis

Termite resistance was determined by the Gulfport scale (Hardie and Wood 1968; Wagner *et al.* 2009) and percentage weight loss (WL) rating (Kaakeh 2005).

Damage rating by termites on the Gulfport scale was designated by A_{gps} (very durable); no attack, B_{gps} (durable); evidence of surface damage without significant incision, C_{gps} (moderately durable); definite grooving without evidence of colonisation, D_{gps} (slightly durable); deep grooving with runnels inside specimens, and E_{gps} (not durable); total destruction.

Termite resistance rating, *i.e.* damage rating index (DRI), on the basis of WL was recorded as DRI 0-1, 2-3, and 4-5. The percentage WL for each specimen was calculated as a fraction of estimated initial oven dry weight of wood material in ground contact. Table 1 shows the termite resistance indices corresponding to the natural durability classes.

Table 1. Termite Resistance Indices Corresponding to Natural Durability Classes of Wood in BS EN 350-1 (1994)

Damage Rating Index	Weight Loss (%)	Natural Durability Class
0 to 1	0 to less than 5	D_{nd} (Durable)
2 to 3	5 to less than 20	M_{nd} (Moderately durable)
4 to 5	more than 20	S_{nd} (Susceptible)

The natural durability classes for *E. grandis* and *B. madagascariensis* were determined as D_{nd} if DRI was 0 to 1; M_{nd} if DRI was 2 to 3, and S_{nd} if DRI was 4 to 5, respectively.

One-way analysis of variance (ANOVA) at 5 % level of significance was applied to detect differences in the basic density of *B. madagascariensis* and *E. grandis*.

RESULTS AND DISCUSSION

Subterranean Termite Species

Although two groups of subterranean termites were involved in this study, only one aggressive group was important. Further analysis was performed on this aggressive group. Subterranean termite soldiers of the aggressive group had teardrop-shaped to rectangular-shaped heads while their alates were yellowish brown and 13.5 mm long. The wings of these termites were more hairy compared with other groups under the light microscope. These morphological characteristics were typical of *Coptotermes formosamus* Shiraki (Isoptera: Rhinotermitidae) as described by Shiraki (1909); this species is commonly referred to as Formosan subterranean termites.

Based on phylogenetic analyses of the COII gene region, termites obtained from Zambia grouped into one group. Heuristic search analysis in PAUP of the COII sequence data resulted in 16 most parsimonious trees (TL = 678, CI = 0.948, RI = 0.852, RC = 0.808, HI = 0.052) (**Fig. 1**).

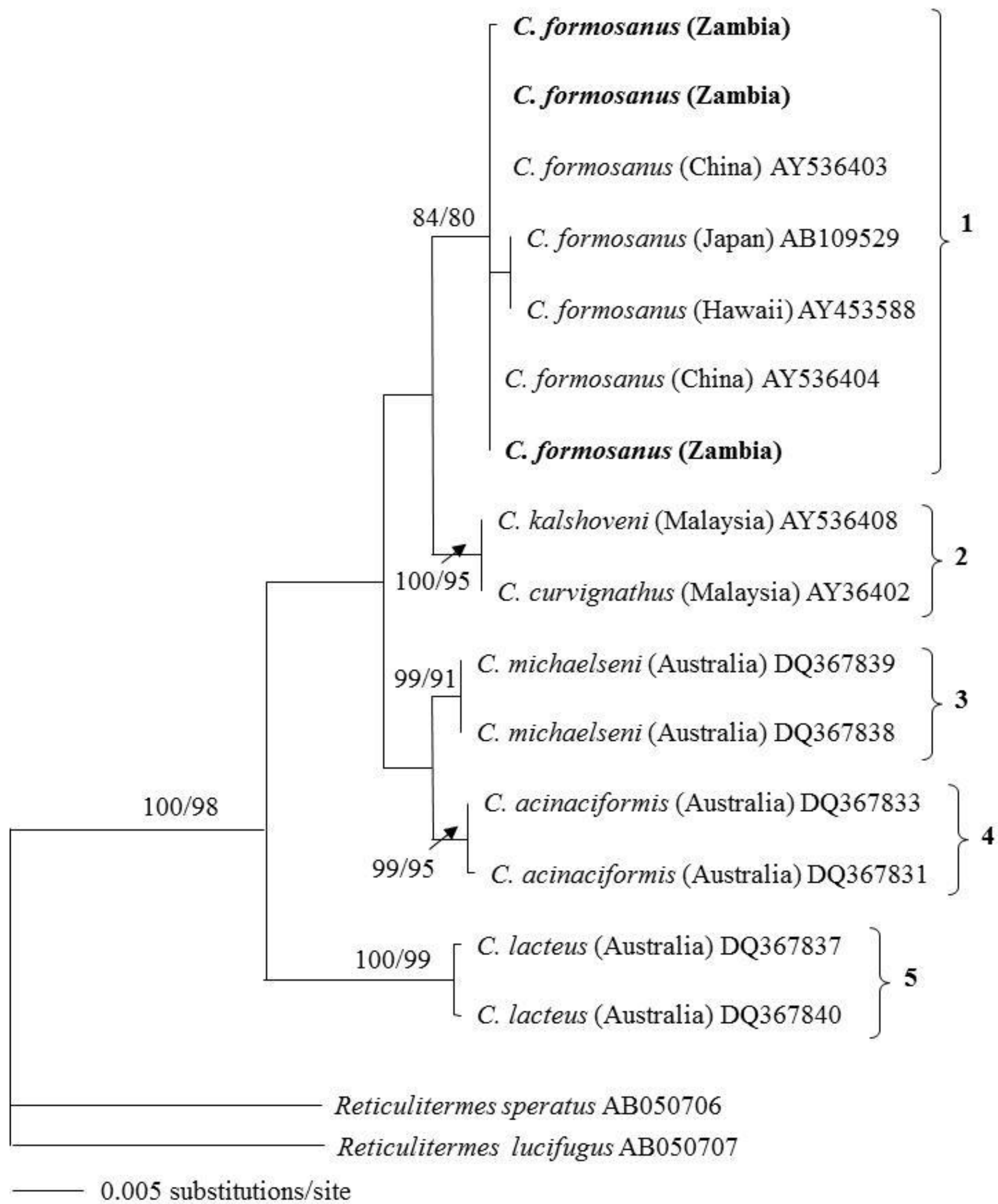


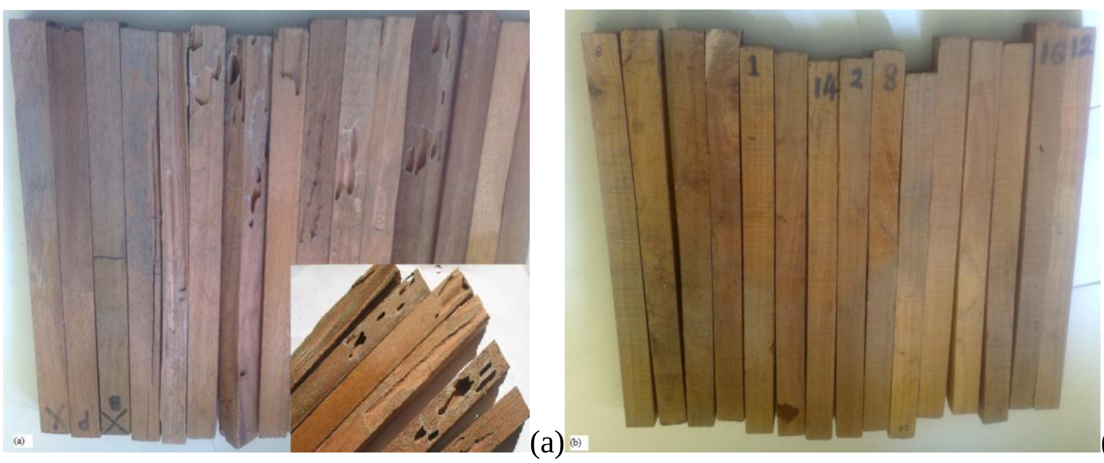
Fig. 1. Phylogram of *Coptotermes* species indicating the phylogenetic placement of *C. formosanus* obtained from the analysis of sequence data of COII gene region of the mitochondria DNA. Values above the branches indicate bootstrap values (TL = 378, CI = 0.828, RI = 0.701, RC = 0.780, HI = 0.063). Isolates sequenced in this study are in bold. *Reticulitermes speratus* and *R. lucifugus* were used as outgroups.

Termites in the phylogenetic tree obtained from the analysis of COII sequence data set resided in 5 sub-clades (1-5) (Fig. 1). Termites from Zambia resided in sub clade 1. Sub-clade 1 represented a previously known group within *Coptotermes* species and comprised of termites from a termite mound in a natural forest in Mwekera. Clades 2-5 represented other species of *Coptotermes* from different countries. Each clade was strongly supported with > 80 % bootstrap value. Termites from Zambia residing in Clade 1 (84 % bootstrap value) were resolved in a well-supported monophyletic single clade, representing *C. formosanus* which was distinct from all other species (Fig. 1).

Coptotermes may be the most economically harmful genus of termites worldwide. *C. formosanus*, an Asian species, has become observed in a number of countries, including Zambia, where its economic impact is in millions of dollars of damage annually (Su and Scheffrahn 2000). In Zambia, distribution and biology of termites is poorly understood and provides opportunity for future studies.

Termite Damage Rating by Gulfport Scale

Termite damage on *E. grandis* was characterized by grooving runnels inside the wood specimens. Termite damage rating by the Gulfport Scale was designated as ranging between being moderately durable (C_{gps}) and slightly durable (D_{gps}) (Fig. 2). There was no evidence of significant attack on *B. madagascariensis* specimens, indicating that this wood is very durable (A_{gps}), in agreement with Hardie and Wood (1968).



(a) (b)

Fig. 2. Termite damage incurred by *Eucalyptus grandis* (a) and *Bobgunnia madagascariensis* (b) wood specimens after 32 days of field exposure in ground contact

Weight Loss and Natural Durability Rating

After 32 days of exposure, wood specimens were removed from the field, and the extent of termite damage was determined by weight loss (Table 2). Two *B. madagascariensis* measurements detected as outliers (*i.e.* their modified z-scores were greater than three and half standard deviations) were not used in the data analysis (Fallon and Spada 1997).

Table 2. Basic Density, Estimated Service Life Including the Weight Loss Incurred by Two Hardwoods after 32 Days of Field Exposure to Subterranean Termites (Standard deviation is shown in brackets)

Wood Species	Basic Density (kg/m3)	Weight Loss (g)	Weight Loss (%)	Estimated Service Life (years)
Eucalyptus grandis	812.4[a] (126.8)	6.3 (1.5)	8.3 (1.5)	1.1 (0.2)
Bobgunnia madagascariensis	955.1[b] (137.1)	1.9 (0.6)	2.2 (0.8)	8.5 (2.7)
NOTE: Values with different superscripts are significantly different at 5 % level.				

Termites achieved slight to moderate feeding and penetration, DRI of 2 to 3, on *E. grandis* specimens (Fig. 2), which concurs with the natural durability rating of M_{nd} in the Prota database (2012). The in-ground life expectancy for *E. grandis* at 50 % weight losses was projected as 1 year or 403 days. Impregnation of *E. grandis* with preservatives is a requirement for improving termite resistance. However, since heartwood treatability is very poor, transmission wood poles are not adequately protected. Ninety to one hundred and forty wood poles are replaced monthly, i.e. between April and November, in Kitwe urban because the entire heartwood core has been gouged out (per. comm.).

Termite damage on *B. madagascariensis* wood specimens ranged from nil to superficial, DRI of 0-1, which agrees with a natural durability rating of D_{nd} (Table 2), and concurs with the rating conferred by the Gulfport scale. The life expectancy for *B. madagascariensis* in ground contact was projected as 8.5 years or 3,090 days with 50 % weight loss. This is consistent with results for natural durability obtained after 8.5 years of field exposure at Mwekera National Forest in the late 1950s (Hardie and Wood 1968). The higher basic density of *B. madagascariensis* possibly enhanced natural durability and significantly differed from *E. grandis* as revealed by one-way analysis of variance (F_{calc} 9.44 > F_{crit} 4.11, P < 0.05) (Table 2). These results demonstrate the potential for short span field test as a reliable alternative for initial screening of lesser-known wood species.

Adeyemi and Adebote (2010) found that methanol extracts of *B. madagascariensis* stem bark has high contact mortality of 65.5 % at 100 mg/mL, and feeding inhibition rate against the red flour beetle (*Tribolium casteneum*) of 70.66 % at 0.10 ppm concentration compared to 73.88 % at 0.05 ppm for the control, with Phostoxin (aluminium phosphide 57 %) a standard storage pesticide. The high termite resistance of *B. madagascariensis* wood indicates its antitermitic properties. Adfa *et al.* (2010) and Boué and Raina (2003) have observed *C. formosanus* termites mortality of 15 and 40 % (d. b.), respectively, in no-choice feeding tests using quercetin. Further work is underway to verify effects of quercetin on termite resistance of *B. madagascariensis*.

CONCLUSIONS

1. The most aggressive subterranean termites in this study were identified as *Coptotermes formosamus.* These termites cause damage to wood in service and were thus employed in the test for natural durability of various wood species.
2. Based on weight loss incurred by wood specimens after 32 days of field exposure, *Eucalyptus grandis* was rated as slightly durable, while *Bobgunnia madagascariensis* was rated as very durable using the Gulfport scale and damage rating indices.

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