Research Article

Received: 13 June 2017 Revised: 9 October 2017 Accepted article published: 2 November 2017

(wileyonlinelibrary.com) DOI 10.1002/ps.4785

Tropical wood resistance to the West Indian drywood termite *Cryptotermes brevis*: If termites can't chew …

Lírio Cosme Jr,[a] Marcelo M Haro,[a,b] Nelsa Maria P Guedes,[a,c] Terezinha Maria C Della Lucia[d] and Raul Narciso C Guedes[a*]

Abstract

BACKGROUND: The importance and impact of invasive species are usually considered based on their economic implications, particularly the direct damage that they cause. The West Indian drywood termite *Cryptotermes brevis* (Walker) is an example and is a concern in structural lumber, furniture, and other wood products. Despite its importance, its tropical wood preferences and the wood physical characteristics contributing to resistance have not been investigated to date. Here, we developed wood testing units to allow the X-ray recording of termite colonization and then subsequently tested tropical wood resistance to the termite through free-choice and no-choice bioassays using these wood testing units. The relevance of wood density and hardness as determinants of such resistance was also tested, as was termite mandible wear.

RESULTS: The wood testing units used allowed the assessment of the termite infestation and wood area loss, enabling subsequent choice bioassays to be performed. While pine (*Pinus* sp.), jequitiba (*Cariniana* sp.) and angelim (*Hymenolobium petraenum*) exhibited the heaviest losses and highest infestations; cumaru (*Dipteryx odorata*), guariuba (*Clarisia racemosa*), and purpleheart (*Peltogyne* sp.) showed the lowest losses and infestations; courbaril (*Hymenaea courbaril*), eucalyptus (*Eucalyptus* sp.), and tatajuba (*Bagassa guianensis*) exhibited intermediary results.

CONCLUSION: Wood hardness and in particular wood density were key determinants of wood resistance to the termites, which exhibited lower infestations associated with greater mandible wear when infesting harder high-density wood.

Keywords: wood preference; wood area loss; termite mandible wear; termite choice; invasive termites; Isoptera; Khalotermitidae

1 INTRODUCTION

Economic costs are prevailing determinants in the recognition of the relative importance of and priorities associated with invasive species,[1] costs that are indeed high, even if underestimated.[2,3] The direct costs attributable to losses caused by the invasive species infestation and its management costs are the core components determining the impact and importance of such species.[1,3] This potential economic impact and the arrival likelihood of the invasive species are the usual focus of attention in invasive biology, but the species establishment and subsequent spread should not be neglected.[1,4] This emphasis is a likely contributor in explaining why termites (Dictyoptera: Isoptera) are frequently overlooked as invasive species and have only recently been recognized in this category.[5,6]

The difficulties in recognizing the natural habitat of termite species prevent the identification of their native range, and thus their recognition as invasive species,[5] which is further impaired by problems of taxonomic misidentification.[5,7] Nonetheless, some 28 termite species, particularly Kalotermitidae (mainly *Cryptotermes*) and Rhinotermitidae (mainly *Coptotermes* and *Heterotermes*), are currently recognized as invasive species worldwide, all of which are wood-eating and considered important pest species of structural lumber, furniture, and other wood products.[5,8,9] Among them, the West Indian drywood termite *Cryptotermes brevis* (Walker) (Isoptera: Kalotermitidae) is the most widespread species whose endemic origin was recently recognized as the Pacific coastal desert of South America extending from Chile to Peru.[6] This species is arguably the main termite pest species in the tropics, except in Asia, probably as a consequence of the mutually exclusive distribution of the competing *Cryptotermes domesticus* (Haviland) in the region.[6,7] Although widespread, the distribution range of *C. brevis* is still expanding.[6,10–12]

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* *Correspondence to: RNC Guedes, Departamento de Entomologia, Universidade Federal de Viçosa, Viçosa, MG 36570-900, Brazil. E-mail: guedes@ufv.br*

a *Departamento de Entomologia, Universidade Federal de Viçosa, Viçosa, Brazil*

b *Empresa de Pesquisa Agropecuária e Extensão Rural de Santa Catarina (Epagri), Estação Experimental de Itajaí, Itajaí, Brazil*

c *ENTO$^+$ Soluções & Pesquisa, CENTEV-UFV, Viçosa, Brazil*

d *Departamento de Biologia Animal, Universidade Federal de Viçosa, Viçosa, Brazil*

The West Indian drywood termite exhibits essentially post-Colombian anthropogenic release and dispersion beyond its natural range.[6] In fact, anthropogenic dispersal resulting from international trade, transportation, and recreation prevails among invasive species.[13,14] Shipboard infestation and transport of wood and wood products infested with *C. brevis* during the establishment and development of early Spanish colonies in South America apparently provided the initial means of dispersal and invasion of this pest species,[6] which was probably aided by its attraction to light (during crepuscular and nocturnal flights) and cryptic behavior.[5,6,15] The characteristics of wood eating, wood nesting, and frequent production of secondary reproductives conspire to produce effective transported propagules, reinforcing the invasive nature of the West Indian drywood termite. Kalotermitidae in general, and *C. brevis* in particular, are single wood piece nesters, allowing their transportation and dispersal as viable propagules in small wood pieces.[5,7,16] The consequence is an invasive tramp species (i.e. a species anthropogenically and inadvertently spread throughout the world) well adapted to survival and reproduction even in wood pieces as small as 8 cm^3.[5]

Wood size does affect the inter-individual interactions and production of secondary reproductives,[17,18] which are also affected by wood anatomy and volatile emissions.[19,20] This is important not only for dispersal and establishment, but also for the potential economic losses in urban areas caused by infestation of wood and wood products by termites.[8,12,20] Termites also favor wood decomposition,[21] and are even a recognized threat to historic buildings and (wooden) artworks representative of the colonial period in the Americas.[7,22] *Cryptotermes brevis*, like the other 27 invasive termite species, nests in wood and produces secondary (or supplementary) reproductives, traits that increase their invasiveness potential.[5] Regarding this latter trait, the primary reproductives are from alates (i.e. standard imago adults), while secondary reproductives from *C. brevis* are mainly from pseudergates (or pseudoworkers; i.e. immatures that regressively molted from nymphs, losing their wing buds).

Despite the economic importance of *C. brevis* throughout its (pan)tropical distribution, tropical wood preferences and wood physical characteristics contributing to resistance to this species have been understudied, with only studies focusing on limited screening of wood resistance having been carried out, without exploring the underlying types and mechanisms involved.[23,24] Wood preferences, including those for pine and eucalypt, have been reported for other termite species (mainly subterranean),[8,25] although not their underlying causes, and insecticidal treatments prevail in their control.[8,26] Wood physical characteristics such as density and hardness are likely important determinants of termite wood preferences, but have not been studied in this context to date. Here, we aimed to address this shortcoming while developing wood testing units to allow digital X-ray recording of wood colonization by, and loss attributable to, West Indian drywood termites (*C. brevis*) for subsequent use for testing the resistance of tropical wood species to this termite through both free-choice and no-choice bioassays. Wood density and hardness were also recorded to test their role in the resistance to termite infestation and damage aided by the characterization of the termite's mandible wear. We expected *C. brevis* to adjust well to our designed wood testing unit and also expected variation in its response to different tropical woods, anticipating that the termites would favor softer material as they may not be able to chew on harder wood, as the popular perception reflected in the title suggests.

2 MATERIALS AND METHODS

2.1 Insects

The insects were collected from pieces of furniture and other wooden objects in the campus of the Federal University of Viçosa (Viçosa, state of Minas Gerais, Brazil) and established as colonies within plastic buckets using the original nest as a food source, in addition to smaller pieces of wood and cardboard. Winged adults were also collected between September and December 2014, sexed and paired in 500-mL containers with wood and cardboard to start new colonies, which provided individuals for the experiments. The colonies were maintained under controlled conditions of 27 ± 3 °C temperature, 60 ± 10% relative humidity, and a 10:14 h (light:dark) photoperiod; the same conditions were used in the experiments. The insects were sent to Prof. Reginaldo Constantino (Department of Zoology, University of Brasília, Brasília, DF, Brazil) for identification and they were confirmed be the West Indian drywood termite *Cryptotermes brevis*.

2.2 Termite colonization of wood testing units

As the insects seek refuge in covered hiding places, a wood testing unit was developed to allow termite colonization and subsequent testing of wood resistance to *C. brevis*. This wood testing unit was constructed from two identical wood pieces (0.50 cm thick x 2.00 cm wide x 8.00 cm long) glued together at their long edges with polyvinyl acetate (PVA; HEXION, Curitiba, PR, Brazil) to form an inverted "V" (i.e. a triangular tunnel). Each testing unit was placed over the bottom portion of a Petri dish and deposited inside a transparent plastic container (500 mL), the upper part of which was perforated below the lid. The set-up allowed free insect movement, particularly within the Petri dish, and eventual selection of a penetration site. Newly collected winged (reproductive) adults were placed in experimental units at densities ranging from one to six insects (paired as male and at least one female from the density of two, three, four and six insects per unit) and allowed to colonize testing units made of pine (*Pinus* sp., which is known to be promptly attacked); between six and seven replicates were used for each insect density. The colonies and experimental units were inspected at regular intervals (at 1, 4, 8, 12, 16, 20, 24, 28, 60, 75, 90, 120, 150, and 210 days) using an LX-60 specimen radiography system equipped with a digital camera (Faxitron X-Ray Corp., Wheeling, IL, USA) allowing recording of the colonization of the wood testing units. The projected area of tunneling was used as a proxy for the termite colonization of each testing unit, which was determined from the X-ray images using the software IMAGE PRO PLUS (Media Cybernetics, Rockville, MD, USA). A set of wood testing units infested with termites was maintained without exposure to X-rays to detect differences in termite development and tunneling caused by X-ray exposure, but no variation was observed at the end of the experiment.

2.3 Wood resistance to termites

Wood testing units were made from the heartwood of nine different and representative tropical species with diverse common uses in the neotropics, ranging from furniture to floor and wall paneling, frames, and internal and external structures, among others. They included: angelim (*Hymenolobium petraenum* Ducke), courbaril (or jatobá; *Hymenaea courbaril* L.), cumaru [*Dipteryx odorata* (Aubl.) Willd.], eucalyptus (*Eucalyptus* sp.), guariuba (or oity; *Clarisia racemosa* Ruiz & Pav.), jequitiba (*Cariniana* sp.), pine (*Pinus* sp.), purpleheart (*Peltogyne* sp.), and tatajuba (*Bagassa guianensis* Aubl.).

2.3.1 Free-choice bioassays

The free-choice tests were run in bioassays adapted from earlier methods.[27,28] Each bioassay contained two experimental units of each wood species randomly distributed as a circle over a white plastic tray (35 cm diameter) with inner walls coated with Teflon PTFE (DuPont, Paulínia, SP, Brazil) to prevent the insects from escaping; the bioassays were replicated six times. Nine hundred insects (pseudergates) were released within each tray and allowed free choice, and were observed for 120 days. The number of insects colonizing each wood testing unit was recorded at the end of the 120 days, wood tunneling was analyzed using the X-ray digital system, and the testing units were weighted to record the wood consumption in the period. As the natural variation in wood weight was negligible during the experiment, wood consumption was calculated based on the wood dry weight before and after the infestation following the formula: wood consumption (or loss) = wood initial dry weight − final weight. The wood dry weight was determined by drying the wood testing units in an oven maintained at $200 \pm 2\,^{\circ}\mathrm{C}$ until a constant weight was obtained. The wood units were maintained in a desiccator until weighing on a precision balance (BG 440; Gehaka, São Paulo, SP, Brazil).

2.3.2 No-choice bioassays

As in the free-choice bioassays, the no-choice tests were also run with two testing units of each wood species and six replicates. However, in this case, each wood testing unit was placed within a 500-mL transparent glass container as in the colonization experiment. Thirty insects, 29 pseudergates and one soldier, were released within each container and after 120 days the number of insects and wood loss were recorded as described for the free-choice bioassays. In addition, respiration rate and mandible wear were also recorded at the end of the experiment (i.e. 120 days) for the insects subjected to no-choice bioassays.

Respiration rate, which is a measure of both stress and food conversion efficacy, was determined through respirometry bioassays using a TR3C respirometer equipped with a CO_2 analyzer (Sable Systems International, Las Vegas, NV, USA) following general methods described elsewhere.[29,30] Briefly, three groups of ten insects maintained in each wood species for 120 days were weighed and subsequently enclosed in 25-mL glass respirometric chambers connected to a completely closed system. The amount of carbon dioxide produced was determined by injecting CO_2-free air into the chambers and directing the insect-produced CO_2 to an infrared reader. The CO_2 production in a control chamber without insects was also determined to calibrate the system.

Twenty pairs of mandibles were removed from the termites (pseudergates) colonizing each wood species and placed over parafilm on Petri dishes for measurements under a stereomicroscope (Leica MZ 7.5 equipped with a Leica Qwin Lite image capture system; Leica, Göttingen, Germany). The mandible wear was recorded by measuring the distance from the edge of the apical tooth to the base of the mandible, and also measuring the distance from the longest apical tooth to its insertion.[31] After such measurements, the mandibles were individually weighed in an analytical balance (AUW220D; Shimadzu, Kyoto, Japan) and three pairs of termite mandibles obtained from each wood species were prepared for scanning electron microscopy. The mandibles were fixed in paraformaldehyde (4%) in phosphate-buffered saline (PBS; 0.1 M, pH 2.4), washed in PBS, dehydrated in ascending acetone solutions (from 50 to 100%), dried in CO_2, mounted in aluminum stubs, and metalized with gold using a putter coating device. The samples were visualized in a LEO VP1430 scanning electron microscope (LEO Electron Microscopy Ltd, Cambridge, UK) at the Microscopy and Microanalysis Center of the Federal University of Viçosa.

2.4 Wood density and hardness

The wood density was determined using Archimedes's principle, which is based on the liquid displacement by the wood block. Briefly, each wood testing unit was weighed on an analytical balance and subsequently immersed in metallic mercury (13.5 kg cm^{-3} density); the buoyancy is equal to the weight of the displaced mercury, which was determined with an analytical balance. This value divided by the mercury density provides the wood volume, which gives the wood density when divided by the wood weight. The determination was carried out three times for each wood testing unit and the average was used as the testing unit density. Three replicates were performed for each wood species.

Wood hardness was determined using a commercial hardness testing unit (Hardmatic HH-401; Mitutoyo Sul Americana, Santo Amaro, SP, Brazil), whose principle is based on the Janka hardness test. The procedure measures the force required to drive a 100° diamond cone into the wood testing sample. Three assessments were performed for each wood testing unit and the average of the two smaller values was used as the hardness, expressed in Vickers (HV). This was done to circumvent the effect of the wood deformation by the diamond cone; three independent replicates were performed for each wood species.

The wood species were also histologically characterized using 1.0 x 1.0 x 1.5 cm wood samples transversally cut. The wood samples were immersed in glycerin-water solution and boiled for 3 h to soften them. The wood samples were subsequently fixed in the sliding microtome and a PVC plastic resin and butyl acetate mixture (2:1 proportion) was applied to its surface and allowed to dry for 5 min before proceeding to obtain the 20-μm histological slices, which were placed in ethanol. The wood samples were then clarified with sodium hypochlorite (20%), washed in acetic acid (1%), dehydrated in an alcohol series (30-50-80-90-100%), and stained with safranin. The wood slices were transferred to glass slides, coated with resin, and covered with glass coverslips for inspection under a microscope.

2.5 Statistical analyses

The estimated area of nest gallery obtained in the pine testing units subjected to colonization for 210 days by increasing numbers of termites was subjected to regression analysis using the curve-fitting procedure from TABLECURVE 3D (Systat, San Jose, CA, USA); the significant regression models ($P < 0.05$) were tested from the simplest (linear and quadratic) to more complex peak models, and model selection was based on parsimony, high F values (and mean squares), and steep increases in R^2 with model complexity.

The results from the free-choice test were subjected to a Kruskal–Wallis H test ($P < 0.05$), as samples within each bioassay were interdependent, preventing the use of analysis of variance (ANOVA). In contrast, the results of the no-choice test and the wood physical characterization were subjected to ANOVA and Tukey's honest significant difference (HSD) test ($P < 0.05$), when appropriate (PROC GLM in SAS; SAS Institute, Cary, NC, USA), after normality and homoscedasticity assumptions had been tested (PROC UNIVARIATE in SAS). Wood differences in mandible wear were also subjected to ANOVA and Tukey's HSD test ($P < 0.05$) (PROC GLM in SAS) after the required assumptions had been ascertained to have been met, while left–right side differences in

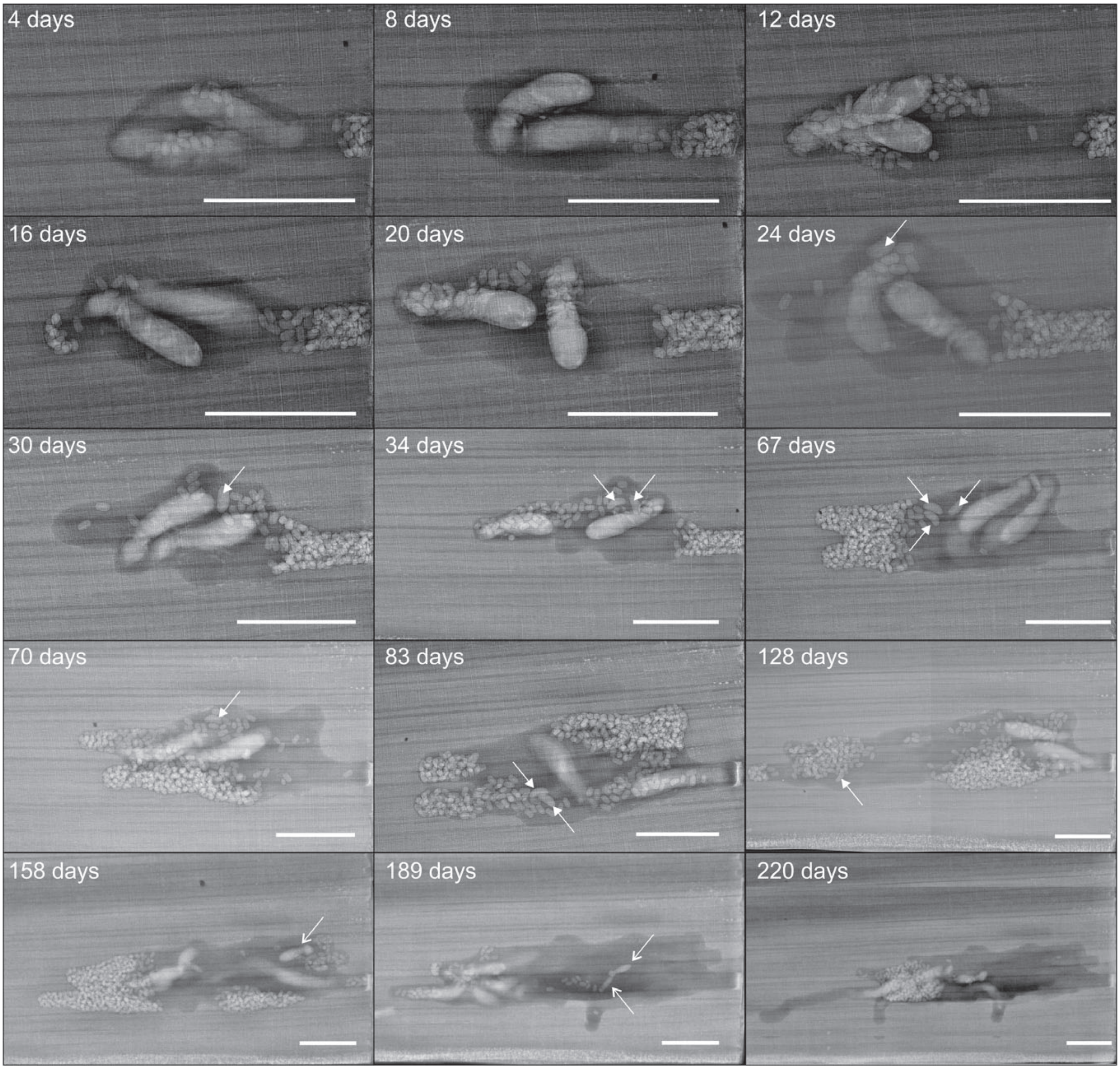

Figure 1. Digitalized X-ray images of pine testing unit colonization by the West Indian drywood termite *Cryptotermes brevis*. The sequential images indicate the colonization by a couple of termites up to 220 days. The arrows indicate either eggs (up to 128 days) or immatures (after 218 days). White bar, 0.5 cm.

mandible wear were subjected to a χ^2 randomness test ($P < 0.05$) (PROC FREQ in SAS).

Path analysis was used to test and summarize the hypothesized relationships among wood physical properties, termite mandible wear and respiration rate, wood consumption and termite colonization. This analysis was performed using the procedures REG and CALIS in SAS (SAS Institute), following guidelines provided by Mitchell.[32]

3 RESULTS

3.1 Termite colonization of wood testing units

Production of winged reproductives, as well as of the other termite castes, in addition to egg-laying and population increase took place successfully in the laboratory colonies and the developed pine testing units. The X-ray used to record colony development and tunneling in the wood testing units did not affect the termites, allowing their successful development throughout the 220 days of observation, which is representatively illustrated in Fig. 1. Eggs started appearing on about the 24th day of colonization, while immatures were present after 128 days. The increase in termite tunneling in the wood testing units was a directly correlated to the increase in insect colony size over time, with the amount of tunneling, and thus wood loss, increasing with the duration of colonization for wood testing units in which there were initially two or more insects, the colony size being dependent on the duration of colonization (Fig. 2).

3.2 Termite free-choice preferences

The differences in number of termites colonizing the different wood species were significant only at $P = 0.15$ (Fig. 3A), but wood

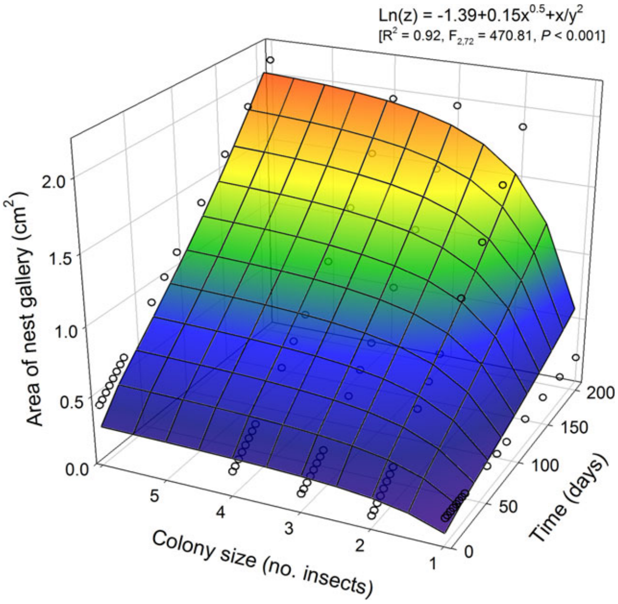

Figure 2. Effect of colony size (x) and time (y) on the tunneling activity [i.e. area of nest gallery (z)] of the West Indian drywood termite *Cryptotermes brevis*. The symbols represent the observed values.

consumption exhibited greater variation among wood species, with pine and jequitiba exhibiting greater losses and contrasting mainly with cumaru and guariuba (Fig. 3B). The other wood species exhibited intermediate levels of wood loss (Fig. 3B).

3.3 Wood resistance to termites

The wood species showed significant differences in termite response for all assessed traits ($F_{8,45} \geq 2.45$; $P < 0.05$). Insect colonization varied significantly among wood species, being lowest on guariuba and tatajuba and highest on courbaril, pine and purpleheart, with the other wood species having intermediate values (Fig. 4A). Pine consumption was highest, while consumption of cumaru and guariuba was lowest; a few wood species with high insect numbers exhibited low loss by termite consumption, such as purpleheart (Fig. 4B). The termites colonizing courbaril and guariuba exhibited significantly higher body mass than those on jequitiba (Fig. 4C), while the termite respiration rate exhibited a somewhat distinct (inverted) trend with higher respiration rate in insects on jequitiba and lower in those on guariuba (Fig. 4D), indicating difficulties in conversion of the latter two wood substrates.

Wood physical properties, particularly density and hardness, are potential contributors to termite resistance, and these properties varied significantly among wood species ($F_{8,18} \geq 13.13$; $P < 0.001$). Pine exhibited the lowest density, while courbaril, cumaru and purpleheart exhibited the highest densities and the remaining wood species were in the intermediate density range (Fig. 5A). Regarding hardness, again courbaril exhibited the highest levels followed by cumaru and purpleheart, with again pine, plus jequitiba, guariuba and eucalyptus exhibiting the lowest levels (Fig. 5B). Representative aspects of wood colonization by termites are shown in Fig. 6(A, B), which also shows representative histological slides for the different wood species, indicating smaller and sparser intercellular spaces in harder, high-density wood species than in softer, low-density wood (Fig. 6C).

A potential result of the effects of harder and higher density woods on termites is higher mandible wear. Therefore, mandible wear was assessed among termites colonizing the different wood species, and significant variations in mandible area and mass were evident (Fig. 7A, B). Again, colonization of pine and jequitiba led to the lowest mandible wear, while colonization of courbaril, cumaru and purpleheart led to the highest levels of wear (Fig. 7A, B). Curiously, mandible wear was left-biased, with consistently higher levels of wear on the left side of the mandible (Fig. 7A, B) and lower wear on the right side (Fig. 7C). Representative mandibles are illustrated in Fig. 8, but termite feeding on harder, high-density woods had consequences beyond wear, with cracks and breaks being found in the mandible teeth. Feeding on the intermediate and hardest wood species led to significant wear which was easily perceptible in the mandible teeth, mainly the marginal ones, and frequent breakage of the apical tooth (Fig. 7C).

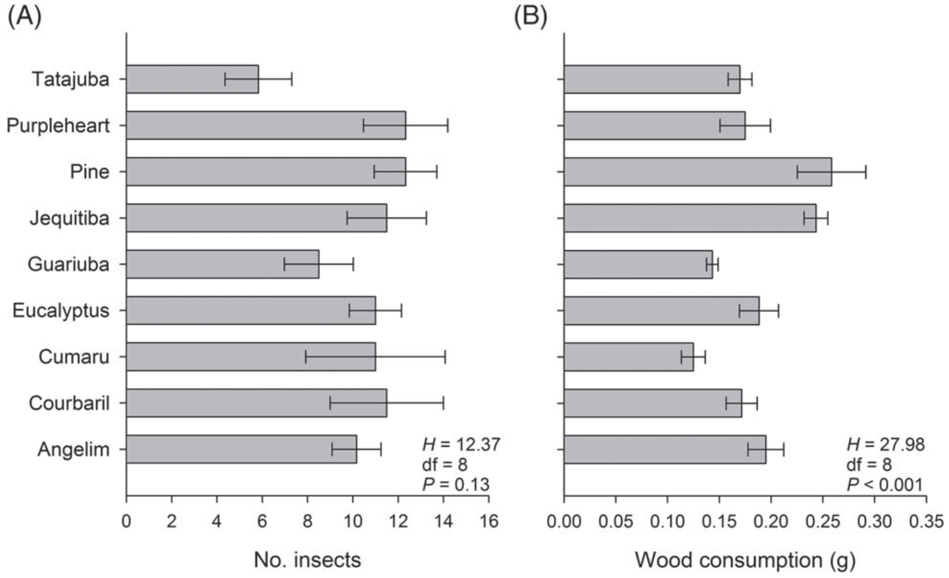

Figure 3. Number of termites (A) and wood consumption (B) in free-choice bioassays of tropical wood preference of the West Indian drywood termite *Cryptotermes brevis*. The horizontal lines of each histogram bar indicate standard error of the mean and the results of Kruskal–Wallis H test are indicated at the bottom right of each respective panel.

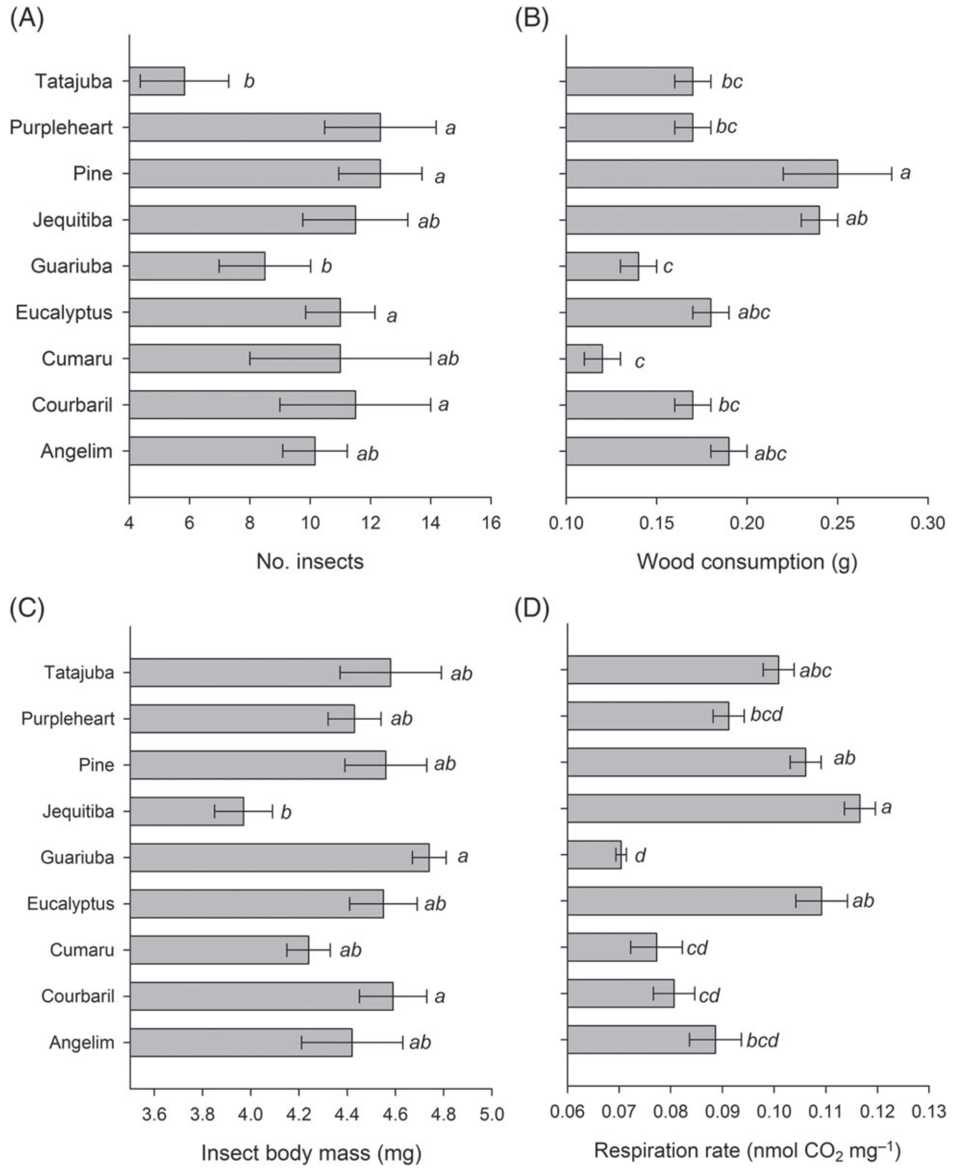

Figure 4. Number of termites (A), wood consumption (B), insect body mass (C) and respiration rate (D) in no-choice bioassays of tropical wood resistance to the West Indian drywood termite *Cryptotermes brevis*. The horizontal lines of each histogram bar indicate standard error of the mean; histogram bars with the same lowercase letters do not differ significantly by Tukey's HSD test ($P < 0.05$).

3.4 Path summarized trends

The effects of wood density and hardness on termite infestation were hierarchically tracked and structured as a path diagram subjected to path analysis (Fig. 9). The path model proposed was deemed valid based on the low χ^2 and high P-value obtained (Fig. 9). Wood density and hardness were significantly correlated and also significantly affected mandible wear, although with a much higher contribution of density than hardness (Table 1). Wood density and hardness also exhibited indirect effects (higher for density) on wood consumption, which was directly affected by mandible area and correlated with termite respiration rate (Table 1); thus, higher mandible area (lower wear) led to higher consumption, which is also associated with a higher respiration rate reflecting a high feeding rate. Respiration rate directly and negatively affected termite body mass, a consequence of poor food conversion, with a negligible direct contribution from wood consumption, and indirect contributions of mandible wear and wood density and hardness (Table 1; Fig. 9). In contrast, termite infestation was (positively) affected by wood consumption only at $P < 0.10$ (Fig. 9), with indirect contributions of mandible wear and wood density and hardness (Table 1; Fig. 9); there was no significant correlation between number of termites infesting the wood testing units and termite body mass (Fig. 9).

4 DISCUSSION

Wood preferences have been reported for a few termite species (mainly subterranean termites).[8,25] Curiously, the underlying causes determining such preferences are seldom investigated and little is known about the whole subject in the invasive drywood termite *C. brevis*. Therefore, we developed wood testing units to allow digital X-ray recording of wood colonization and loss, and subsequently tested tropical wood resistance to drywood termites. We also tested if wood density and hardness

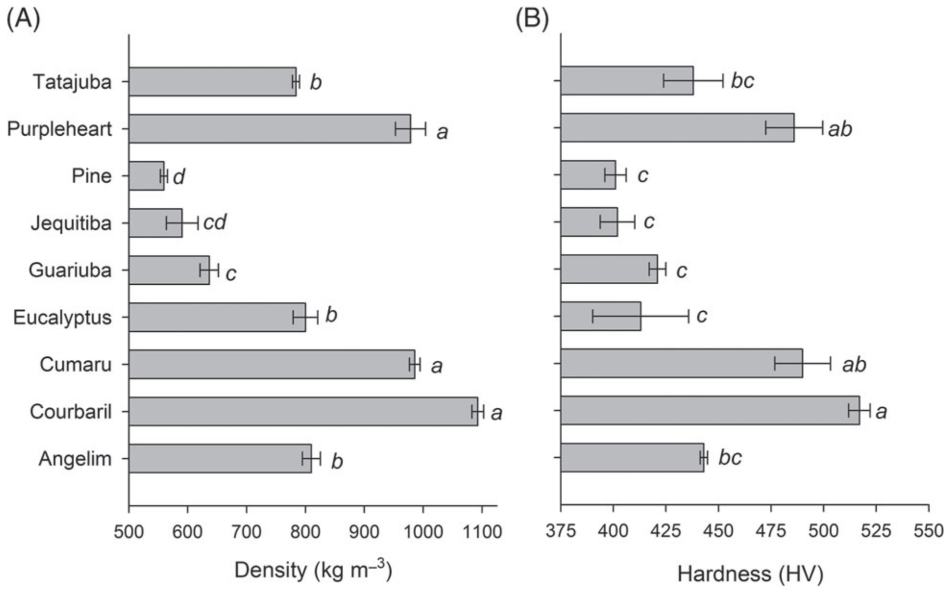

Figure 5. Density (A) and hardness (B) of the tropical wood species used in the bioassays of resistance to the West Indian drywood termite *Cryptotermes brevis*. The horizontal lines of each histogram bar indicate standard error of the mean; histogram bars with the same lowercase letters do not differ significantly by Tukey's HSD test ($P < 0.05$).

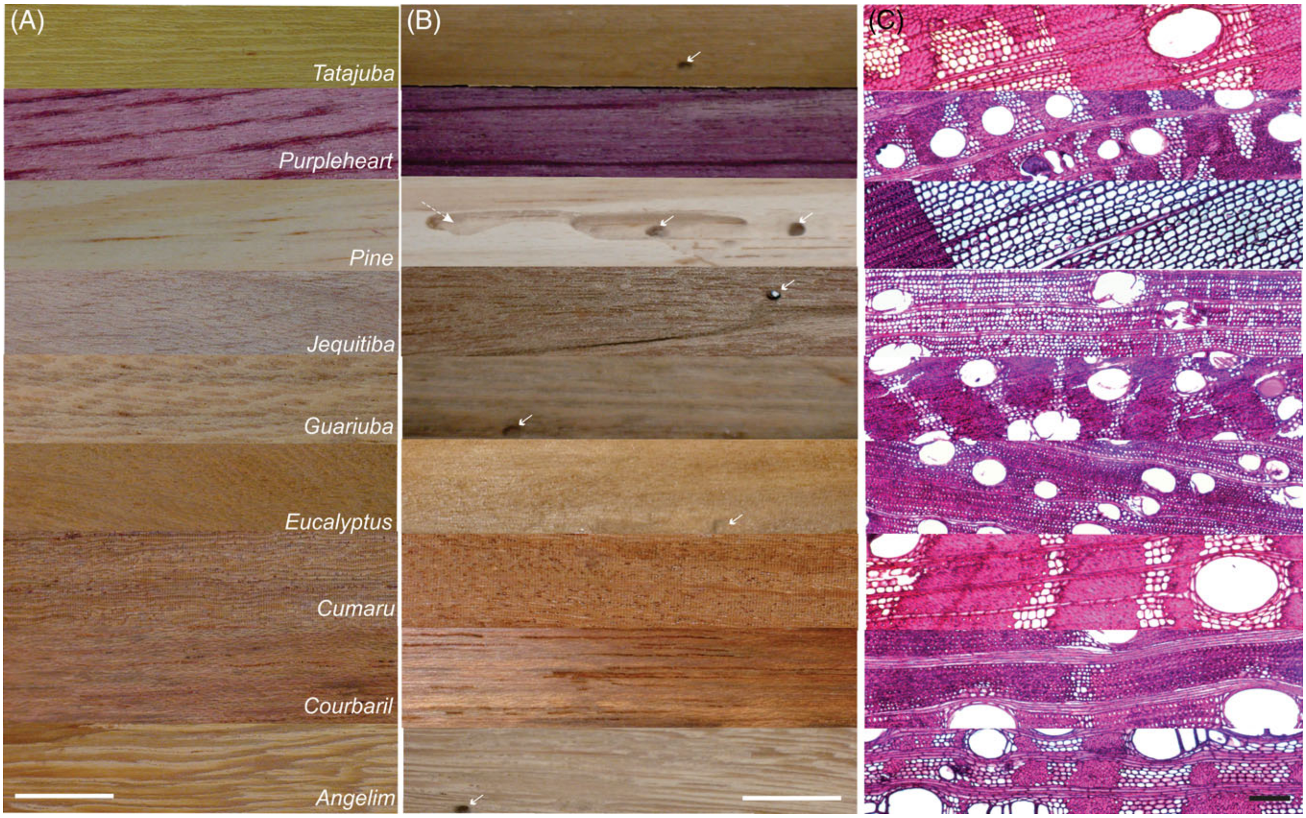

Figure 6. Images of the surface of the wood testing units used in the bioassays of resistance to the West Indian drywood termite *Cryptotermes brevis*. The testing units are shown before (A) and after (B) termite colonization. The solid white arrows in (B) indicate termite perforation holes found in soft, low-density wood species and the dashed white arrow indicates surface tunneling by the termites. Panel (C) shows histological sections of the heartwood of the different tropical wood species exhibiting their respective intercellular spaces. The white scale bar at the bottom right of panels (A) and (B) represents 2 cm, while the black scale bar of panel (C) represents 20 μm.

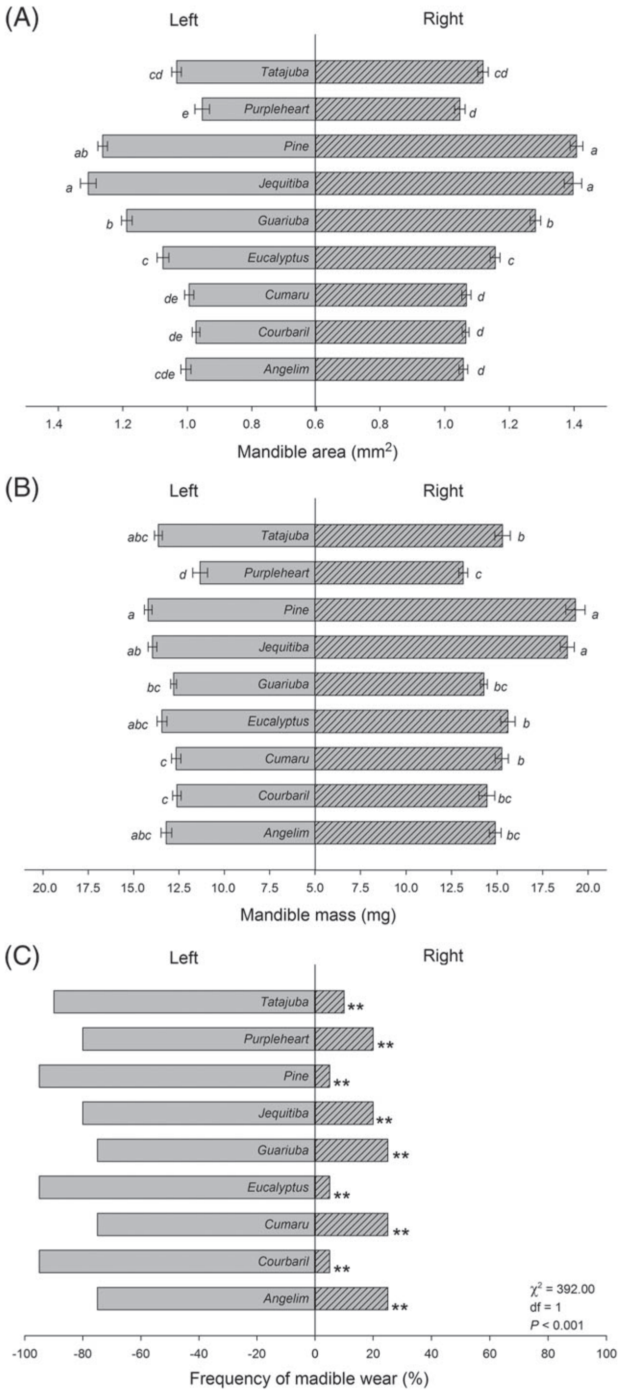

Figure 7. Results of mandible wear of West Indian drywood termites (*Cryptotermes brevis*) maintained on different tropical wood species. The mandible area (A) and mass (B) of each side of the mandible are indicated and were compared using a χ^2 randomness test ($P < 0.05$) (C). The histogram bars for each mandible side with the same lowercase letters in (A) and (B) do not differ significantly by Tukey's HSD test ($P < 0.05$).

are determinant factors in wood resistance to the West Indian drywood termite, which we expected to be the case. Indeed, this is so, but there also seems to be more to it.

The West Indian drywood termite is a single wood piece nester recognized as an invasive tramp species.[7,16,18] Its nesting behavior allowed the development and testing of wood testing units constructed from two 8-cm^3 pieces of wood joined together, enabling the development and reproduction of newly established termite colonies, with better results than those obtained with alternative units (e.g. Harris).[33] The wood testing unit developed has an important added benefit – it allows the recording of the rate of colony increase, the evolution of nesting and tunneling in the wood unit, and determination of wood loss. In our case, for the colonies that started with six individuals, the volume of wood loss was roughly estimated at 3.3 cm^3 in 210 days, which corresponds to a significant proportion of the wood testing unit. The X-ray did not interfere with termite reproduction and colony development, which might have been a problem, despite the low level of radiation emitted by the equipment, in contrast to gamma radiation sources.[34]

The suitability of the wood testing units for termite development allowed subsequent free-choice and no-choice bioassays to be performed to investigate insect preferences and tropical wood resistance to the West Indian drywood termite. Variation in such traits was expected based on previous results with other wood and termite species,[8,25] and was also found in our study. Curiously, colony size did not vary significantly among wood species in the free-choice bioassay, but wood consumption exhibited significant variation, indicating differential termite preference for the tropical wood species tested. This was further confirmed in the no-choice bioassays, where significant differences were apparent for termite colony size and wood consumption. Although the ranking of wood species resistance to *C. brevis* was similar in the free- and no-choice bioassays, with cumaru and guariuba exhibiting the highest resistance and pine exhibiting the highest susceptibility, some wood species such as purpleheart and intermediate-ranked species varied in their resistance to termites. Therefore, wood (chemical) attractiveness also seems to play a role in termite resistance.

The no-choice bioassays also allowed us to explore the potential role of wood physical properties, namely density and hardness, in termite resistance. Again, the significant variation observed among the tropical wood species tested allows their rough grouping into species with the hardest, intermediate, and softest wood, with the termites usually exhibiting the lowest levels of infestation and damage on the first group and the highest levels on the last group. Cumaru, guariuba, and purpleheart proved resistant to termite damage, although purpleheart allowed higher colony numbers, and angelim, jequitiba and in particular pine were the most susceptible wood species suffering the greatest damage, with eucalyptus following close behind. The recorded termite body mass and respiration rate were also consistent with the wood resistance pattern.

Mandible wear was another trait assessed to provide evidence of the potential role of wood physical properties in termite resistance. Again, mandible wear varied significantly with the wood species, and when termites were feeding on harder woods the effect went beyond wear, with the mandibles exhibiting cracks and breaks in addition to extensive tooth wear in the chewing region. Such wear was impressive, especially considering the reported metal accumulation in termite mandibles, enhancing by up to 20-fold their resistance to this problem.[35] The asymmetric wear of the mandibles, which is common among insects as a consequence of the mandible's close fit on both sides, reflects their chewing mechanics.[36–38] Such asymmetry in mandible wear was also significant among West Indian drywood termites, with wear being greater on the left side of the mandible and in termites feeding on harder and denser wood species. Although the inversion of wear from left to right might be possible, this did not minimize the

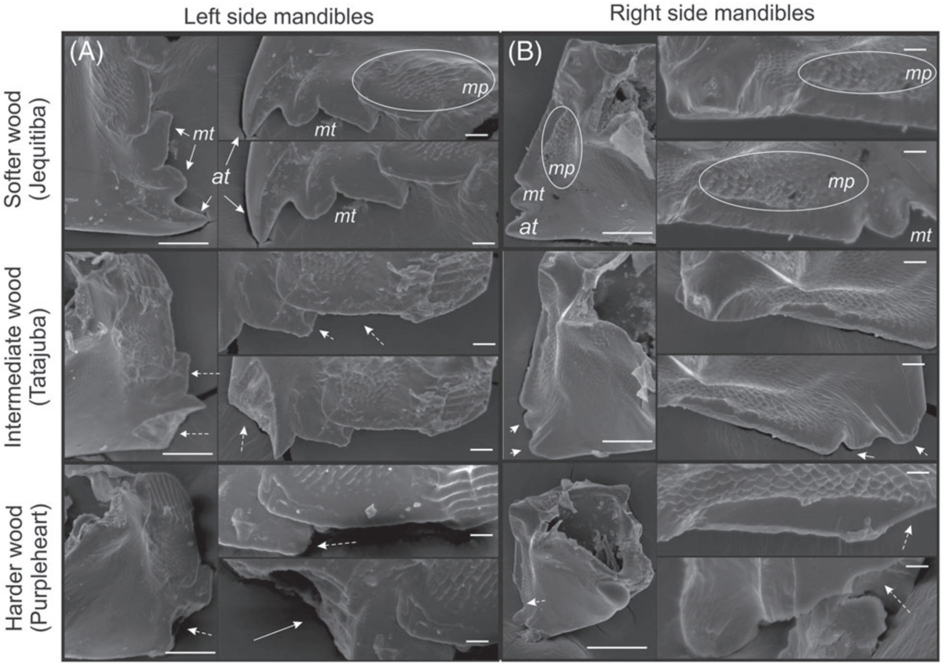

Figure 8. Representative left- and right-side mandible wear in West Indian drywood termites (*Cryptotermes brevis*) maintained on different tropical wood species. The marginal teeth (mt), apical teeth (at), and molar plaques (mp; delimited by a circle) are indicated. The dashed arrows indicate sites of significant wear or breaks. White solid bar, 20 μm.

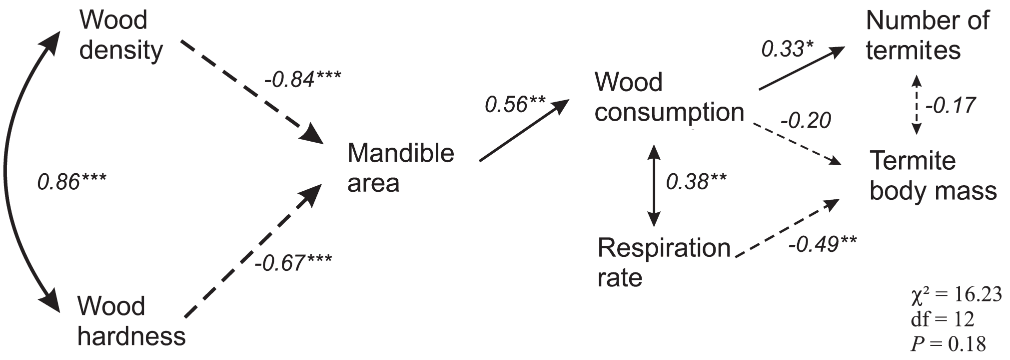

Figure 9. Path analysis diagram for the influence of wood physical properties on the resistance of tropical wood species to the West Indian drywood termite (*Cryptotermes brevis*). The results of χ^2 goodness-of-fit for the path model are indicated. Single-headed arrows indicate causal relationships (regression), while double-headed arrows indicate correlation between the variables. Significance levels are represented by asterisks (*$P < 0.10$; **$P < 0.05$; ***$P < 0.001$), and the thickness of each line is proportional to the strength of the relationship. Solid arrows indicate positive relationships, while dashed arrows indicate negative relationships. Direct, indirect and total values for path coefficients are presented fully in Table 1.

overall mandible wear nor reduced wood consumption recorded with harder woods.

Combining the different trends observed, particularly in no-choice bioassays, for tropical wood resistance to termites allowed a more complete picture to be obtained and our hypothesis to be tested regarding the relationships among wood physical properties, termite mandible wear and termite infestation. Wood density and hardness were correlated, which comes as no surprise as density is a significant determinant of hardness, as illustrated by the sparser and smaller intercellular spaces observed in the histological slides of denser woods. Wood physical characteristics, particularly density, were closely related to mandible wear which determines wood consumption by the West Indian termites. Wood consumption was positively correlated with respiration rate, as expected because of the oxygen demand for wood ingestion and degradation. Respiration rate was negatively correlated with termite body mass, which again is expected because a high respiration rate indicates difficulties in food conversion, which compromises gain in body mass. Curiously, wood consumption did not exhibit a significant effect on body mass, although it affected termite colony numbers. Therefore, this finding reinforces the suggestion that, although wood hardness and in particular wood density are important determinants of wood resistance to termites, their effect is expressed in termite colony numbers, not termite body mass. Thus, other resistance factors are also likely to be involved as the correlation between wood consumption and termite infestation was only significant at $P < 0.10$, which warrants further attention.

In brief, and as the saying goes, if termites can't chew, they can only lick … This, however, does not seem to be due only to wood density and hardness. Our results suggest that, although such physical traits are important determinants of termite infestation, there is more to it. Additional wood resistance factors are expected

Table 1. Direct (DE), indirect (IE), and total (TE) effects in the path diagram of Fig. 9 for the model of the influences of wood density and hardness on termite mandible wear, wood consumption, respiration rate, termite body mass and colony size of the drywood termite *Cryptotermes brevis*

	Mandible area (mm^2)			Wood consumption (g)			Termite body mass (mg)			Termite colony size (no. of termites)		
Variable	DE	IE	TE	DE	IE	TE	DE	IE	TE	DE	IE	TE
Wood density (kg m^{-3})	-3.35	-	-3.35	-	-0.32	0.32	-	0.06	0.06	-	-0.27	-0.27
Wood hardness (HV)	0.001	-	0.001	-	0.0001	0.0001	-	-0.00003	-0.0003	-	0.0001	0.0001
Mandible area (mm^2)	-	-	-	0.09	-	0.09	-	-0.02	-0.02	-	0.08	0.08
Wood consumption (g)	-	-	-	-	-	-	-0.18	-	-0.18	083	-	0.83
Respiration rate (nmol CO_2 mg^{-1})	-	-	-	-	-	-	-0.19	-	-0.19	-	-	-
Termite body mass (mg)	-	-	-	-	-	-	-	-	-	-	-	-
R^2	0.72			0.32			0.24				0.11	
P	< 0.001***			0.002**			0.03**				0.08*	

Asterisks indicate significant regression models at $P < 0.10$ (*), < 0.05 (**), and <0.01 (***).

to be present, such as wood chemical characteristics, which remain to be investigated.

ACKNOWLEDGEMENTS

We thank Prof. R. M. Della Lucia for assistance with experiments and Prof. R. Constantino for identifying the termites used in this study. The financial support provided by the CAPES Foundation, the National Council of Scientific and Technological Development (CNPq), and the Minas Gerais State Foundation for Research Aid (FAPEMIG) is greatly appreciated.

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