LÍRIO COSME JÚNIOR

TROPICAL WOOD SPECIES, THE WEST INDIAN DRYWOOD TERMITE, AND THE GAME INSECT WORLD

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Doctor Scientiae*.

Orientador: Raul Narciso Carvalho Guedes

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APROVADA: 26 de julho de 2019.

Lírio Cosme Júnior

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Autor

Raul Narciso C.

Raul Narciso Carvalho Guedes
Orientador

“Milagres não são coisas que acontecem, são coisas que as pessoas fazem acontecer”

Neon genesis evangelion.

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Muito obrigado!

SUMÁRIO

RESUMO

COSME JÚNIOR, Lírio, D.Sc., Universidade Federal de Viçosa, julho de 2019**. Espécies de madeira tropical, cupim de madeira seca e o jogo *Insect World*.** Orientador: Raul Narciso Carvalho Guedes.

O cupim de madeira seca, *Cryptotermes brevis* (Walker) é espécie essencialmente social que vive e forrageia junto com seus coespecíficos dentro da madeira, onde eles realizam um conjunto de caracteríscas comportamentais individuais e em grupo (grooming, autogrooming, escavação dos túneis, limpeza e etc). Dentro deste contexto, os cupins também exibem outros relevantes comportamentos (alarme de defesa e cuidado parental) incluindo a seleção do substrato para o estabelecimento da colônia, o que é conhecido por ser mediado pelo tamanho e qualidade da madeira, porém outros fatores podem também estar envolvidos, como fatores sociais, os quais tem recebido pouca atenção. Portanto, aqui nós testamos a hipótese que as pistas vibracionais (capítulo 1) e os constituintes químicos da madeira (capítulo 2) influenciam o comportamento de escolha do substrato por *C. brevis*. Para isso, testes com e sem chance de escolha foram realizados, onde se avaliou a colonização pelos cupins, bem como os parâmetros de sucesso de colônia (consumo e sobrevivência). Foram identificados dois comportamentos realizados pelos cupins no interior da madeira – a caminhada e a mastigação, mas apenas a mastigação foi caracterizada e usada em teste de playback. Em geral, os cupins exibiram uma escolha aleatória entre as unidades de madeira, indicando que a pista de mastigação não é usada como um gatilho social para mediar o recrutamento coespecífico. Apesar disso, resultados contrastantes foram observados no consumo de madeira e na sobrevivência dos cupins entre as colônias estabelecidas em madeiras infestadas e não infestadas, o que provavelmente está relacionado ao custo para o estabelecimento de uma nova colônia. Em seguida foram testados se os componentes químicos (i.e., lignina, holocelulose e extrativos) de nove tipos de madeiras tropicais poderiam influenciar a escolha e a colonização do substrato por *C. brevis*. Aqui, a colonização e consumo da madeira variou entre as espécies de madeira apenas no teste de livre escolha. Além disso, em ambos os casos, não houve correlação significativa entre a colonização por cupins e os componentes químicos da madeira, o que sugere pequena contribuição destes componentes sobre o comportamento de escolha do substrato pelos cupins. Por último, foi desenvolvida uma atividade didática utilizando um jogo desenvolvido como ferramenta para o ensino de entomologia. Nós aplicamos um jogo entomológico (Insect World) para avaliar se ele pode ser uma ferramento para o ensino de entomologia, o que foi testado pelo score de aprendizagem alcançada e pelo feedback (funcional e pessoal) dos estudantes de graduação. O jogo recebeu feedback positivo sob uma perspectiva funcional (i.e., conteúdo, manual e designer) e pessoal (i.e., divertido, rápido, competitivo, instrutivo) dos estudantes, embora algumas dificuldades também foram apontadas pelos alunos. Mesmo assim, a atividade aumentou a aprendizagem dos estudantes, destacando a sua relevância como uma ferramenta para promover uma prática didática em sala de aula que encoraja a criatividade e competição saudável entre os estudantes. Tudo isso sob a óptica de um integrativo e dinâmico para professores e alunos, que vai além dos métodos de ensino tradicional.

Palavras-chaves: Cupins. Vibroacústica. Escolha de madeira. Aprendizagem baseada em jogos. Jogos Educacionais.

ABSTRACT

COSME JÚNIOR, Lírio, D.Sc., Universidade Federal de Viçosa, July, 2019**. Tropical wood species, the west indian drywood termite and the game Insect World.** Advisor: Raul Narciso Carvalho Guedes.

The West Inndian drywood termite *Cryptotermes brevis* (Walker) is an essentially social species that live and forage with their conspecific inside wood pieces, where they perform a suite of behavioral individual and group traits (grooming, autogrooming, tunnel excavation, cleaning and etc). Within context, this termite species exhibits other relevant behaviors (defense alarm and parental care), including substrate selection for colony establishment, which is mediated by the size and quality of the wood, but other factors may be involved, as a social factor, which has received little attention. Therefore, here we tested the hypothesis that vibrational cues and wood chemical components influence the wood choice behavior by *C. brevis*. For this purpose, free- and no-choice tests were performed to assess termite colonization, as well as the parameters of colony success (consumption and survival). We identified two behaviors performed by termites inside the wood walking and chewing, but only chewing was characterized and used in the playback experiments. Overall, drywood termite exhibits random choice between wood-testing unities, indicating that the chewing cues are not used as a social trigger to mediate conspecific recruiting. Nevertheless, contrasting outcomes in wood consumption and termite survival were observed from the colonies established in wood infested and non-infested, which is likely related to the cost of establishing of a new colony. We also tested whether the wood-chemical components (i.e., lignin, holocellulose, and extractives) from nine tropical wood types could influence the drywood termite choice. Here, the colonization and wood-consumption varied with wood species, but only under free-choice testing. Furthermore, no significant correlation between termite colonization and the wood chemical components was observed, which suggests a negligible role of wood chemical components in the substrate choice behavior by drywood termites. Lastly, we used an entomological game to assess whether it can be a tool for teaching entomology, which was tested by scoring the learning achieved and feedback (functional and personal) provided by undergraduate students. The game (Insect World) received positive feedback from a functional (i.e., content, manual and designer) and personal (i.e., fun, fast, competitive and, instructive) perspective provided by students, although some difficulties also were pointed. Although the game activity increased student learning highlighting its importance as a teaching tool activity in the classroom, encouraging creativity and healthy competition among students. Besides, the game also subjects students and teachers to an integrative and dynamic teaching-mode shifting from the traditional learning method.

Keywords: Termite. Vibroacoustic. Wood-choice. Game-based learning. Educational games.

INTRODUÇÃO GERAL

O mundo dos insetos contém a maior biodiversidade conhecida do planeta, onde os organismos interagem em complexas relações ecológicas e são reconhecidos pelo seu protagonismo na prestação de serviços ecológicos e na alimentação humana (Stork, 1988; Schowalter, 2016; Van-Huis et al. 2014). Apesar disso, em uma perspectiva antropogênica, os insetos são vistos como não carismáticos, por serem vetores na transmissão de doenças e também pragas urbanas e agrícolas (Albert, 2011; Stenseth et al. 2008). Um bom exemplo desta visão ambígua são os cupins, tal como o cupim de madeira seca, *Cryptotermes brevis* (Walker) (Blattodea: Isoptera: Kalotermitidae), que embora desempenhe um importante papel na decomposição e ciclagem de nutrientes, também é considerado uma grande praga urbana. Este enquadramento se baseia no fato de se alimentarem de móveis, acervos históricos e produtos derivados da madeira, causando perdas econômicas e patrimoniais com elevados custos e sérios impactos ecológicos em escala local (Guerreiro et al., 2014; Govorushko, 2019).

Esses organismos exibem comportamentos singulares, pois são insetos essencialmente sociais que vivem e forrageiam junto com seus coespecíficos dentro da madeira. Assim, um fator determinante para o sucesso de *C. brevis* deve estar relacionado a escolha do substrato para o estabelecimento de novas colônias. Dentro deste contexto, o tamanho e a qualidade da madeira são geralmente apontados como características importantes para o forrageamento por cupins (Cosme et al., 2018; Evans et al., 2005, 2007; Inta et al., 2007a, 2007b; Oberst el al., 2018). Contudo, outros fatores podem também estar associados à preferência da madeira (odor, textura), mas são ainda negligenciados.

Alguns estudos têm apontado o processo de comunicação nos cupins como uma importante característica adaptativa. Para cupins, o processo de comunicação é realizado através de pistas olfativas, táteis e acústicas, já que eles exibem comportamento críptico onde as pistas visuais são pouco favorecidas (Bagnères & Hanus, 2015; Cristaldo et al., 2015; Delattre et al., 2015). Neste contexto, a vibração no substrato (pista acústica) tem seu papel reconhecido por disseminar a informação rapidamente dentro e fora da colônia (Howse, 1965; Hunt & Richard, 2013), o que tem desempenhado importante papel na história de vida dos cupins estando envolvidos com o comportamento de defesa (Evans et al., 2009; Oberst et al., 2017) e alimentação (Evans et al., 2005, 2007; Inta et al., 2007a, 2007b). Além disso, outro fator que pode potencialmente influenciar a colonização e consumo por térmites relaciona-se as características intrínsecas da madeira, o que foi demonstrado para propriedades físicas, como a densidade e dureza (Cosme et al. 2018; Oberts et al. 2018). No entanto ainda não foram escrutinadas as propriedades químicas da madeira, tal como os teores de lignina, holocelulose, e extrativos totais (Brebu & Vasile, 2010).

Dito isto, aqui o esforço foi direcionado a explorar o comportamento de escolha do substrato para colonização por cupins de madeira seca. Procurou-se ainda desconstruir a visão não carismática do mundo dos insetos por meio de uma abordagem de aprendizagem baseada em jogo. Para isso, nós testamos as seguintes hipóteses: 1) Pistas vibroacústicas produzidas por cupim dentro de madeira agem como um gatilho social, promovendo uma resposta fonotática para madeira infestada ou madeiras com pistas vibroacústicas (playback) via comportamento de atração (fonotaxia positiva) ou evasão (fonotaxia negativa); 2) Existe uma associação dos constituintes químicos da madeira (i.e., lignina, holocelulose e extrativos) com o padrão de preferência e colonização de *C. brevis* em diferentes tipos de madeiras tropicais; 3) Uma atividade baseada em jogos é uma estratégia capaz de ensinar entomologia, potencialmente beneficiando a aprendizagem dos estudantes.

REFERÊNCIAS

Aberth, J. Plagues in world history. Rowman & Littlefield Publishers, 2011.

Bagnères, A. G., Hanus, R. (2015). Communication and social regulation in termites. In Social recognition in invertebrates. pp. 193-248. Springer, Cham.

Bianchi, F. J. J. A.; Booij, C. J. H.; Tscharntke, T. (2006). Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. Proceedings of the Royal Society B: Biological Sciences, 273, 1715-1727; DOI 10.1098/rspb.2006.3530.

Brebu, M., Vasile, C. (2010). Thermal degradation of lignin—a review. Cellulose Chemistry & Technology, 44, 353-363.

Cosme, L., Haro, M. M., Guedes, N. M., Della-Lucia, T. M., Guedes, R. N. C. (2018). Tropical wood resistance to the West Indian drywood termite *Cryptotermes brevis*: If termites can't chew…. Pest Management Science. 74, 914-924. DOI: 10.1002/ps.4785.

Cristaldo, P. F., Jandak, V., Kutalova, K., Rodrigues, V.B., Brothanek, M., Jiricek, O., DeSouza, O., Sobotnik, J. (2015). The nature of alarm communication in *Constrictotermes cyphergaster* (Blattodea: Termitoidea: Termitidae): the integration of chemical and vibroacoustic signals. Biology open, 4, 1649-1659. DOI: 10.1242/bio.014084.

Delattre, O., Sillam-Dusses, D., Jandak, V., Brothanek, M., Rucker, K., Bourguignon, T., Vytiskova, B., Cvacka, J., Jiricek, O., Sobotnik, J. (2015). Complex alarm strategy in the most basal termite species. Behavioral ecology and sociobiology, 69, 945-1955. DOI 10.1007/s00265-015-2007-9.

Evans, T. A., Inta, R., Lai, J. C. S., Lenz, M. (2007). Foraging vibration signals attract foragers and identify food size in the drywood termite, *Cryptotermes secundus*. Insectes Sociaux, 54:374-382. DOI: 10.1007/s00040-007-0958-1.

Evans, T. A., Lai, J. C., Toledano, E., McDowall, L., Rakotonarivo, S., Lenz, M. (2005). Termites assess wood size by using vibration signals. Proceedings of the National Academy of Sciences, 102, 3732-3737. DOI: 10.1073/pnas.0408649102.

Govorushko, S. (2019) Economic and ecological importance of termites: A global review. Entomological science, 22, 21-35. DOI: 10.1111/ens.12328.

Guerreiro, O., Cardoso, P., Ferreira, J. M., Ferreira, M. T. (2014). Potential distribution and cost estimation of the damage caused by *Cryptotermes brevis* (Isoptera: Kalotermitidae) in the Azores. Journal of Economic Entomology, 107, 1554-1562. DOI: 10.1603/EC13501.

Howse, P. E. (1965) On the significance of certain oscillatory movements of termites. Insectes Sociaux, 12, 335-345.

Hunt, J. H., Richard, F. J. (2013). Intracolony vibroacoustic communication in social insects. Insectes Sociaux, 60, 403-417. DOI: 10.1007/s00040-013-0311-9.

Inta, R., Evans, T. A., Lai, J. C. S., Lenz, M. (2007a). What do vibrations have to do with termites' food choice?. Acoustics Australia, 35, 73-77.

Inta, R., Lai, J. C., Fu, E. W., Evans, T. A. (2007b). Termites live in a material world: exploration of their ability to differentiate between food sources. Journal of The Royal Society Interface, 4, 735-744. DOI: 10.1098/rsif.2007.0223.

Oberst, S., Bann, G., Lai, J. C. S., Evans, T. A. (2017). Cryptic termites avoid predatory ants by eavesdropping on vibrational cues from their footsteps. Ecology Letter, 20, 212-221. DOI: 10.1111/ele.12727.

Oberst, S., Lai, J. C. S, Evans, T. A. (2018). Key physical wood properties in termite foraging decisions. Journal of The Royal Society Interface, 15, e20180505, DOI: 10.1098/rsif.2018.0505.

Schowalter, T. D. (2016) Insect ecology: an ecosystem approach. Academic Press.

Stenseth, N. C., Atshabar, B. B., Begon, M., Belmain, S. R., Bertherat, E., Carniel, E., Gage, K. L., Leirs, H., Rahalison, L. (2008). Plague: past, present, and future. PLoS medicine, 5, e3. DOI: 10.1371/journal.pmed.0050003.

Stork, N. E. (1988) Insect diversity: facts, fiction and speculation. Biological Journal of the Linnean Society, 35, 321-337. DOI: 10.1111/j.1095-8312.1988.tb00474.x.

Van-Huis, A. (2013) Potential of insects as food and feed in assuring food security. Annual Review of Entomology, 58, 563-583. DOI: 10.1146/annurev-ento-120811-153704.

CHAPTER 1

Rasp, rasp, rasp… Do chewing cues from drywood termites mediate recruiting for wood colonization?

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Original research paper

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Short title: Do chewing cues mediate termite wood choice?

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ABSTRACT

Drywood termites are essentially social insects living and foraging with conspecifics inside wood pieces, where they perform a suite of behavioral traits ranging from individual to coordinated group activities. Within this context, vibroacoustic cues play important roles in behavioral mediation explored mainly in defense and foraging activities. However, termite societies also exhibit other relevant contexts, including substrate selection for colony establishment, which received little attention. Therefore, we tested the hypothesis that vibrational cues produced by termites inside drywood pieces influence wood-choice recruiting of conspecifics using dichotomous choice tests. Besides, we also assessed whether the decision-making outcome affects colony success (i.e., termite survival and wood consumption). Two behavioral activities were recognized among drywood termites colonizing wood galleries – walking and chewing. However, only the chewing cue overcomes background noise allowing acoustic signal characterization and playback tests; thus, they were the focus of our study. Overall, drywood termite groups exhibit random choice among wood-testing unities indicating that the chewing cues from the natural and/or playback signals are not used as a social trigger to mediate conspecific recruiting favoring wood preference and colonization. Nonetheless, the establishment of termite colonies in infested and non-infested wood pieces led to contrasting results in termite wood consumption and survival rate.

Keywords: substrate-borne vibration, invasive termites, Kalotermitidae, tropical wood, eusocial

INTRODUCTION

The complexity of social insects is certainly a curious and intriguing phenomenon in nature, not only due to their social organization, but also due to the exchange of information among them, or in other words, *how they communicate*. In fact, the communication process is recognized as an important trait in behavioral mediation and comprises an assumption for social activities because effective communication requires the emission of a signal by an organism imparting a response in another (con or heterospecific) organism (Cocroft, 2001; Greenfield, 2002; Hill, 2001).

The above-mentioned process has been studied on different insect orders (Cocroft and Rodriguez, 2005; Hill, 2001, 2009), and on different behavioral contexts, including sexual, alarm and defense, feeding and social interaction (Hill, 2001, 2009). Among termites, and particularly on drywood termites, the individuals are essentially social living and foraging with conspecifics inside wood pieces. Their process of communication occurs through olfactory, tactile and acoustic cues associated with their cryptic behavior, where the visual cues do not play relevant roles (Bagnères and Hanus, 2015; Cristaldo et al., 2015; Delattre et al., 2015). As substrate vibrations disseminate information quickly inside and outside termite colonies, these vibroacoustic cues are important for termite communication (Howse, 1965; Hunt and Richard, 2013). They arguably play significant role in termite life-history with the studies in the subject usually emphasizing defense behavior – as alarm signals or eavesdropping their competitors and predators (Evans et al., 2009; Oberst et al., 2017), and feeding behavior – in the assessment of food resource size and quality (Evans et al., 2005, 2007; Inta et al., 2007a, 2007b).

The West Indian drywood termite *Cryptotermes brevis* (Walker) (Blattodea: Isoptera: Kalotermitidae) is a voracious urban pest with great invasive potential and worldwide distribution that feeds on wooden structures, household furniture, books and museum collections causing economic and patrimonial losses. The associated costs of this species infestation are indeed high (see Guerreiro et al., 2014), and with serious ecological impacts on a local scale (e.g., biodiversity losses) (Govorushko, 2019). Furthermore, the West Indian termite exhibits a suite of behavioral traits encompassing from individual activities (e.g., walking, chewing, autogrooming) to coordinated group activities (e.g., build the nest, alarm for defense, etc.), which make them an interesting experimental model for communication studies. Wood size and quality are generally regarded as important foraging traits for drywood termites (Cosme et al., 2018; Evans et al., 2005, 2007, 2011; Inta et al., 2007a, 2007b; Oberst el al., 2018). However, other factors may also be associated with wood preference and remain neglected.

A curious question that remains unanswered is whether acoustic cues performed by termite inside wood (e.g., chewing, walking) mediate decision regarding wood preference by conspecific drywood termites outside the wood piece. Therefore, we characterized the vibroacoustic cues performed by termites inside the wood pieces and investigated whether they mediate wood choice by conspecifics. We aimed to test the hypothesis that vibroacoustic cues act as social trigger with the external conspecific termites exhibiting phonotaxis in response to termite-infested wood or wood emitting vibroacoustic cues (playback) either via attraction (positive phonotaxis) or avoidance (negative phonotaxis). The lack of response to vibroacoustic cues emitted by the feeding termite within the wood piece is the alternative hypothesis with the external conspecifics exhibiting random choice of wood substrate when exposed to a choice of infested and non-infested cues.

We also assessed whether the output from making-decision (group or individual) affects termite survival and wood consumption. We expected a higher survival and wood consumption from the insects that exhibit a positive phonotaxis response to previously infested wood because this response will likely optimize colony size and resource use. In contrast, negative phonotaxis is more likely in heavily infested wood where dispersal would be favored, and lack of phonotaxis will demand a higher initial cost for establishing a new colony.

MATERIAL AND METHODS

Insect and wood testing unit

Pseudogaters of the West Indian drywood termite (*Cryptotermes brevis* (Walker)) were used in the experiments since they are the individuals especially active in the colony playing important roles in nest building, waste management and nymph feeding (Constantino, 2002; Evans et al., 2013). The insects were collected from pieces of pine wood (*Pinus* sp.) of ten termite colonies previously established in the laboratory, as described by Cosme et al. (2018). Briefly, the colonies are maintained within a plastic box with smaller wood and cardboard pieces under controlled conditions of 27 ± 3 °C temperature, 60 ± 10% relative humidity, and 10:14 h (L:D) photoperiod. The individuals used in the experiments were of mixed parentage (i.e., from different colonies), which were subsequently arranged in smaller groups containing ten individuals each and left to acclimatize and interact for 24h before the bioassay; the same above-mentioned controlled conditions were used.

The wood testing unit was developed to allow termite colonization and test the wood-preference of *C. brevis*. These testing units consisted of wood pieces (0.50 cm thick x 2.00 cm wide x 8.00 cm long) made from pine heartwood (Cosme et al., 2018). Each wood testing unit was placed over the bottom portion of a Petri dish (9 cm diameter) allowing free insect movement and eventual selection of penetration site (i.e., colonization).

Audio and video recording

The natural vibroacoustic signals were recorded using a laser-doppler vibrometer (PVD-100, Polytec Inc., Germany) set with the velocity of 500 mm.s^{-1} and perpendicularly pointed to a reflective surface (2 x 2 mm) attached to the wood testing unit. Signal velocity was determined by measuring voltages on the oscilloscope (TDS-2012C, Tektronix, USA) directly obtained from the analog output of the laser vibrometer. The bioassay trials were simultaneously audio-recorded (FR-2, Fostex, USA) and videotaped with a digital high-resolution camcorder (EOS Rebel T6; Canon, Tokyo, Japan). All the bioassays were performed on top of a vibration isolating table (63-500 Series Micro-g, TMC, USA) to reduce external interference (i.e., background noise).

Drywood termite behavior and vibroacoustic cues

The recognition of the vibroacoustic cues produced by termites inside the wood piece was achieved by artificially colonizing a testing unit with one termite, where the wood testing unit contained an artificial gallery sealed with glass simultaneously allowing the recording of behavior and the corresponding vibration cue. This procedure was replicated four times using 15 min recording on each. The output of the laser vibrometer was synchronized with the video (and audio) recordings to recognize the relationships between behavior and vibroacoustic cues. Two behaviors were easily recognized in the recorded videos - walking and chewing. Twenty recording segments containing each behavior were used for temporal, spectral and amplitude characterization of the vibrational cues.

Playback

The playback signals were set according to temporal, spectral and amplitude parameters obtained from the naturally recorded vibroacoustic cues obtained as described above. The vibroacoustic cues were subsequently generated artificially, amplified, and replicated on a wood testing unit via a wave generator (WW5062, Tabor Electronics Ltd., Israel) connected to a power amplifier (Type 2718, Brüel & Kjaer, Denmark) and mini-shaker (Type 4810, Brüel & Kjaer, Denmark). We proceeded a fine calibration for each wood testing unit, as they exhibit particular physical characteristics (e.g., density, hardness, etc.). Briefly, the mini-shaker amplitude was readjusted by shifting the power amplifier settings until matching that calculated for the trial (i.e., 6 mm.s^{-1} amplitude peak). The artificial vibrations (i.e., playback) were recorded using a laser-doppler vibrometer and audio-recording on a vibration isolating table, as above mentioned, but without video monitoring. This procedure was replicated four times with a 15 min recording time each for a total 1h audio recording. Subsequently, twenty recording segments containing each behavior were once again used for acoustic characterization of the playback.

Termite choice bioassay

Dichotomous free-choice bioassays were performed to investigate whether natural and playback cues mediate wood-choice by termites. The treatments subjected to pairwise tests consisted of: a) non-infested wood testing pieces without any termites and free of any acoustic signal; b) termite infested wood testing units containing five pseudorgates per unit; and c) wood testing unit without any termites, but subjected to artificial wood vibration (playback). These treatments were organized in five dichotomous trial arrangements, as depicted in figure 1. Suitable controls were included, except for the dual provision of artificial signals due to equipment limitations.

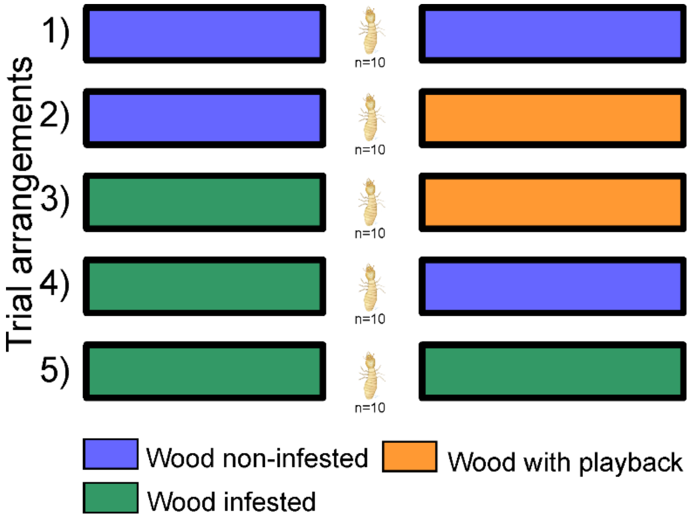

Figure 1. Sets of dichotomous free-choice bioassays carried out with the West Indian drywood termites (*Cryptotermes brevis*). The depicted termite between wood treatments represents a termite group of ten individuals subjected to the free-choice test.

All bioassays and trials were carried out under controlled conditions of temperature (28 ± 3ºC) and photoperiod (24 h dark, as the drywood termites living inside wood under total darkness). A total of 20 replicates were carried out for each bioassay, where a group of insects (n = 10 pseudogaters/replicate) was subjected to a free-choice test for 15 min. Subsequently, both wood-testing unities were isolated in plastic pots (500 mL). The number of insects colonizing each wood-testing unit was recorded and weekly monitored through an X-ray digital system (LX-60, Faxistron, X-Ray Corp. Illinois, USA) to record colony survival. Furthermore, wood tunneling was determined to record the total wood consumption (cm^2) in each trial arrangement at the end the 220 days, which was determined using the software Image-J Pro Plus version 4.5 (Media Cybernetics, Rockville, MD, USA).

Statistical analyses

The sound files (wav.) from natural and playback recordings were used for temporal, spectral and amplitude characterization of the vibroacoustic cues, which were performed using the R packages *tuner*, *seewave*, *warbleR*, and *phonTools*, according to procedures described by Sueur (2018). The assumptions of data normality and homoscedasticity were checked using Shapiro-Wilk and Bartlett tests, respectively. The average of acoustic parameters of signal duration (s), dominant frequency (kHz), bandwidth at -3dB (kHz), and root mean square (RMS) amplitude ($mm.s^{-1}$) from natural and playback vibroacoustic cues were contrasted using F-test with *stats* package. The average termite preference or choice (i.e., proportion data) and wood consumption (continuous data) were compared by Student's *t*-test, while the data of colony survival through time were subjected to survival analyses using Kaplan-Meier estimators with the package *survival*. All analyses were performed using the R-software version 3.5.1 (R Core Team, 2019). The graphical illustrations were produced with Wacom creative table (Intuos S, Japan) using Corel Painter (Essential 6, Canada).

RESULTS

Drywood termite behavior and vibroacoustic cues

The drywood termites are highly active within the wood, but curiously inconspicuous in their acoustic behavior. From wood-testing unities artificially colonized, we identified two behaviors regularly performed by pseudogaters of *C. brevis* – walking and chewing. Although recorded in video, the walking behavior could not produce vibroacoustic cues able to overcome the background noises compromising their acoustic characterization. However, the chewing was easily identified in the video and audio recordings (see Supplementary video S1), which allowed acoustic characterization and the setting for playback bioassays.

The chewing cue (i.e., event) was characterized by an average duration of 690 ± 330 $ms.event^{-1}$ with a broad range of variation ranging from 5.73 – 37.89s. Chewing was also characterized by four peaks with an average duration of 42 ± 23 $ms.peak^{-1}$ and interval of 220 ± 360 ms between them, which is correlated with the mandibular movement of opening and closing the mouthparts (Pearson correlation; n= 12; r= - 0.59; *P* = 0.04) (see Supplementary video S2). These peaks exhibit a dominant frequency of 182 ± 50 Hz with a bandwidth of 94 ± 30 Hz at -3 dB, and amplitude peak-to-peak ranged from -6.28 to 6.24 $mm.s^{1}$ (Figure 2).

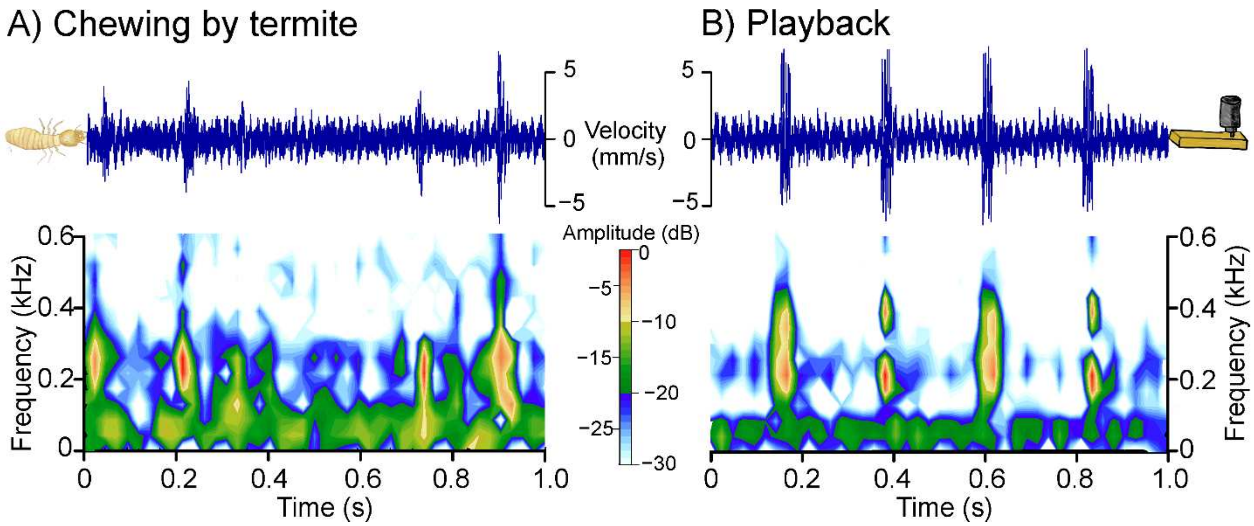

Figure 2. Oscillogram and spectrogram of termite chewing on wood (A) and their respective playback signals (B) recorded with a laser vibrometer. The oscillograms indicate the amplitude velocity (mm.s^{-1}), and the spectrograms represent the frequency (kHz) through time; the respective power spectra based in the amplitude dB is indicated by the heat scale.

We used the above-mentioned parameters to design the playback signal applied to the wood testing unit, which exhibited an average duration of 680 ± 00 ms.event^{-1} with a symmetric interval of 18s among events (i.e., defined by average inter-event interval). Throughout the event, four peaks were generated, each peak showing an average duration of 36 ± 06 ms.peak^{-1} and inter-peak-interval of 200 ms. Theses peaks exhibited a dominant frequency of 199 ± 10 Hz with a bandwidth of 62 ± 50 Hz at -3 dB, and amplitude peak-to-peak ranging from -8.60 to 9.93 mm.s^{-1} (Figure 2). The termite chewing and respective playback signals were similar in duration (F$_{1,38}$ = 0.03; P = 0.86) and dominant frequency (F$_{1,38}$ = 2.03; P = 0.16). However, they differed in root mean square (RMS) amplitude (F$_{1,38}$ = 10.67; P = 0.002) and bandwidth (F$_{1,38}$ = 6.41; P = 0.01). These latter results were expected because these parameters are strongly related to wood proprieties, in particular the resonance potential and hardness (Table 1).

Table 1. Characteristics (mean ± SD) of chewing vibroacoustic cues by West Indian drywood termites (*Cryptotermes brevis*) in pine wood pieces, and their respective playback signals recorded with laser vibrometer

Signal vibrational	n	Characteristics				
		Duration of signal (s)	Dominant frequency (kHz)	Bandwidth (kHz) at -3dB	Amplitude RMS (mm.s^{-1})	Amplitude range (mm.s^{-1})
Termite chewing	20	0.69 ± 0.33	0.182 ± 0.05	0.094 ± 0.03 a	0.86 ± 0.28 b	(-6.28 - 6.24)
Playback	20	0.68 ± 0.00	0.199 ± 0.01	0.062 ± 0.05 b	1.06 ± 0.05 a	(-8.60 - 9.93)
F$_{1,38}$	-	0.03	2.03	**6.41**	**10.67**	-
P	-	0.86	0.16	**0.01**	**0.002**	-

Bold highlight significance difference (P < 0.05).

Termites free-choice bioassays

Groups of drywood termites subjected to dichotomous free-choice bioassays exhibited a random choice among wood-testing unities regardless of trial arrangements and treatments involved. These results indicate that the chewing cues from natural and playback signals are not social triggers that mediate wood substrate choice leading to the rejection of the first and second of hypotheses of (positive and/or negative) phonotaxis mediating this interaction (Figure 3A).

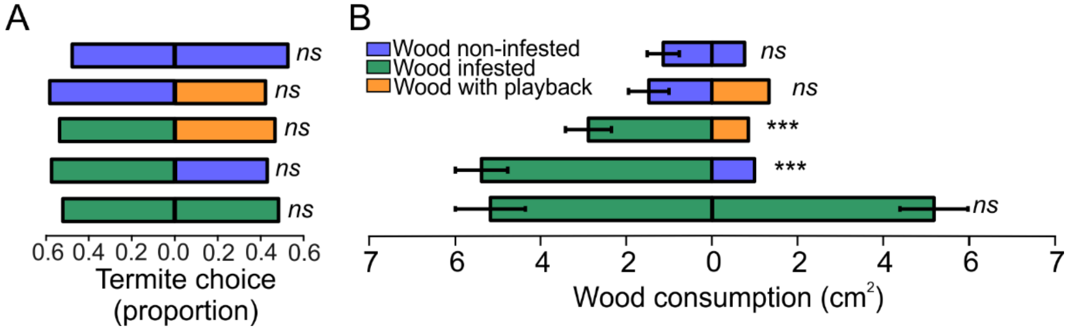

Figure 3. Dichotomous termite colonization (A) and wood consumption (B) resulting from termite wood choice. The asterisk indicates significant differences by Student's *t*-test (*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$) and ns indicates lack of significant difference ($P > 0.05$).

Regarding wood consumption, the termites subjected to the dichotomous bioassays of non-infested vs non-infested wood ($t = 0.84$; d.f. = 3,11; $P = 0.41$), non-infested vs artificially vibrating wood ($t = 0.22$; d.f. = 37,5; $P = 0.83$), and infested vs infested wood ($t = 0.002$; d.f. = 37,9; $P = 0.99$) did not exhibit significant difference (Figure 3B). However, the termite consumption of wood-infested pieces was proportionally higher rather than that of other treatments. This result is more evident when considering the bioassays of infested vs artificially vibrating woods ($t = 3.27$; d.f. = 30,6; $P = 0.003$), and infested vs non-infested woods ($t = 6.18$; d.f. = 30,5; $P < 0.001$), where the consumption of non-infested and artificially vibrating woods were significantly lower rather than infested woods (Figure 3B).

Likewise, termite survival reflected the results of those of wood consumption (non-infested vs non-infested wood: $\chi^2 = 0.06$; d.f.= 1,39; $P = 0.81$; non-infested vs artificially vibrating woods: $\chi^2 = 0.23$; d.f.= 1,39; $P = 0.62$; and infested vs infested woods: $\chi^2 = -9.15$; d.f.= 1,39; $P = 1.00$; Fig.4). Nonetheless, the differences in termite survival were significant in the bioassays of infested vs artificially vibrating woods ($\chi^2 = 4.37$; d.f.= 1,39; $P = 0.04$; Fig.4C), and infested vs non-infested woods ($\chi^2 = 27.79$; d.f.= 1,39; $P < 0.001$; Fig.4D). Survival was significantly lower in non-infested and artificially vibrating woods than in termite infested wood (Figure 4).

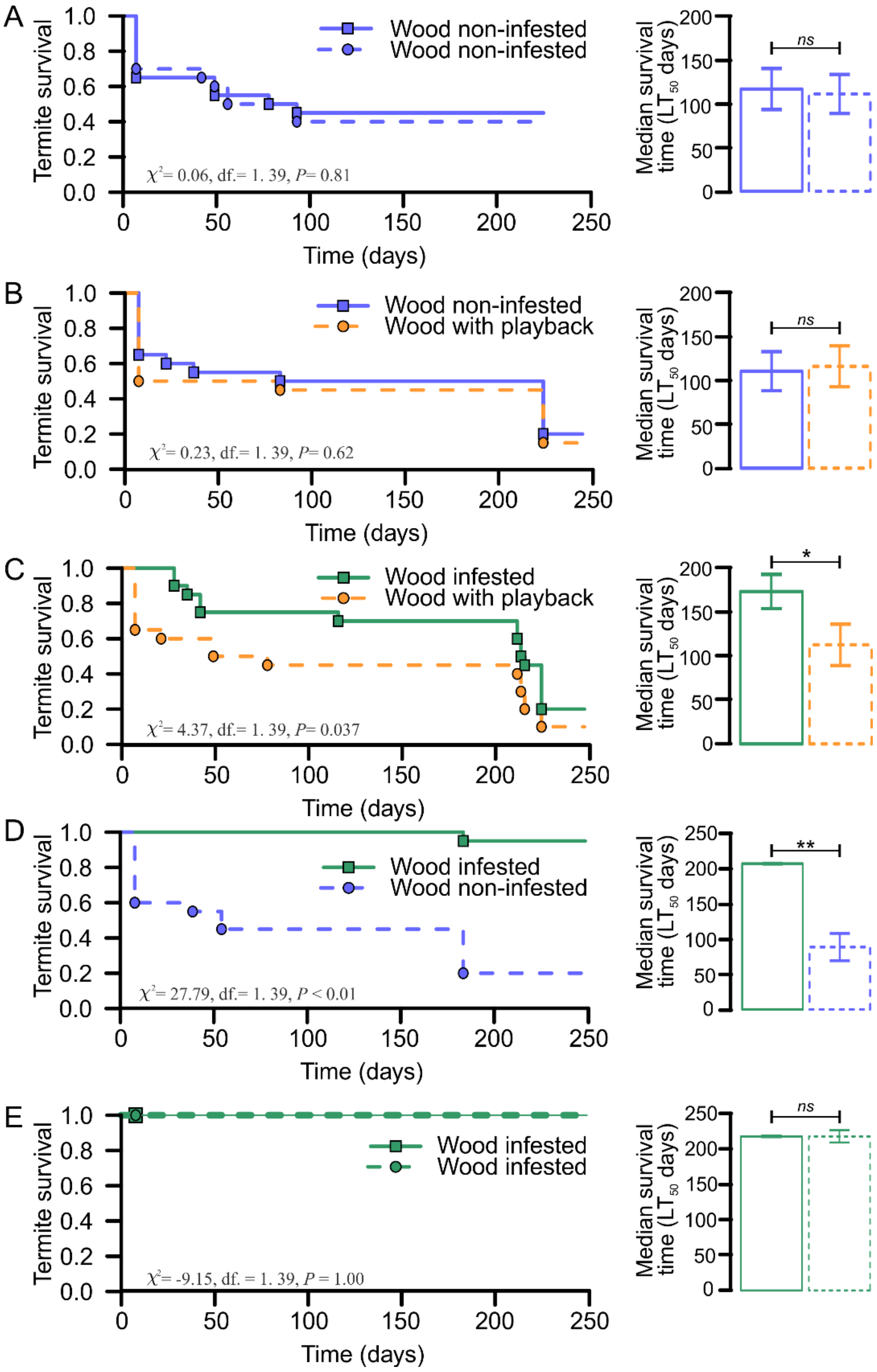

Figure 4. Termite survival curves and median time (LT_{50}) for wood-choice. The asterisk indicates significant differences by Kaplan-Meier estimators (*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$) and *ns* indicates lack of significant difference ($P > 0.05$).

DISCUSSION

The rasp, rasp, rasp papery rustling sound of the chewing drywood termites was the vibroacoustic cue identified from video and audio recordings (see Supplementary video S1). They were subsequently used for acoustic signal characterization and tested whether they could be used as a cue mediating termite behavior in wood choice through natural and playback assays. Our results did not support the hypothesis that chewing (i.e. natural or playback) was used as a social trigger mediating the foraging behavior of termites because the chewing cue, although distinct from background noise, did not elicit phonotaxis response (Figure 3A). Nonetheless, this cue may still be relevant in other contexts and interactions not assessed here, as a cue for eavesdropping competitors or predators (Barber and Conner, 2007; Romer et al., 2010).

Thereby, we accepted the hypothesis that wood choice is an individual decision not associated with chewing cues. This foraging decision though is determined by the wood physical proprieties, which are the prominent drivers of feeding-preference for drywood termites (Cosme et al. 2018). These wood physical characteristics also have significant life-history consequences for these organisms (Carere and Eens 2005; Réale et al. 2007; Sih et al. 2004). A few other wood traits, like size and quality, have also been recognized as important in directing wood choice among drywood termites (Evans et al., 2005, 2007; Cosme et al. 2018; Inta et al. 2007a; Oberst et al. 2018). However, these traits were standardized in our study not playing any relevant role in the obtained results.

Although wood chewing was not a significant cue mediating wood-choice by the drywood termites, the termite choice itself exhibited serious consequences for the insect survival and wood consumption. Overall, the choice of infested wood pieces favored the termite colonization increasing the colony size and optimizing the exploration of the (wood) resource (see wood infested in Figure 3B). In addition, this pattern of choice reduces the initial cost of establishment of a new colony (e.g., mechanical damage in mandibles, desiccation, and replacement of individual), which have a negative impact on the insect survival rate, as was observed in the non-infested and in the artificially vibrating wood (Figure 4C and 4D, respectively).

The undamaged wood, either non-infested or artificially vibrating, negatively affected the wood colonization reducing consumption and survival (Figure 3B), a likely consequence of the initially higher cost for establishing a new colony. Such observations are consistent with predictions of resource-use hypothesis, particularly for termite societies where the individuals living in central pathways and through of a collective process (i.e., work division) are able to better explore the food resource and exhibit lower impact in colony fitness (Korb and Roux, 2012; Korb and Thorne, 2017).

In summary, termite chewing did not play a significant role in wood-choice. Therefore, this potential vibroacoustic cue does not configure into signal mediating this interaction. Nonetheless, infested woods were more successfully colonized by the choosing drywood termites leading to higher rates of survival and wood consumption (and loss) than non-infested and artificially vibrating wood not infested by these insects. Thus, wood chewing by termites may only provide acoustic cues in other contexts, if any. So far, the rasp, raps papery rustling sound of the chewing by drywood termites are just that, soft rustling noises that, although interesting, do not seem to mediate effective conspecific communication.

ACKNOWLEDGMENTS

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REFERENCES

Bagnères, A.G., Hanus, H. 2015. Communication and social regulation in termites. In: Social recognition in invertebrates. Springer, Cham, pp. 193-248. https://doi.org/10.1007/978-3-319-17599-7_11

Barber, J. R., Conner, W. E. 2007. Acoustic mimicry in a predator–prey interaction. Proc. Natl. Acad. Sci., 104, 9331-9334. https://doi.org/10.1073/pnas.0703627104

Carere, C., Eens, M. 2005. Unravelling animal personalities: how and why individuals consistently differ. Behav. 142, 1149-1157.

Cocroft, R.B. 2001. Vibrational communication and the ecology of group-living, herbivorous insects. American Zoologist 41, 1215-1221. https://doi.org/10.1093/icb/41.5.1215

Cocroft, R.B., Rodriguez R.L. 2005. The behavioral ecology of insect vibrational communication. Bioscience 55, 323-334. https://doi.org/10.1641/0006-3568(2005)055[0323:TBEOIV]2.0.CO;2

Constantino, R. 2002.The pest termites of South America: taxonomy, distribution and status. J. Appl. Entomol. 126, 355-365. https://doi.org/10.1046/j.1439-0418.2002.00670.x

Cosme, L., Haro, M.M., Guedes, N.M., Della-Lucia, T.M., Guedes, R.N.C. 2018. Tropical wood resistance to the West Indian drywood termite *Cryptotermes brevis*: If termites can't chew…. Pest Manag Sci. 74, 914-924. https://doi.org/10.1002/ps.4785

Cristaldo, P. F., Jandak, V., Kutalova, K., Rodrigues, V.B., Brothanek, M., Jiricek, O., DeSouza, O., Sobotnik, J. 2015. The nature of alarm communication in *Constrictotermes cyphergaster* (Blattodea: Termitoidea: Termitidae): the integration of chemical and vibroacoustic signals. Biology Open 4, 1649-1659. https://doi.org/10.1242/bio.014084

Delattre, O., Sillam-Dusses, D., Jandak, V., Brothanek, M., Rucker, K., Bourguignon, T., Vytiskova, B., Cvacka, J., Jiricek, O., Sobotnik, J. 2015. Complex alarm strategy in the most basal termite species. Behav Ecol Sociobiol 69, 945-1955. https://doi.org/10.1007/s00265-015-2007-9

Evans, T.A., Lai, J.C., Toledano, E., McDowall, L., Rakotonarivo, S., Lenz, M. 2005. Termites assess wood size by using vibration signals. Proc. Natl. Acad. Sci. 102, 3732-3737. https://doi.org/10.1073/pnas.0408649102

Evans, T.A., Inta, R., Lai, J.C.S., Lenz, M. 2007. Foraging vibration signals attract foragers and identify food size in the drywood termite, *Cryptotermes secundus*. Insec Soc 54:374-382. https://doi.org/10.1007/s00040-007-0958-1

Evans, T.A., Inta, R., Lai J.C.S., Prueger, S, Foo, N.W, Fu, E.W., Lenz M. 2009. Termites eavesdrop to avoid competitors. Proc. R. Soc. B 276, 4035-4041. http://doi.org/10.1098/rspb.2009.1147

Evans, T.A., Inta, R. Lai, J.C.S. 2011. Foraging choice and replacement reproductives facilitate invasiveness in drywood termites. Biol Invasions 13, 1579-1587. https://doi.org/10.1007/s10530-010-9915-4

Evans, T.A., Brian T.F., Kenneth J.G. 2013. Biology of invasive termites: a worldwide review. Annu. Rev. Entomol. 58, 455-474. https://doi.org/10.1146/annurev-ento-120811-153554

Govorushko, S. 2019. Economic and ecological importance of termites: A global review. Entomol. Sci. 22, 21-35. https://doi.org/10.1111/ens.12328

Greenfield M.D. 2002 Signalers and receivers: mechanisms and evolution of arthropod communication. Oxford University Press

Guerreiro, O., Cardoso, P., Ferreira, J.M., Ferreira, M.T. 2014. Potential distribution and cost estimation of the damage caused by *Cryptotermes brevis* (Isoptera: Kalotermitidae) in the Azores. J. Econ. Entomol. 107: 1554-1562. https://doi.org/10.1603/EC13501

Hill, P.S.M. 2001. Vibration and animal communication: A review. Amer. Zool. 41, 1135-1142. https://doi.org/10.1093/icb/41.5.1135

Hill, P.S.M. 2009. How do animals use substrate-borne vibrations as an information source? Naturwissenschaften. 96, 1355-1371. https://doi.org/10.1007/s00114-009-0588-8

Howse, P.E. 1965. On the significance of certain oscillatory movements of termites. Insect. Soc. 12, 335-345. https://doi.org/10.1007/BF02222723

Hunt, J.H., Richard, F.J. 2013. Intracolony vibroacoustic communication in social insects. Insect. Soc. 60: 403-417. https://doi.org/10.1007/s00040-013-0311-9

Inta, R., Evans, T.A., Lai, J.C.S., Lenz, M. 2007a. What do vibrations have to do with termites' food choice? Acoust. Aust. 35, 73-77.

Inta, R., Lai, J.C.S., Fu, E.W., Evans, T.A. 2007b. Termites live in a material world: exploration of their ability to differentiate between food sources. J. Royal Soc. Interface 4, 735-744. https://doi.org/10.1098/rsif.2007.0223

Korb, J., Roux, E.A. 2012. Why join a neighbour: fitness consequences of colony fusions in termites. J. Evo. Biol. 25, 2161-2170. https://doi.org/10.1111/j.1420-9101.2012.02617.x

Korb, J., Thorne, B. 2017. Sociality in termites. In: Rubenstein, D.R., Abbot, P. (Eds.), Comparative social evolution, Cambridge University Press, pp. 124-153.

Oberst, S., Lai, J.C.S., Evans, T.A. 2016. Termites utilise clay to build structural supports and so increase foraging resources. Sci. Rep. 6, e20990. https://doi.org/10.1038/srep20990

Oberst, S., Bann, G., Lai, J.C.S., Evans, T.A. 2017. Cryptic termites avoid predatory ants by eavesdropping on vibrational cues from their footsteps. Ecol. Lett. 20, 212-221. https://doi.org/10.1111/ele.12727

Oberst, S., Lai, J.C.S, Evans, T.A. 2018. Key physical wood properties in termite foraging decisions. J. R. Soc. Interface 15, e20180505. https://doi.org/10.1098/rsif.2018.0505

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Réale, D, Reader, S.M., Sol, D., McDougall, P.T., Dingemanse, N.J. 2007. Integrating animal temperament within ecology and evolution. Biol Rev 82:291-318. https://doi.org/10.1111/j.1469-185X.2007.00010.x

Römer, H., Lang, A., Hartbauer, M. 2010. The Signaller's Dilemma: a cost–benefit analysis of public and private communication. PLoS ONE 5, e13325. https://doi.org/10.1371/journal.pone.0013325

Sih, A., Bell, A., Johnson, J.C. 2004. Behavioral syndromes: an ecological and evolutionary overview. Trends Ecol Evol 19, 372-378. https://doi.org/10.1016/j.tree.2004.04.009

Sueur, J. (2018). Sound analysis and synthesis with R. Culemborg, the Netherlands: Springer. https://doi.org/10.1007/978-3-319-77647-7

CHAPTER 2

Chemical constituents of tropical woods and resistance to the invasive drywood termite *Cryptotermes brevis*

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Original research paper

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ABSTRACT

Wood-feeding, nesting, and production of secondary reproductives are key determinant traits of invasive species of drywood termites, and the West Indian drywood termite *Cryptotermes brevis* (Walker) is one of their major examples of worldwide concern as pest species of structural lumber, furniture, and other wood products. The problem and losses by this species are as serious as the prevailing wood characteristics. However, despite the current widespread occurrence of this species in the tropics, except Asia, tropical wood resistance and underlying mechanisms of resistance against this termite are scarcely known. Nonetheless, wood hardness and particularly wood density were recently recognized as important underlying traits for termite resistance in tropical woods, but the chemical wood constituents were not considered. Here, we assessed tropical wood resistance to the invasive termite species *C. brevis* and tested the relevance of their holocellulose, lignin, and (total) extractive contents preventing termite infestation. Indeed, resistance to the West Indian termite varied with wood species in terms of both colonization and consumption, but only under free-choice testing because without choice no significant difference was detected among wood species. Regardless, none of these traits were significantly correlated with wood resistance to *C. brevis*. Therefore, wood physical resistance, particularly wood density, seems the main recognized determinant of tropical wood resistance against the West Indian drywood termite. The pattern of termite movement on the surface of soft, mid, and hard wood was also consistent with this conclusion.

Keyword: wood preference, wood area loss, termite choice, invasive termites, Isoptera, Kalotermitidae

INTRODUCTION

If the wood is the tree memoir, it may also be the termite´s dream, or nightmare. The outcome would be essentially a function of this interaction and the physicochemical properties of the wood counteracting termite species and infestation. Thus, structural lumber, furniture, and other wood products are all at the mercy of such interaction potentially leading to economic losses (Constantino, 2002; Guerreiro et al., 2014; Rust & Su, 2012). The species of both interacting agents are important and the drywood termites are a focus of concern because they encompass the most important and prevalent invasive termites. This is so because of their wood-nesting and wood-feeding habits, besides of their frequent capacity to produce secondary reproductive either through neoteny of nymphs, of workers or pseudergates, and through the retention of alates (Bourguignon et al., 2015; Evans et al., 2011, 2013). The West Indian drywood termite *Cryptotermes brevis* (Walker) fits the description and is one of the most prominent of these invasive pest species (Constantino, 2002; Evans et al., 2013; Rust & Su, 2012).

The endemic origin of the West Indian drywood termite (Isoptera: Kalotermitidae) extends from Chile to Peru covering the Pacific coast desert of South America, and this species distribution range remains in expansion (Borges et al., 2014; Nunes et al., 2005; Scheffrahn et al., 2009). The dispersal of this species was post-Columbian and anthropogenic likely resulting from international trade, transportation, and recreation, as frequently is the case for invasive species in general (Banks et al., 2015; Yemshanov et al., 2014). The trade among Spanish colonies in South America likely led to the initial spread of the West Indian termite via shipboard infestation and infested wood transportation during the early colonial period on the continent (Scheffrahn et al., 2009). Light attraction, cryptic behavior and capacity of colonizing small wood propagules as small as 8 cm^3 probably aided this species spread throughout most of the (pan) tropical world (Bourguignon et al., 2015; Evans et al., 2011, 2013; Korb, 2006; Minnick, 1973; Scheffrahn et al., 2009). As a consequence, not even historic buildings and (wooden) artworks representative of the American colonial period are free from such a threat (Constantino, 2002; Mello et al., 2014).

Wood type and size affect conspecific interactions and production of secondary reproductives of drywood termites (Evans et al., 2011; Korb, 2006), as do wood anatomy and volatile emissions (Himmi & Yoshimura, 2016; Xu et al., 2015). Curiously, resistance of tropical wood and its underlying causes were seldom investigated and the studies carried out so far focus on recognizing preferences and mainly by subterranean termite species with few exceptions (Cornelius & Osbrink, 2015; Lee et al., 2008; Lee & Forschler, 2016; Manzoor et al., 2015; Oberst et al., 2018; Rust & Su, 2012). Nonetheless, varied resistance to the West Indian drywood termite exists (Cosme et al., 2018; Gonçalves & Oliveira, 2006), and the wood physical properties, namely hardness and specially density, are important determinants of colonization and wood consumption by this invasive pest species (Cosme et al., 2018). The chemical constituents of tropical woods and their potential relationship with drywood termites as determinants of their infestation were not yet investigated.

The main chemical components of drywood species are holocellulose, lignin, and extractives (Pettersen, 1984), which are relevant determinants of wood decomposition (Poletto et al., 2012; Ulyshen, 2016; Ulyshen et al., 2016). The former two are structural components, while the latter are mostly low-molecular-mass compounds (Pettersen 1984; Ritter & Kurth, 1933). Holocellulose corresponds to the water-insoluble fraction of total polysaccharide obtained from wood, straw or the like that is made up of cellulose and all of the hemicelluloses acquired through the removal of lignin and extractives from the natural material (Pettersen, 1984). Lignin is an organic polymer in support tissues of vascular plants particularly important in forming the plant cell wall. This phenolic substance consists of an irregular array of bonded hydroxyl- and methoxy-substituted phenylpropane units important in papermaking, among others (Pettersen, 1984). In contrast, wood extractives are not structural wood chemical components, are soluble in neutral solvents, and encompasse a variety of organic compounds involved in tree metabolism, protection against microbial attack, and energy reserves contributing to wood properties such as color, odor, and decay resistance (Pettersen, 1984; Telmo & Lousada, 2011; Ulyshen et al., 2016). Thus, their potential role in termite protection is worth assessing.

Here we assessed the resistance of tropical wood species to the West Indian drywood termite and quantified the chemical components of these wood species to assess their potential role mediating this interaction. We also recorded the movement from termites on wood surface attempting to recognize potential patterns of preference associated with the physicochemical type of wood. We were expecting significant variation in wood resistance to *C. brevis* based on our previous study focusing on underlying physical resistance of tropical woods. We also expected a variation on the chemical components and expected their supporting association with termite resistance contribution with the wood physical properties.

MATERIAL AND METHODS

Tropical wood types and chemical characterization

Wood testing units were made of heartwood as earlier described and tested against the West Indian drywood termite (Cosme et al., 2018). Nine distinct and representative tropical wood species were used, which are commonly employed in a diverse range of uses including furniture, floor and wall paneling, frames, house structure, etc. They were: 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.).

These nine wood species were chemically characterized using two independent samples of each, which were ground to powder using a knife mill and subsequently sieved in 40 and 60 mesh sieves. The obtained powder (60 g/sample) was used to quantify the contents of lignin, total extractives, and holocellulose. Insoluble lignin was determined by Klason´s method (Dence, 1992; Kirk & Obst, 1988), as modified by Gomide and Demuner (1986) from the TAPPI T 264 om-88 standard (TAPPI, 1998). Briefly, the samples were digested and subsequently diluted on sulfuric acid to hydrolyze and solubilize the polysaccharide; the insoluble residue was then dried and weighed as lignin. Soluble lignin was determined by spectrometry where the above filtrate is placed in a fused silica absorption cell and absorbance is measured at 205 nm using sulfuric acid as blank (Goldschimid, 1971). The quantification of total extractives was carried out following the TAPPI 264 om-88 standard method (TAPPI, 1998) where the dried samples were extracted with an ethanol-toluene mixture (1:2 v/v), the solvent was subsequently evaporated, the residue was oven-dried, cooled in a desiccator, and then weighted. The holocellulose content was obtained by subtracting the percent contents of total lignin and total extractives from 100.

Free-choice bioassays

The free-choice bioassays were carried out following early methods (Cosme et al., 2018). Briefly, three hundred insects (pseudergates) were released within a circle over a white plastic tray (35 cm diameter) containing two experimental units of each wood species randomly distributed; the insects were allowed freedom of choice for wood colonization for 120 days. The proportion of insects colonizing each wood testing unit was then recorded and the testing units were weighted to record the wood consumption. 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 = wood initial dry weight – final weight. The wood dry weight was determined by drying the wood testing units in an oven maintained at 200 ± 2°C until constant weight. The wood units were maintained in a desiccator until weighting on a precision balance (BG 440, Gehaka, São Paulo, SP, Brazil). Each bioassay was replicated three times.

No-choice bioassays

The no-choice tests were also performed with two testing units of each wood species and three replicates. In this case, each wood experimental unit was placed within a 500 mL transparent glass container where 30 insects, 29 pseudergates and one soldier were released. The proportion of insects and wood loss (i.e., wood consumption) were recorded after 120 days, as described for the free-choice bioassays, again following methods by Cosme et al. (2018).

Walking bioassay

The walking activity was recorded for 10 min on wood arenas, where each arena contained a mixture of wood types qualitatively representing three densities/hardness - soft, mid, hard woods (i.e., jequitiba, tatajuba, and purpleheart, respectively). Each wood experimental unit was 0.50 cm thick x 2.00 cm wide x 8.00 cm long and arranged side-by-side within of a glass box to prevent insect scape. A single insect (pseudergate) was released in the center of the arena, and its movement was recorded and digitally transferred to a computer using an automated video tracking system equipped with a charge-coupled device (CCD) camera (ViewPoint Life Sciences, Montreal, Canada). This procedure was replicated fifteen times. The individual traits recorded included the number of stops (no.), resting time (s), time walking (s), total distance walked (mm), and walking velocity (mm/s; calculated based on the time the insect spent walking).

Statistical analyses

The assumptions of normality and homoscedasticity were checked using Shapiro-Wilk and Bartlett tests, respectively. The proportion and continuous data were subjected to analyses of deviance and generalized linear model (GLM) with binomial and Gaussian distribution, respectively. When required, the overdispersion of models was adjusted and significant treatments were compared by the test of contrasts ($P < 0.05$). Pairwise correlation analyses were also used to test the association between individual wood chemical constituents and termite colonization. The R software (version 3.5.1) was always used for the analyses with the packages stats and mass (R Core Team, 2019).

RESULTS

Wood chemical characterization

All of the wood chemical constituents characterized differed significantly among wood species. Holocellulose was more abundant in guariuba and less abundant in angelim, courbaril, and cumaru ($\chi^2 = 0.35$; d.f. = 8, 9; $P < 0.001$; Figure 1). The levels of total lignin (i.e., soluble + insoluble) exhibited opposite trend with angelim, courbaril, and cumaru, besides of eucalypt, showing highest levels and guariuba exhibiting the lowest ($\chi^2 = 0.07$; d.f. = 8, 9; $P < 0.001$; Figure 1). The levels of total extractives were the lowest among the chemical constituents, but again exhibiting significant differences among wood species with equally higher contents in angelim, courbaril, cumaru, and purpleheart, and the lowest content in guariuba ($\chi^2 = 0.46$; d.f. = 8, 9; $P < 0.001$; Figure 1).

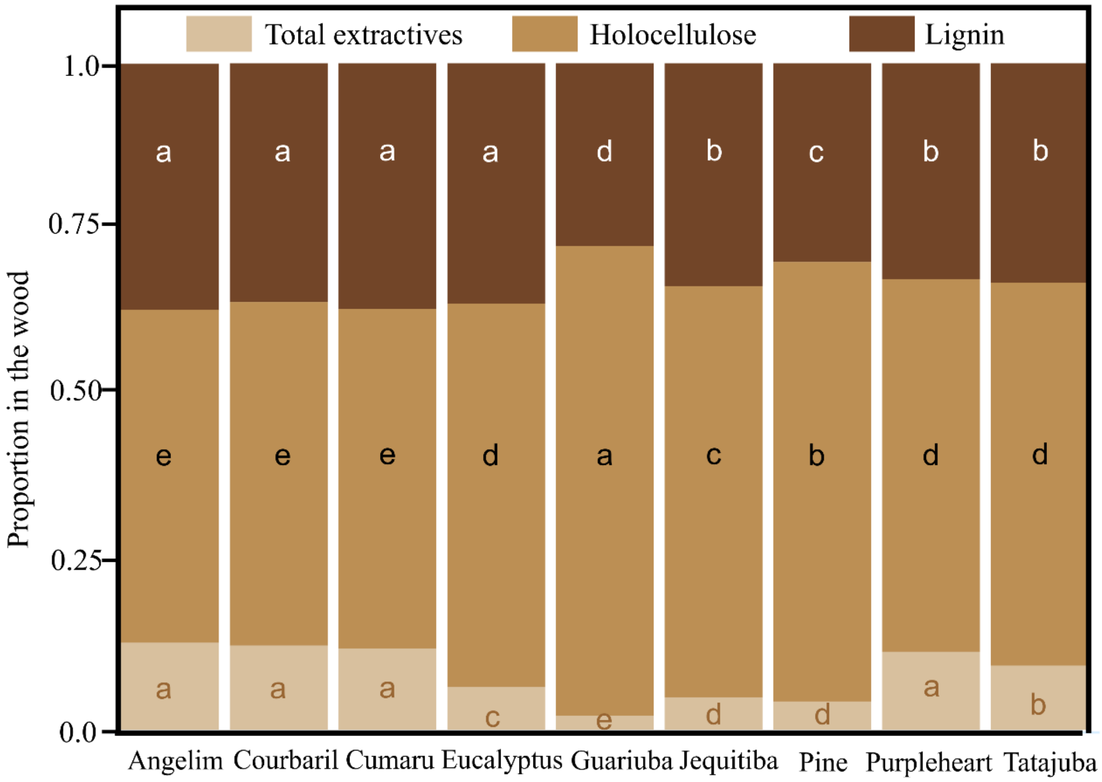

Figure 1. Level of total extractives, holocellulose and lignin from tropical wood species. Different letters within each histogram indicate significant differences among treatments by the test of contrasts ($P < 0.05$).

Termite free-choice wood preference

The drywood termites exhibited significant differences in colonization preference among tropical wood species in free-choice bioassays ($\chi^2 = 4.37$; d.f. = 8, 18; $P < 0.001$). Termite colonization was significantly higher in jequitiba (0.32 ± 0.12) and pine (0.17 ± 0.11) (Figure 2A). Similarly, the wood consumption also exhibited contrasting results ($F_{8,18} = 4.75$; $P = 0.002$) and, as expected, it was higher in the colonized woods (i.e., jequitiba and pine) (Figure 2B). Curiously, the non-colonized wood species also exhibited variation in their final weight, a consequence of their differing hygroscopic potential, whose overall average is indicated in Figure 2B.

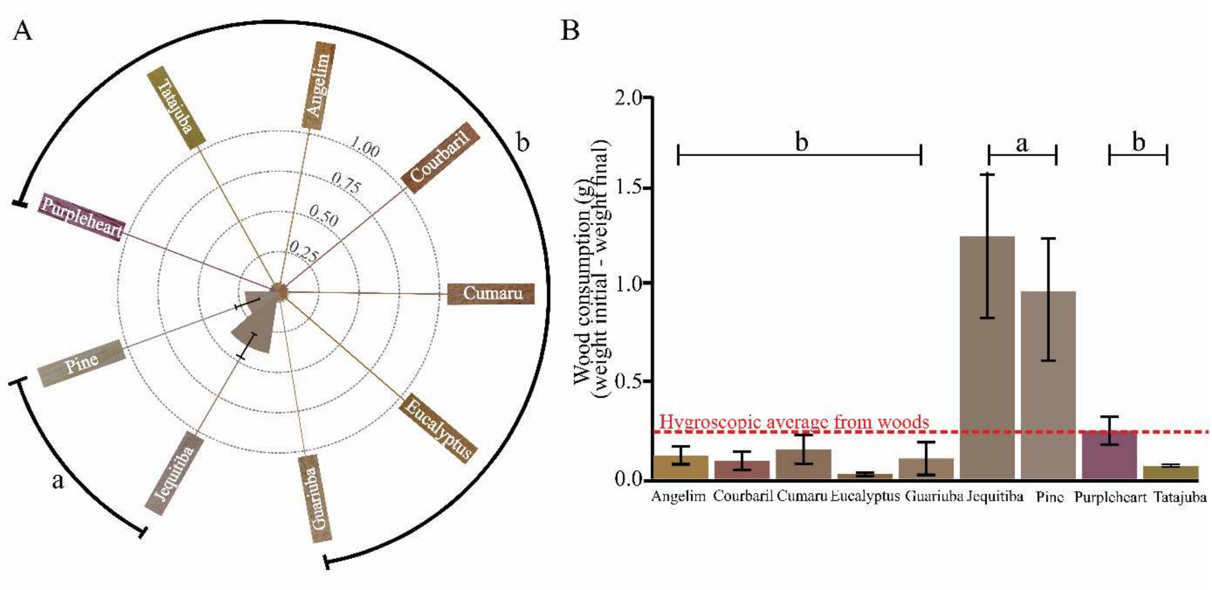

Figure 2. The proportion of termites (A) and wood consumption (B) resulting from free-choice wood preference by the drywood termite *Cryptotermes brevis*. In the polar plot, the circles with dotted-line are indicating the proportion scale and each histogram pie-slice indicates average proportion (± standard error). In the barplot, the histogram bars are indicating the average consumption by termites (± standard error). The horizontal red dotted line in (B) refers to the potential hygroscopic average from wood. The differing lowercase letters, in both plots, indicate significant difference among wood types by test of contrasts (*P* < 0.05; ns = not significant).

Termite no-choice wood colonization

Drywood termites subjected to no-choice bioassays did not exhibit significant difference in colonization (χ^2 = 1.84, d.f. = 8,18, *P* = 0.28; Figure.3A); likewise, the tropical wood did not exhibit significant weight loss by termite consumption ($F_{8,18}$ = 0.15; *P* = 0.06; Figure 3B).

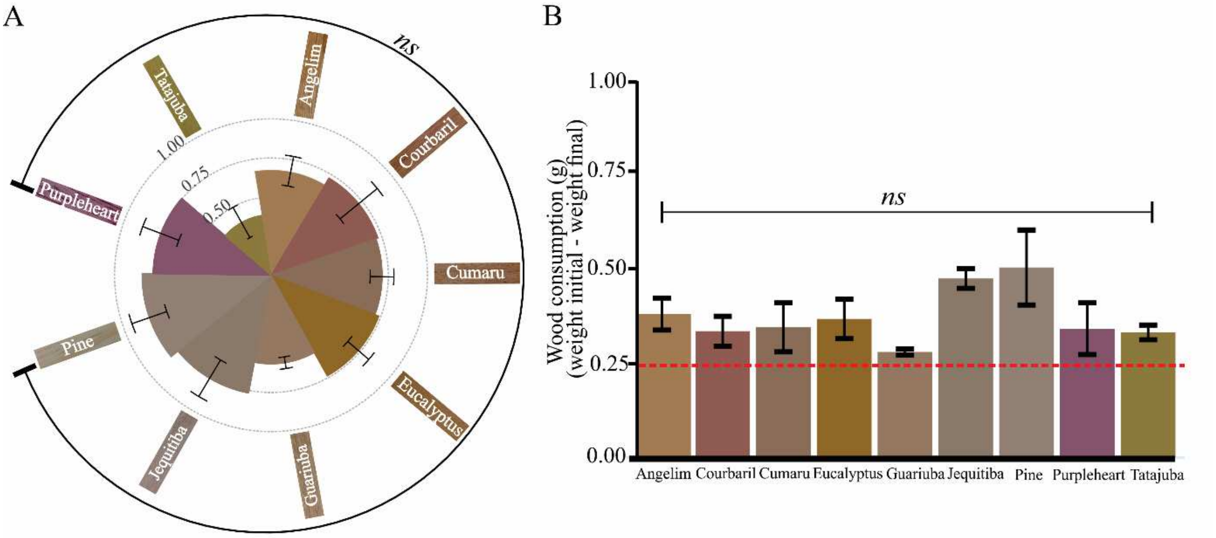

Figure 3. The proportion of termites (A) and wood consumption (B) resulting from no-choice bioassays with tropical wood species and the drywood termites *Cryptotermes brevis*. In the polar plot, the circles with dotted-line are indicating the proportion scale and each histogram pie-slice indicates average proportion (± standard error). In the barplot, the histogram bars are indicating the average consumption by termites (± standard error). The horizontal red dotted line in (B) refers to the potential hygroscopic average from wood. The differing lowercase letters, in both plots, indicate significant difference among wood types by test of contrasts ($P < 0.05$; ns = not significant).

Relationship between wood chemicals constituents and termite infestation

The single (pairwise) correlations testing the association between the levels of each wood chemical constituents and termite infestation on no-choice bioassays were carried out, but there was no significant correlation (n = 9; $P > 0.50$). Again, no significant correlations existed between each wood chemical constituents and wood consumption from the no-choice test either (n = 9; $P > 0.55$). Correlations with data from the free-choice bioassays also did not provide significant results (n = 9, $P > 0.41$).

Termite walking activity

Termite walking activity on the surface of three wood species representative of the three different classes of density and hardness (i.e., soft, mid and hard) was significantly different for number of stops ($F_{2,42} = 13.65$; $P < 0.001$), resting time ($F_{2,42} = 10.52$; $P < 0.001$), distance walked ($F_{2,42} = 10.42$; $P < 0.001$), and walking velocity ($F_{2,42}= 19.48$; $P < 0.001$). While jequitiba and tatajuba, our representatives of soft and mid wood, led to higher number of stops (Figure 4A), resting time (Figure 4B), time walking (Figure 4C), and distance walked (Figure 4D), they led to lower walking velocity (Figure 4E), particularly the former (soft) wood species. The general walking pattern is visually summarized in Figure 4F indicating a substantial persistence and movement (and permanence) on the soft wood (i.e., jequitiba), then on tatajuba and little activity and persistence on purpleheart, which is a much denser and a hard wood type.

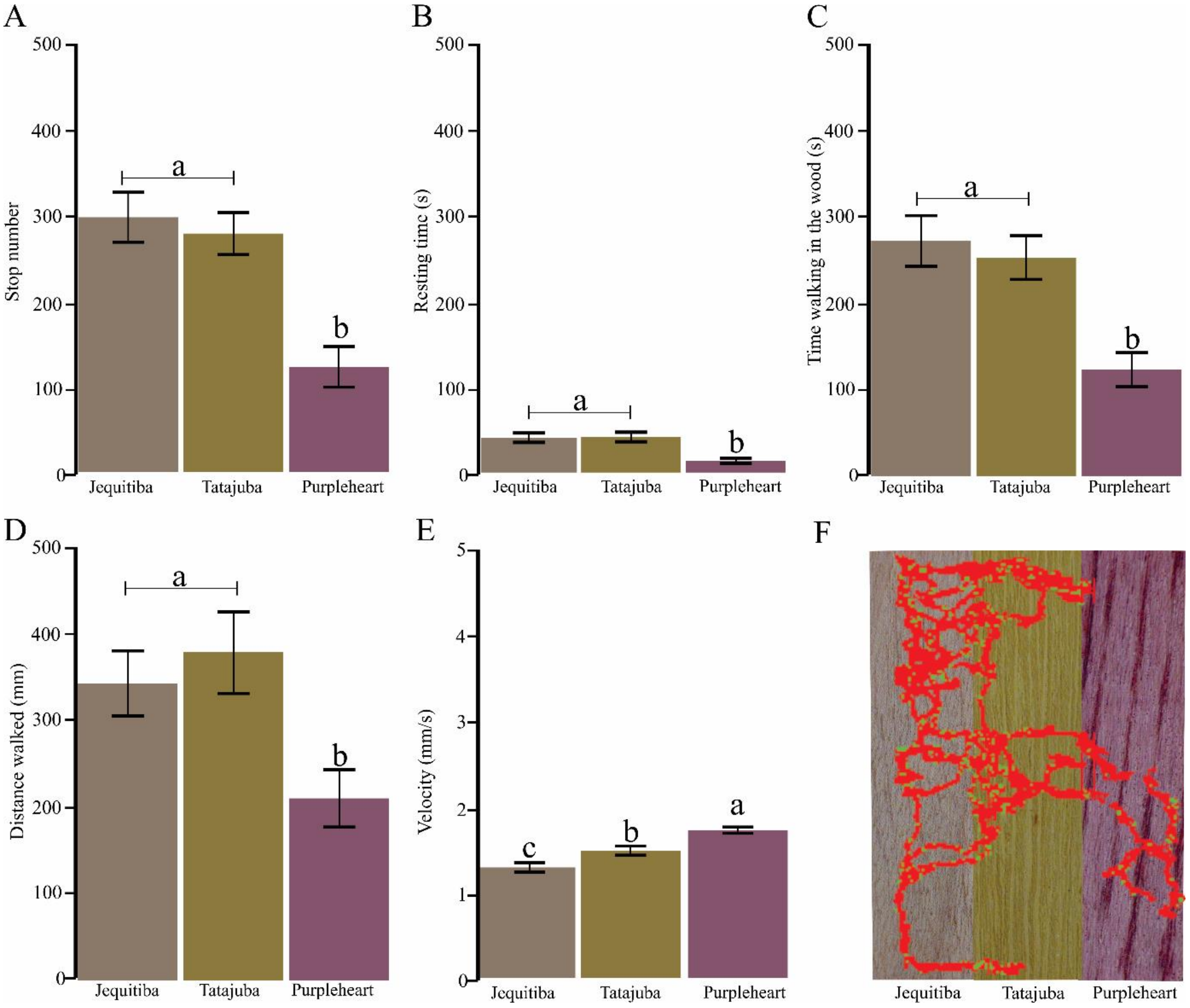

Figure 4. Number of stops (±SE; A), resting time (±SE; B), time walking (±SE; C), distance walked (±SE; D), and walking velocity (±SE; E) of the drywood termites *Cryptotermes brevis* in free-choice bioassay on three tropical wood types. Histogram bars with the same lower case letter are not significantly different by test of contrasts ($P < 0.05$). Panel F shows a representative 10-min track of an individual termite on the three side-by-side placed wood experimental units.

DISCUSSION

The resistance of tropical wood species to the West Indian drywood termite *C. brevis* was earlier recognized and associated with wood physical characteristics, particularly density and hardness (Cosme et al., 2018). However, wood chemical constituents may also play a role in wood resistance to termites as two of them, holocellulose and lignin, are structural components of wood (Pettersen 1984; Ritter & Kurth, 1933), and extractives are frequently involved in wood protection and decay resistance (Pettersen, 1984; Telmo & Lousada, 2011; Ulyzhen et al., 2016). Therefore, the present study was carried out expecting a significant role of wood chemical constituents in wood resistance. Nonetheless, our results did not provide support for this expectation.

The levels of wood chemical constituents varied among wood species with holocellulose as the main component with contents ranging from 49 to 72%, followed by total lignin varying from 26 to 38%, and total extractives ranging from 1.1 to 11.5%. These ranges of content are within the expected range from other wood species (Gomide & Demuner, 1986; Polleto et al., 2012; Ritter & Kurth, 1933; Telmo & Lousada, 2011). However, their variation differed significantly among the tested tropical wood species suggesting that they might be involved in termite resistance if the wood variation for these traits is significant and correlated with the levels of these wood chemical constituents. Variation in termite resistance was indeed significant particularly in the free-choice bioassays performed in our study.

Guariuba, for instance, exhibited the highest levels of holocellulose and the lowest levels of both lignin and (total) extractives, in contrast with angelim, courbarial, and cumaru. Nonetheless, guariuba resembled angelim, courbarial and cumaru on termite colonization and consumption under free-choice and the wood performance variation against termite infestation was even smaller under no-choice conditions. These findings suggest that the levels of wood chemical constituents in tropical wood species is not important for termite resistance. Indeed, correlations between the wood chemical constituents and termite colonization and losses were not significant leading us to reject our initial hypothesis of the involvement of wood chemical constituents on termite resistance, even if secondary. Thus, the wood physical properties, hardness and mainly density, remain the sole recognized determinants of wood resistance to the West Indian drywood termite (Cosme et al., 2018), and also for other termite species (Oberst et al., 2018).

A further investigation was carried out on wood experimental units of three wood species with contrasting levels of density and hardness – soft, mid, and hard wood, respectively represented by jequitiba, tatajuba, and purpleheart. The findings give further credence for the major involvement of these characteristics in termite resistance. The walking activity of drywood termites on the surface of the wood experimental units of the above-related species indicates that the permanence and movement of the insects on the soft wood surface significantly outlasted that on mid and hard woods. The termites remained for longer on the soft jequitiba, where they walked more and at a lower velocity than in tatajuba and purpleheart. Thus, soft wood exhibiting lower density and hardness were favored by the termites likely leading to their higher colonization and consumption, as earlier reported (Cosme et al., 2018).

In summary, among the physicochemical properties potentially involved on (tropical) wood resistance to the West Indian drywood termite, only the physical properties of density and hardness play major roles in such interaction. Despite the recorded variability in the contents of holocellulose, lignin and extractives among the tropical wood species tested, such variation was uncorrelated with termite infestation and wood consumption by these insects. The results of termite walking activity also reinforce the importance of the wood physical characteristics for termite resistance. Therefore, the tree memoir is better reflected on the termite physical scars rather than on its chemical memory. Besides, if termites can't chew, how will they damage?

Author contribution

LCJr and RNCG conceived and designed the study. LCJr and RNCG established the experimental protocols, and RNCG provided reagents and analytical tools. LMT and LCJr performed the experiments. LMT and RNCG analyzed the data. LCJr and RNCG wrote the manuscript, which was read, corrected, and approved by all of the authors.

Compliance with ethical standards

The authors declare that they have no conflict of interest.

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REFERENCES

Banks, N. C., Paini, D. R., Bayliss, K. L., & Hodda, M. (2015). The role of global trade and transport network topology in the human-mediated dispersal of alien species. *Ecology letters*, 18, 188-199. DOI: 10.1111/ele.12397

Borges, P. A. V, Guerreiro, O., Ferreira, M. T., Borges, A., Ferreira, F., Bicudo, N., Nunes, L., Marcos, R. S., Arroz, A. M. Scheffrahn, R. H. & Myles, T. G. 2014. *Cryptotermes brevis* (Isoptera: Kalotermitidae) in the Azores: Lessons after 2yr of monitoring in the archipelago. *Journal of Insect Science*, 14: 1–7. DOI: 10.1093/jisesa/ieu034

Bourguignon, T., Lo, N., Cameron, S. L., Šobotník, J., Hayashi, Y., Shigenobu, S., Watanabe, D., Roisin, Y., Miura, T. & Evans, T. A. (2014). The evolutionary history of termites as inferred from 66 mitochondrial genomes. Molecular Biology and Evolution, 32, 406-421. DOI: 10.1093/molbev/msu308

Constantino, R. (2002). The pest termites of South America: taxonomy, distribution and status. *Journal of Applied Entomology,* 126: 355–365. DOI: 10.1046/j.1439-0418.2002.00670.x

Cornelius, M. L., & Osbrink, W. L. (2015). Natural resistance of exotic wood species to the Formosan subterranean termite (Isoptera: Rhinotermitidae). *International biodeterioration & biodegradation*, 101, 8-11. DOI: 10.1016/j.ibiod.2015.03.016

Cosme Jr, L., Haro, M. M., Guedes, N. M. P., Della Lucia, T. M. C., & Guedes, R. N. C. (2018). Tropical wood resistance to the West Indian drywood termite *Cryptotermes brevis*: If termites can't chew…. *Pest management science*, 74, 914-924. DOI: 10.1002/ps.4785

Dence C. W. (1992). The Determination of Lignin. pp. 33-61. In: S.Y. Lin and C.W. Dence (eds) Methods in Lignin Chemistry. Springer Series in Wood Science. Springer, Berlin, Heidelberg.

Evans, T. A., Inta, R., & Lai, J. C. (2011). Foraging choice and replacement reproductives facilitate invasiveness in drywood termites. *Biological Invasions*, 13, 1579-1587. DOI: 10.1007/s10530-010-9915-4

Evans, T. A., Forschler, B. T., & Grace, J. K. (2013). Biology of invasive termites: a worldwide review. *Annual Review of Entomology*, 58, 455–474. DOI: 10.1146/annurev-ento-120811-153554

Goldschimid, O. (1971). Ultraviolet spectra. pp. 241-266. In: K. V. Sarkanen and C. H. Ludwig (eds.), Lignin's: occurrence, formation, structure and reactions. John Wiley & Sons, New York, NY, USA.

Gomide J. L. & Demuner, B.J. (1986). Determinação do teor de lignina em material lenhoso: método Klason modificado. *O papel*, 47: 36-38.

Gonçalves, F. G., & Oliveira, J. T. da S. (2006). Resistência ao ataque de cupim-de-madeira seca (*Cryptotermes brevis*) em seis espécies florestais. *Cerne*. 12: 80-83.

Guerreiro, O., Cardoso, P., Ferreira, J. M., Ferreira, M. T., & Borges, P. A. (2014). Potential distribution and cost estimation of the damage caused by *Cryptotermes brevis* (Isoptera: Kalotermitidae) in the Azores. *Journal of Economic Entomology*, 107, 1554-1562. DOI: 10.1603/EC13501

Himmi, S. K., Yoshimura, T., Yanase, Y., Mori, T., Torigoe, T., & Imazu, S. (2016). Wood anatomical selectivity of drywood termite in the nest-gallery establishment revealed by X-ray tomography. Wood science and technology, 50, 631-643. DOI: 10.1007/s00226-016-0800-x

Kirk, T. K., & Obst, J. R. (1988). Lignin determination. pp. 87-101. In: W.A. Wood and S.T. Kellog (eds.), Methods in Enzymology. Academic Press, San Diego.

Korb, J. (2006). Limited food induces nepotism in drywood termites. *Biology letters*, 2, 364-366. DOI: 10.1098/rsbl.2006.0497

Lee, S. H., Bardunias, P., Su, N. Y., & Yang, R. L. (2008). Behavioral response of termites to tunnel surface irregularity. *Behavioural Processes*, 78, 397-400. DOI: 10.1016/j.beproc.2008.02.009

Lee, T., & Forschler, B. T. (2016). Wood preference of *Reticulitermes virginicus* (Blattodea: Rhinotermitidae) using no-, two-, and four-choice designs and seven different measures of wood consumption. *Journal of Economic Entomolology*. 109: 785-791. DOI: 10.1093/jee/tov391

Manzoor, F., Abbas, M. & Latif, M. U. (2015). Comparative study of resistance and feeding preference of 24 wood species to attack by *Heterotermes indicola* (Wasmann) and *Coptotermes heimi* (Isoptera: Rhinotermitidae). *Sociobiology*. 62: 417-425. DOI: 10.13102/sociobiology.v62i3.732

Mello, A. P., da Costa, B. G., da Silva, A. C., Silva, A. M. B., & Bezerra-Gusmão, M. A. (2014). Termites in historical buildings and residences in the semiarid region of Brazil. *Sociobiology*, 61, 318-323. DOI: 10.13102/sociobiology.v61i3.318-323

Minnick, D. R. (1973). The flight and courtship behavior of the drywood termite, *Cryptotermes brevis*. *Environmental Entomology*, 2, 587-592. DOI: 10.1093/ee/2.4.587

Nunes, L., Gaju, M., Krecek, J., Molero, R., Teresa Ferreira, M., & De Roca, C. B. (2010). First records of urban invasive *Cryptotermes brevis* (Isoptera: Kalotermitidae) in continental Spain and Portugal. *Journal of Applied Entomology*, 134, 637-640. DOI: 10.1111/j.1439-0418.2009.01490.x

Oberst, S., Lai, J. C., & Evans, T. A. (2018). Key physical wood properties in termite foraging decisions. Journal of the Royal Society Interface, 15:e20180505. DOI: 10.1098/rsif.2018.0505

Pettersen, R. C. (1984). The chemical composition of water. pp. 57-126. In: R.M. Rowell (ed.), The chemistry of solid wood. American Chemical Society.

Poletto, M., Zattera, A. J., Forte, M. M., & Santana, R. M. (2012). Thermal decomposition of wood: Influence of wood components and cellulose crystallite size. *Bioresource Technology*, 109, 148-153. DOI: 10.1016/j.biortech.2011.11.122

R Core Team. (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Ritter, G. J., & Kurth, E. F. (1933). Holocellulose, total carbohydrate fraction of extractive-free maple wood. *Industrial & Engineering Chemistry*, 25, 1250-1253. DOI:10.1021/ie50287a016

Rust, M. K., & Su, N. Y. (2012). Managing social insects of urban importance. *Annual Review of Entomology*, 57, 355-375. DOI: 10.1146/annurev-ento-120710-100634

Scheffrahn, R. H., Křeček, J., Ripa, R., & Luppichini, P. (2009). Endemic origin and vast anthropogenic dispersal of the West Indian drywood termite. *Biological invasions*, 11(4), 787-799. DOI: 10.1007/s10530-008-9293-3

Technical Association of the Pulp and Paper Industry. 1998. TAPPI test methods T 264 om-88: preparation of wood for chemical analysis. Tappi Technology Park, Atlanta

Telmo, C., & Lousada, J. (2011). The explained variation by lignin and extractive contents on higher heating value of wood. *Biomass and Bioenergy*, 35, 1663-1667. DOI: 10.1016/j.biombioe.2010.12.038

Ulyshen, M. D. (2016). Wood decomposition as influenced by invertebrates. *Biological Reviews*, 91, 70-85. DOI: 10.1111/brv.12158

Ulyshen, M. D., Müller, J., & Seibold, S. (2016). Bark coverage and insects influence wood decomposition: Direct and indirect effects. *Applied Soil Ecology*, 105, 25-30. DOI: 10.1016/j.apsoil.2016.03.017

Xu, K., Feng, J., Zhong, T., Zheng, Z., & Chen, T. (2015). Effects of volatile chemical components of wood species on mould growth susceptibility and termite attack resistance of wood plastic composites. *International Biodeterioration & Biodegradation*, 100, 106-115. DOI: 10.1016/j.ibiod.2015.02.002

Yemshanov, D., Koch, F. H., Lu, B., Lyons, D. B., Prestemon, J. P., Scarr, T., & Koehler, K. (2014). There is no silver bullet: the value of diversification in planning invasive species surveillance. *Ecological Economics*, 104, 61-72. DOI: 10.1016/j.ecolecon.2014.04.024

CHAPTER 3

Game-based learning as a strategy for teaching Entomology

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Original Research Paper

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Short running title: Game-based learning as a strategy for teaching Entomology

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ABSTRACT

Games are recognized to promote interaction and engagement among children through an abstract challenge, which often results in emotional reaction. The use of game activities is spreading in diverse contexts for other reasons, including stimulating teamwork and creativity, and finding common use for candidate selection and as a teaching aid. Herein, we assess whether a game-based activity is useful as a tool for the teaching of entomology, which was investigated based on the learning achieved with the activity and the feedback provided by the students. The student feedback was positive regarding card content, rule manual, game-play design and usage (functional perspective). Likewise, the students also considered the game a fun activity, fast and competitive, and even challenging (personal perspective). Some difficulties were indicated, such as the amount of initial information required to play. Nonetheless, the game activity increased student learning, highlighting its importance as a tool to provide a didactic activity in the classroom, encouraging creativity and healthy competition among students. Besides, the game also subjects students and teachers to an integrative and dynamic teaching-mode shifting from the traditional learning method.

Key Words: Analogic game, Didactic-activity, Education, Insect science, Learning experience.

INTRODUCTION

All grown-ups were once children, but only a few of them remember it (de Saint-Exupéry, 1943). This quote from "*The Little Prince*" illustrates the notion that when aging we integrate into a social position that requires behavior consistent with our biological age, which implies a reduction in playful activities, including playing and enjoying games. However, is the interruption of such activities really necessary? More particularly, is a rupture in game-playing necessary? According to Koster's *Theory of Fun for Game Design*, games are defined as "a system in which players engage in an abstract challenge, defined by rules, interactivity, and feedback that results in a quantifiable outcome often eliciting an emotional reaction" (Koster, 2013). Thus, the questions stated above seem to have a negative answer because, during the last 20 years or so, games and game-playing have been spreading. They have been used in diverse contexts for different reasons, including stimulating teamwork and creativity, job candidate selection and teaching aid, which are all typical adult activities (Kapp, 2012).

The teacher's concerns regarding game-based learning are related with the infrastructure (e.g., physical space, internet), and the effective relationship between the time expended in game-playing and the learning achieved by students, as compared with the traditional teaching-learning experience (Eastwood & Sadler, 2013). However, in the game-based learning approach, the learners are exposed to social interaction dealing with problems and unexpected challenges where they are able to assess their own activities, the consequences of these activities, and see how they are doing, which allows them to find and eventually pursue more than one viable solution to the same problem. Thus, the learners are active participants in knowledge acquisition within a constructivist approach (Piaget, 1952; Freire, 1972; Vygotskiĭ, 1986). Therefore, game-based activities can be a potential teaching tool at different educational levels, with learning feedback provided by their game performance and improvement. Furthermore, the approach brings the teacher closer to the student's world adding dynamics to the classroom interaction.

Entomology or insect science provides a scenario in which teaching is oftentimes related to a context in which the insect is included. Teaching with and/or about charismatic insects (e.g., honeybees) is easy, and the same is true for insects providing ecological services (i.e., biological control). In contrast, teaching with and/or about non-charismatic insects (e.g., cockroaches), or those associated with negative contexts (i.e., disease vectors or pest species), is unappealing making it more difficult. A curious question with serious consequences within the educational perspective, which remains yet unanswered, is how game-based activities close the gap between students and non-liked (or even disliked) subjects. This likely takes place by assimilation through a ludic environment where the game itself involves both charismatic and non-charismatic insects, thus motivating the understanding of the insect world. Therefore, herein we aimed to investigate whether a game-based activity using a role-playing type of cardboard game is an amenable strategy for the teaching of entomology, potentially favoring or benefiting learning among students.

GAME DESIGN

Based on game classifications reviewed by Abdul-Jabbar and Felicia (2015), our game type is a role-playing game (RPG), which was developed to encourage creativity and healthy competition among students. The game subject matter is science fiction allowing the player immersion in diverse and hypothetical entomological topics. The platform is offered as an analogical card-board game where multiple players (2 learners) create a strategy (i.e., card choice) with an interrelation among different card-types subsequently inviting the opponent for the (ludic) battle.

The cardboard game contains 288 cards, which explores the subjects of taxonomy, physiology, morphology, and behavior of insects while offering a ludic environment of learning. The card-types in the entomological deck are: (a) monster-cards, where a realistic photograph is exhibited and describes the order and family of the insect, its morphological traits, skills and power level (attack/defense); (b) gadget-cards, which exhibit an external morphological trait of the insect and offer a supplement that improves given insect skills; (c) trap-cards, which depict entomological instruments (e.g., entomological-net) and can be used to destroy, cancel, steal and block skills from the other cards; (d) ritual-cards, which reports a physiological process (e.g., respiration) that can increase the monster power (attack/defense), invite a new stronger monster, restore life-points or destroy other monster; and (e) place-cards, where the insect's habitat is described (e.g., forest) and provides advantages in the insect skills (Figure 1). The game also contains two boards and manuals (see Supplementary material: Rules of Insect World).

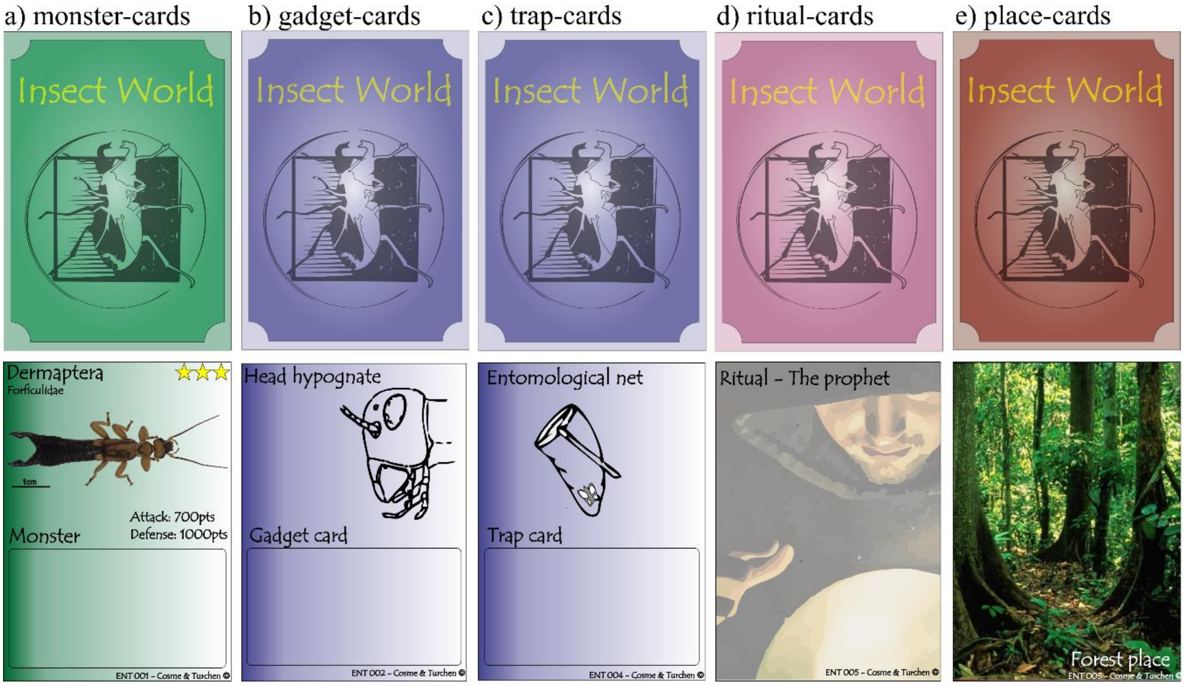
a) monster-cards b) gadget-cards c) trap-cards d) ritual-cards e) place-cards

Figure 1. The card-types illustration on the entomological deck.

DYNAMICS IN CLASSROOM

The activity was developed for undergraduate students in entomology and invertebrate zoology courses, and/or students already familiar with the subject at the high school level. The sample used to test the game encompassed 92 undergraduate students.

The students are invited to read the rules and build a strategy with 30-40 cards, independent of the card-type. The strategy should reflect the knowledge of the student on the game topics, which can be highlighted based on the level of interrelation among different card-types. The heads or tails are used to decide who will start the game and both players begin with 4,000 life-points. Subsequently, the players are able to purchase cards (5-cards/individual) and put them down on the board. The game-play is defined from the count of animal or "monster" card-points (attack/defense) and integration with another card type (i.e., gadget, place, and ritual), confronting the opponent cards on the board. The game winner will either need to suppress all life-points of the opponent (loss of all of his life-points) or finish with the opponent's cards. The enclosed rule book provides additional details and examples (Supplementary material).

DATA COLLECTION

Game feedback

The general student behavior when playing the game (e.g., interaction, perception, difficulties, and enthusiasm) was assessed by direct observation and by audio-recording with the intent of capturing the student's perspectives on the game and on the classroom dynamics. These approaches allowed us to recognize and explore characteristics not usually measurable in experimental tests. In addition, we also applied an electronic form with open and closed questions to obtain feedback from the participating students on the functional performance of the game and the student's personal experience in the activity (https://forms.gle/j21TAtVbA4giEcuq9). Observational and descriptive analyses were used to explore the student's opinion.

Learning outcome and card interaction-index

Student learning was assessed by measuring the learning outcome (i.e., the number of correct answers) through electronic forms, which contained closed questions of entomology exploring the subjects of the game activity. The questions were based in answers quickly provided on the multiple-choice and multiple-choice grid, which are tools for quick assessment of the current status of student learning (https://forms.gle/Pm7pqn6yQvdi9wu57). The electronic forms were applied twice (i.e., pre and post-test) to all participants and the change in the number of correct answers in the post-test provided the desired assessment of the student learning performance with the game. The contrast between the learning outcome between the pre- and post- electronic forms were tested by paired *t*-test ($P = 0.05$).

The card interaction-index, which is a score of all the possible effective interactions among cards, was also measured through a photographic record of each strategy built by the participants. We consider as effective interaction the cards that reflect a real context (e.g., Coleopteran + Elytra wing card; score = 2), whereas the non-effective interactions (e.g., Coleoptera + Hemelytra wing; score =1) were penalized with a reduced score. Subsequently, the card interaction-index was correlated with the learning outcome from the pre-test form to check whether the specific knowledge of the player on entomology exhibits any relationship with the interaction among cards, which could reflect a better strategy. This relationship was tested through linear regression analysis.

OUTCOMES AND INTERPRETATIONS

The opinion of students on the classroom dynamics and their general behavior throughout the game provided feedback with a functional perspective of the game (i.e., Is the game playable?), and a personal assessment of the game-playing experience by the player (i.e., Do you like it?).

Regarding the functional perspective, we verified that 59.8% (n=55) of the students either knows or plays RPG games suggesting the popularity of these games and, consequently, a better acceptance of this didactic activity in the classroom. According to the electronic forms, 95.7% (n=88) of the students had previous knowledge of the game subject and regarded the card contents important to help learning entomology (96.7%, n=89). The rule manual was recognized as informative and easy to understand (83.7%, n=77) with a reduced number of pages and illustrations exemplifying the rules. Another viewpoint expressed refers to the game design, which was considered attractive (94.6%, n=87) exhibiting a variety of colors and information, besides of the easy gameplay establishing a link among color-cards and their place on the board. Likewise, the grasp of the game dynamics from the understanding of the rules up to the final scoring was recognized as ranging from normal to very easy (82.6%, n=76) by the students, which accounts for the positive feedback about the understanding of the game manual and design. (Figure 2)

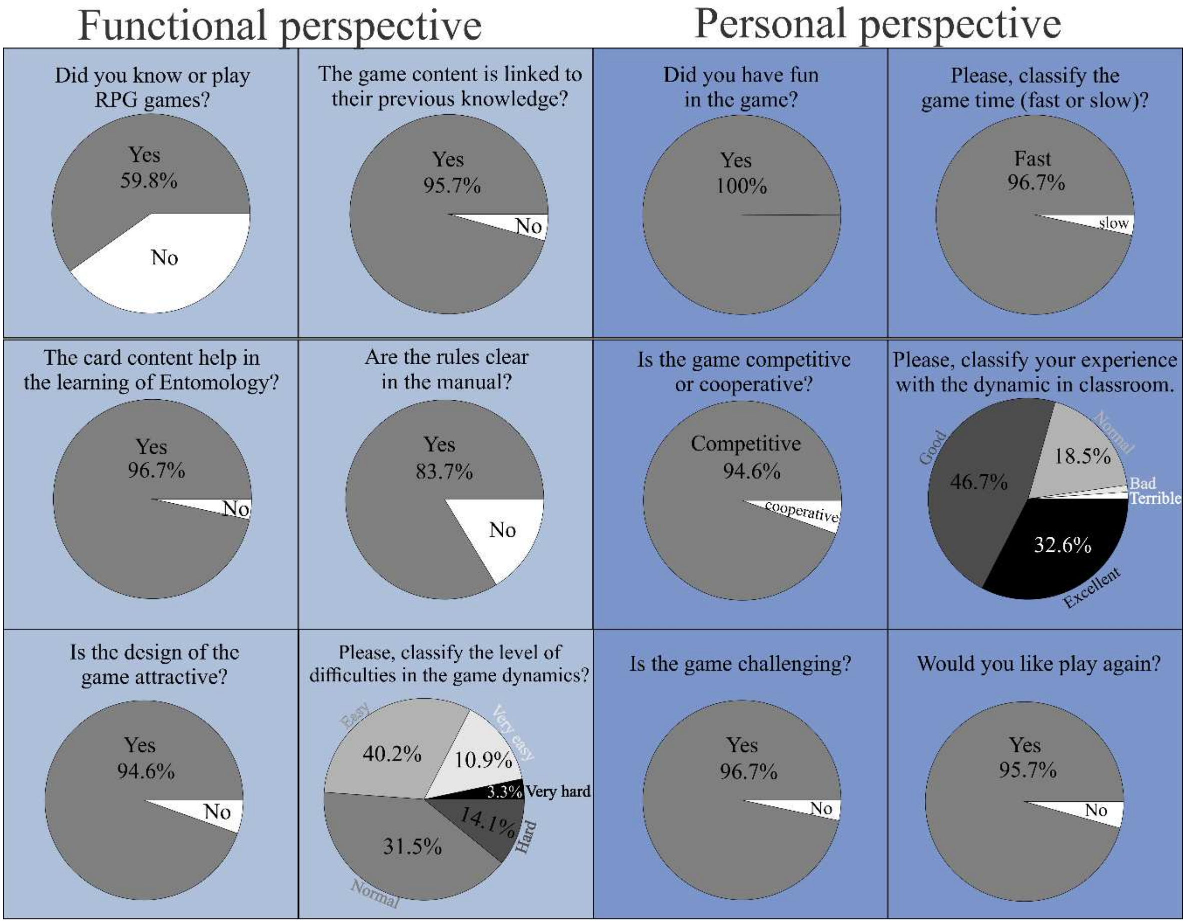

Figure 2. Results of the feedback survey among students participating in the game activity.

The positive feedback on the functional perspective is likely related to the game gender, which offers plasticity in the decision-making through a rich context and makes it possible to solve problems from different challenges. However, the card content, manual of rules, design and easy gameplay also contributed to immersion and engagement leading the students to use the narrative and elements from the cards and manual to make links between an insect and a specific card (e.g., coleopteran with elytral wings), indicating that card choice was not random. This is important because knowledgeable action is required to proceed with the game-play in the game environment, not random action (Barab et al. 2012; Abdul-Jabbar & Felicia, 2015). A frequent suggestion from the students' feedback was to change the game platform from analogical to digital (86.9%, n=80), which will be developed. This feedback agrees with that by Abdul-Jabbar and Felicia (2015) that digital games are more commonly used in game-based learning and preferred by the users. Indeed, we agree with the suggestion and rational recognizing that digital games are promising in game-based learning activities, quickly achieving a broad public. However, there are some limitations related with digital games that led us to choose the analogical card-board approach, which includes the low sociability achieved with the former (i.e., low face-to-face interaction) and eventual difficulties to access the electronic media, which is common particularly in third-world countries (West, 2015).

Regarding a more personal perspective, the students considered the game as fun (100%, n=92), fast (< 20 min; 96.7%, n=89) and competitive (94.6%, n=87), although also recognizing the activity as challenging (96.7%, n=89) because of the required functional strategy to confront the opponent cards (Figure 2). The fun and competition elements are expected in games (Koster, 2013), and their incorporation into the game-play emotionally and cognitively engage learners with significant impact on learning and motivation (Koster, 2013; Abdul-Jabbar & Felicia, 2015). However, the game challenges require a precise balance since too great a challenge may incur in low acceptance by potential players (Bochennek et al., 2007), which is also true for unchallenging games, compromising the continuation of the activity or may even lead to more frustration than enjoyment (Matthias, 2006; Ke & Abras, 2013). In other words, if the game exhibits too much challenge, it can act as a deterrent rather than a motivator. Thus, a more positively-related challenge incites curiosity towards the content and understanding about the topic, as exemplified by Tan et al. (2013), and in accordance with our results.

The main difficulties with the entomology game, as pointed out by the participating students, are the amount of initial information required to play, such as the reading of manual and cards, and the building of strategy with adequate interaction among cards. The students also reported some difficulties related with small misconceptions in the interpretation of the game rules (e.g., position of cards / card-interaction mistakes), which added by the one-time experience, compromises strategy-building and game performance; this can be minimized with the digital format. Two hypotheses can be related with these difficulties: 1) the students do not seem to favor long, text-rich content (Ramirez et al., 2010), or 2) the students exhibit reading difficulties (Ke & Abras, 2013), 3) or the instructions are unclear. As the manual of rules of the entomology game exhibits little text with illustrations to exemplify them, recognized as informative and easy to understand by the students themselves, we reject the first and third hypotheses in favor of the second one – potential reading difficulties. This limitation is mitigated by further exposure to the game, as observed in other surveys (Bochennek et al., 2007; Ke, 2008; Buur et al., 2013). Nevertheless, the occurrence of reading disorders among the students (e.g., dyslexia, autism spectrum disorder and/or attention deficit hyperactivity disorder) (August & Garfinkel, 1990; Pennington & Ozonoff, 1996) will increase the initial level of difficulty of the game.

Even so, the students considered the game as a relevant tool for learning entomology and went beyond suggesting that the game can be expanded to other public audiences (e.g., high school students). Lastly, the students scored the classroom activity as outstanding or excellent (79.3%, n=73) and would like to play again (95.7%, n= 88), including with other people (Figure 2). Additionally, they indicated the following qualities as strong points of the game: the easy game-play and attractive design, a biological context included, a mechanism of science popularization, and a potential game-didactic interface allowing learning and entertainment in a group.

LEARNING OUTCOMES AND CARD INTERACTION-INDEX

Game-based activities are interesting teaching tools within an educational perspective making use of ludic and competitive approaches to retain student attention. However, a major issue to consider is: "Could the students learn with the game?" Here, we observed an increase in learning outcomes when comparing the scores obtained in the pre- and post-test forms (18.09 ± 0.67 and 20.11 ± 0.66, respectively) (t=8.72; d.f.=91; $P < 0.001$), which highlights the better performance achieved when playing the game (Figure 3).

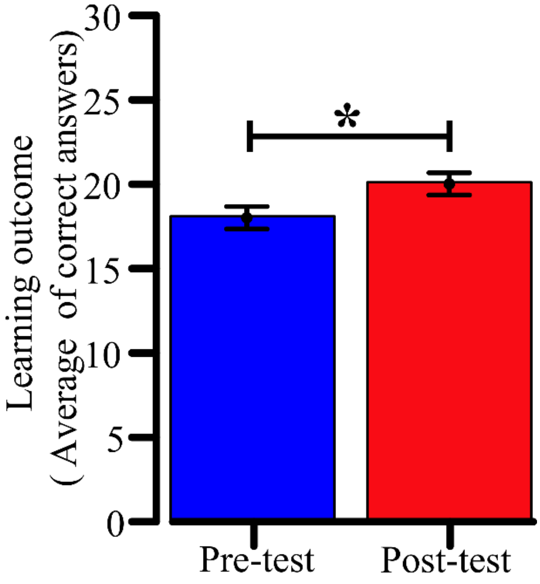

Figure 3. Student learning outcome (± SE) based on the results of pre- and post-tests applied to on the students participating in the game activity (n = 92). The asterisk over the horizontal line indicates significant differences by paired t-test ($P < 0.05$).

The improved student knowledge-based performance after playing the game indicates that the game was able to expose the students to the desired subject enhancing the use of creativity for knowledge construction from an educational perspective. Furthermore, the emotional state of engagement and enjoyment detected among the students while playing the game was arguably an important factor favoring learning (Matthias, 2006; Ke, 2008; Miller et al., 2011; Buur et al., 2013; Ke & Abras, 2013; Koster, 2013; Abdul-Jabbar & Felicia, 2015; Silva et al., 2017). Therefore, our findings further challenge the notion of rigid lecture-based teaching rich in content as a more didactic and integrative approach, what was also recognized by previous studies encompassing diverse areas (Bochennek et al., 2007; Liu & Chen, 2013; Karbownik et al., 2016; Hwang et al., 2016).

Contemporary constructivism defines teaching as "a basic biological capacity, which allows us change and is not solely due to biological maturation or aging. It always comprises the integration of three dimensions: content dimension (i.e., cognitive, skill, knowledge); incentive dimension (i.e., engagement, interest, and motivation); and interaction dimension (i.e., social)" (Illeris, 2018a, b), which can be easily integrated in a game dynamic. Here, the constructivist aspect was based on the student potential of making interactions among the card types, as well as use the knowledge provided by the cards (i.e., content assimilation) to establish a relationship among entomological subjects. Within context, we expected that a strategy with a preference for more interrelation among card-types would reflect the player's knowledge of entomology since the interconnection of card types improves the performance on the game. However, we verify that the relationship between the card interaction index and the pre-test knowledge scores was not significant ($F_{1,79}$= 0.13; R^2=0.002; *P*=0.71) (Figure 4), indicating that the student's previous acquaintance with entomology did not play a significant role developing a game strategy during the activity.

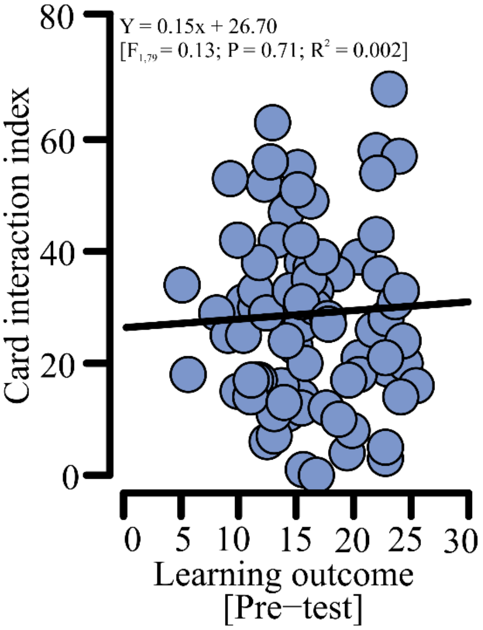

Figure 4. Relationship between the interaction index recorded during the game activity and pre-test results (learning outcome) among students participating in the game activity.

This result suggests that the students were able to create a suitable game strategy with the information provided in the game without the need to rely on previously-acquired information. It could also suggest the learning outcomes measured were only loosely connected to the actual subject, but the improved post-test performance observed rejects this hypothesis. Thus, following Piaget's constructivism (Piaget, 1952), the game-playing allowed the students to correlate previous experience, and thus broaden their knowledge resulting in learning, which can be observed from the learning outcome in the post-test.

In summary, the game was an effective tool to provide a didactic activity in the classroom encouraging creativity and healthy competition among students. Furthermore, the game was a relevant source of learning in a ludic and innovative way to expose content favoring student engagement. Thus, such game-based approach is worthy of incorporation in teaching activities to expose students and teacher to an integrative and dynamic teaching-mode shifting from the traditional learning method and expanding opportunities for creativity and involvement of students in the classroom activities. Furthermore, this initiative can be expanded to other public audiences further benefiting the popularization of science.

ACKNOWLEDGMENTS

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REFERENCES

Abdul-Jabbar, A.I., & Felicia, P. (2015). Gameplay engagement and learning in game-based learning. *Review of Educational Research* 85, 740-779.

August, G.J., & Garfinkel, B.D. (1990). Comorbidity of ADHD and reading disability among clinic-referred children. *Journal of Abnormal Child Psychology* 18, 29-45.

Barab, S., Pettyjohn, P., Gresalfi, M., Volk, C. & Solomou, M. (2012). Game-based curriculum and transformational play: Designing to meaningfully positioning person, content, and context. *Computers & Education* 58, 518-533.

Bochennek, K., Wittekindt, B., & Thomas, S. Y. Z. (2007). More than mere games: a review of card and board games for medical education. *Medical Teacher*, 29, 941-948.

Buur, J.L., Schmidt, P.L., & Barr, M.C. (2013) Using educational games to engage students in veterinary basic sciences. *Journal of Veterinary Medical Education*, 40, 278-281.

de Saint-Exupéry, A.D. (1943). *The little prince*. New York: Harcourt Brace Jovanovich.

Eastwood, J.L., & Sadler, T.D. (2013). Teachers' implementation of a game-based biotechnology curriculum. *Computers & Education*, 66, 11-24.

Freire, P. (1972). *Pedagogy of the Oppressed*. New York: Herder.

Hwang, W.Y., Shih, T.K., Ma, Z.H., Shadiev, R., & Chen, S.Y. (2016). Evaluating listening and speaking skills in a mobile game-based learning environment with situational contexts. *Computer Assisted Language Learning*, 29, 639-657.

Illeris, K. (2018a). *A comprehensive understanding of human learning.* In: Contemporary Theories of Learning, 1-14, Routledge.

Illeris K (2018b) An overview of the history of learning theory. *European Journal of Education*, 53, 86-101.

Kapp, K. M. (2012). *The gamification of learning and instruction*: game-based methods and strategies for training and education. John Wiley & Sons.

Karbownik, M.S., Wiktorowska-Owczarek, A., Kowalczyk, E., Kwarta, P., Mokros, Ł., & Pietras, T. (2016). Board game versus lecture-based seminar in the teaching of pharmacology of antimicrobial drugs—a randomized controlled trial. *FEMS Microbiology Letters*, 363, 1-9.

Ke, F. (2008). A case study of computer gaming for math: Engaged learning from gameplay? *Computers & Education*, 51, 1609-1620.

Ke, F., & Abras, T. (2013). Games for engaged learning of middle school children with special learning needs. *British Journal of Educational Technology*, 44, 225-242.

Koster, R. (2013). *Theory of fun for game design*. O'Reilly Media, Inc..

Liu, E.Z.F., & Chen, P.K. (2013). The effect of game-based learning on students' learning performance in science learning–A case of "Conveyance Go". *Procedia-Social and Behavioral Sciences*, 103, 1044-1051.

Matthias, B.O.P.P. (2006). Didactic analysis of digital games and game-based learning. *The Future of Learning*, 8.

Miller, L.M., Chang, C.I., Wang, S., Beier, M.E., & Klisch, Y. (2011). Learning and motivational impacts of a multimedia science game. *Computers & Education*, 57, 1425-1433.

Pennington, B.F., & Ozonoff, S. (1996). Executive functions and developmental psychopathology. *Journal of Child Psychology and Psychiatry*, 37, 51-87.

Piaget, J. (1952). *The origins of intelligence in children*. New York: International Universities Press.

Ramirez, C.G.R., Almonte, J.B., Tugade, R.R., & Atienza, R.O. (2010). Implementation of a digital game-based learning environment for elementary Education. In*: Education Technology and Computer (ICETC),* 208-212.

Silva, J.N., Lima, M.A.S., Moreira, J.V.X., Alexandre, F.S.O., Almeida, D.M., Oliveira, M.D.F., & Leite, A.J.M. (2017) Stereogame: An interactive computer game that engages students in reviewing stereochemistry concepts. *Journal of Chemical Education*, 94, 248-250.

Tan, J.L., Goh, D.H.L., Ang, R.P., & Huan, V.S. (2013). Participatory evaluation of an educational game for social skills acquisition. *Computers & Education*, 64, 70-80.

Vygotskiĭ, L.S. (1986). *Thought and Language*. MIT Press.

West, D.M. (2015). *Digital divide*: Improving Internet access in the developing world through affordable services and diverse content. Brookings Institution.

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CONCLUSÕES GERAIS

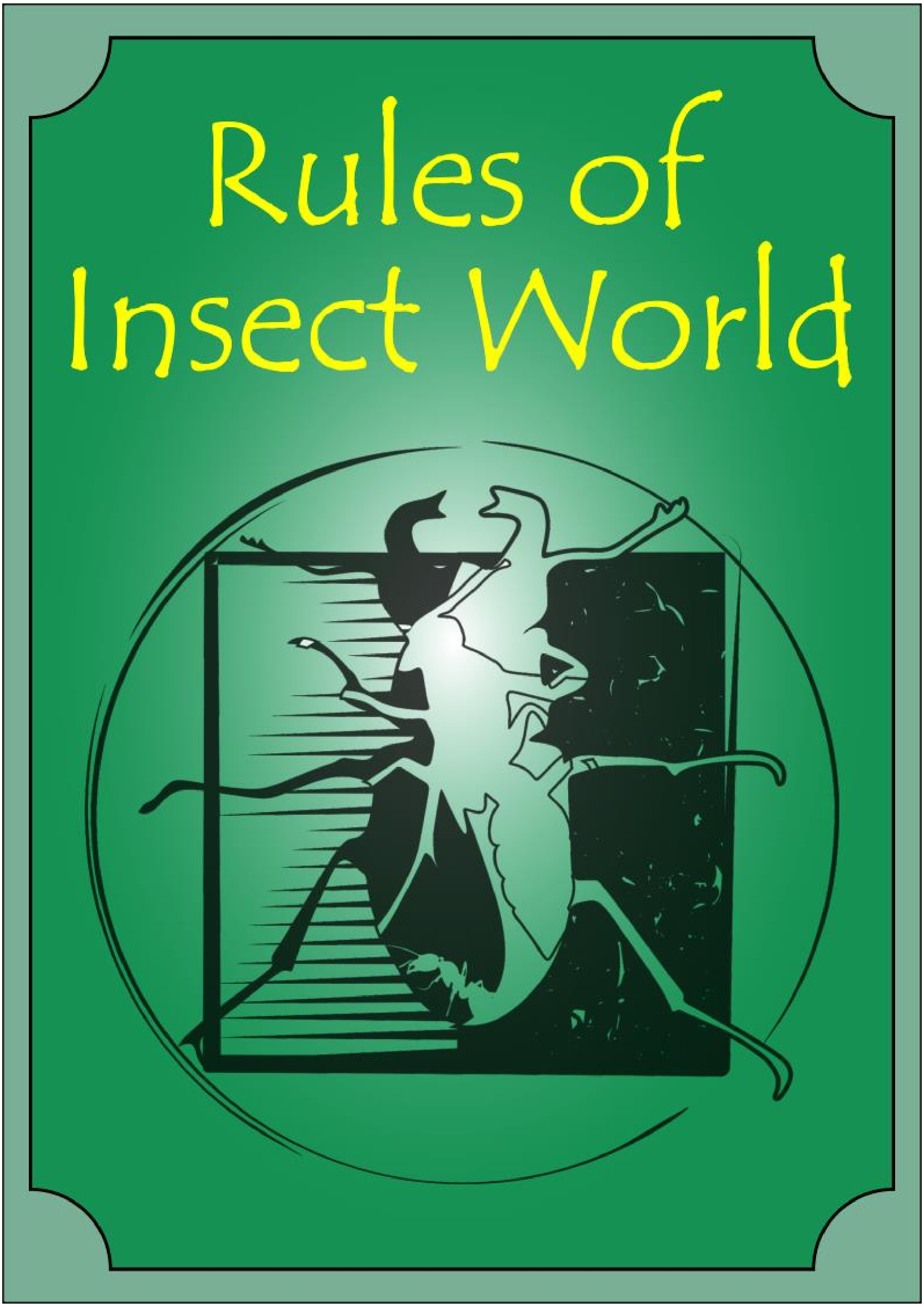
Esta pesquisa teve como proposta explorar o papel de pista vibroacústica e componentes químicos da madeira sobre o estabelecimento de colônias de térmites, bem como investigar o potencial de atividade baseada em jogos como uma ferramenta para o ensino de Entomologia a alunos de graduação, o que foi escrutinado em três capítulos. De modo geral, no primeiro capítulo, dois comportamentos realizados pelos cupins no interior da madeira foram identificados – a caminhada e a mastigação, porém apenas a pista de mastigação sobrepôs-se ao ruído de fundo, permitindo sua caracterização acústica e uso em teste de playback. Embora caracterizada, tal pista não foi capaz de mediar o comportamento dos cupins na escolha do substrato, indicando que ela não é usada como um gatilho social para mediar o recrutamento coespecífico a fim de promover a colonização.

De forma similar, no segundo capítulo, nós caracterizamos os componentes químicos (i.e., lignina, holocelulose e extrativos) de nove espécies de madeiras tropicais e avaliamos se eles poderiam influenciar a escolha e a colonização do substrato por *C. brevis*. Contudo, nenhuma associação/correlação significativa entre a colonização por cupins e os componentes químicos da madeira foi detectada, o que sugere uma contribuição negligenciável dos componentes químicos da madeira sobre o comportamento de escolha do substrato pelos cupins. Em contraponto, no terceiro capítulo, nós aplicamos um jogo entomológico para avaliar se o mesmo pode ser uma ferramenta para o ensino de Entomologia a estudantes de graduação. Neste caso, o jogo (Insect World) exibiu resultados promissores para ser utilizado como uma ferramenta didática no ensino de Entomologia, pois foi capaz de contribuir positivamente para a aprendizagem dos estudantes, ao mesmo tempo que promoveu uma atividade didática em sala de aula, permitindo um ensino integrativo e dinâmico que vai além dos métodos de ensino tradicional.

Por fim esta tese gerou três capítulos que abordaram temas diversificados. A princípio, a coesão entre estes temas é mais evidente no primeiro e o segundo capítulo, devido às similaridades nos objetos de estudo. Contudo, o grande desafio desta pesquisa foi concatenar os temas diversos, construindo um projeto de cunho científico, artístico e educacional.

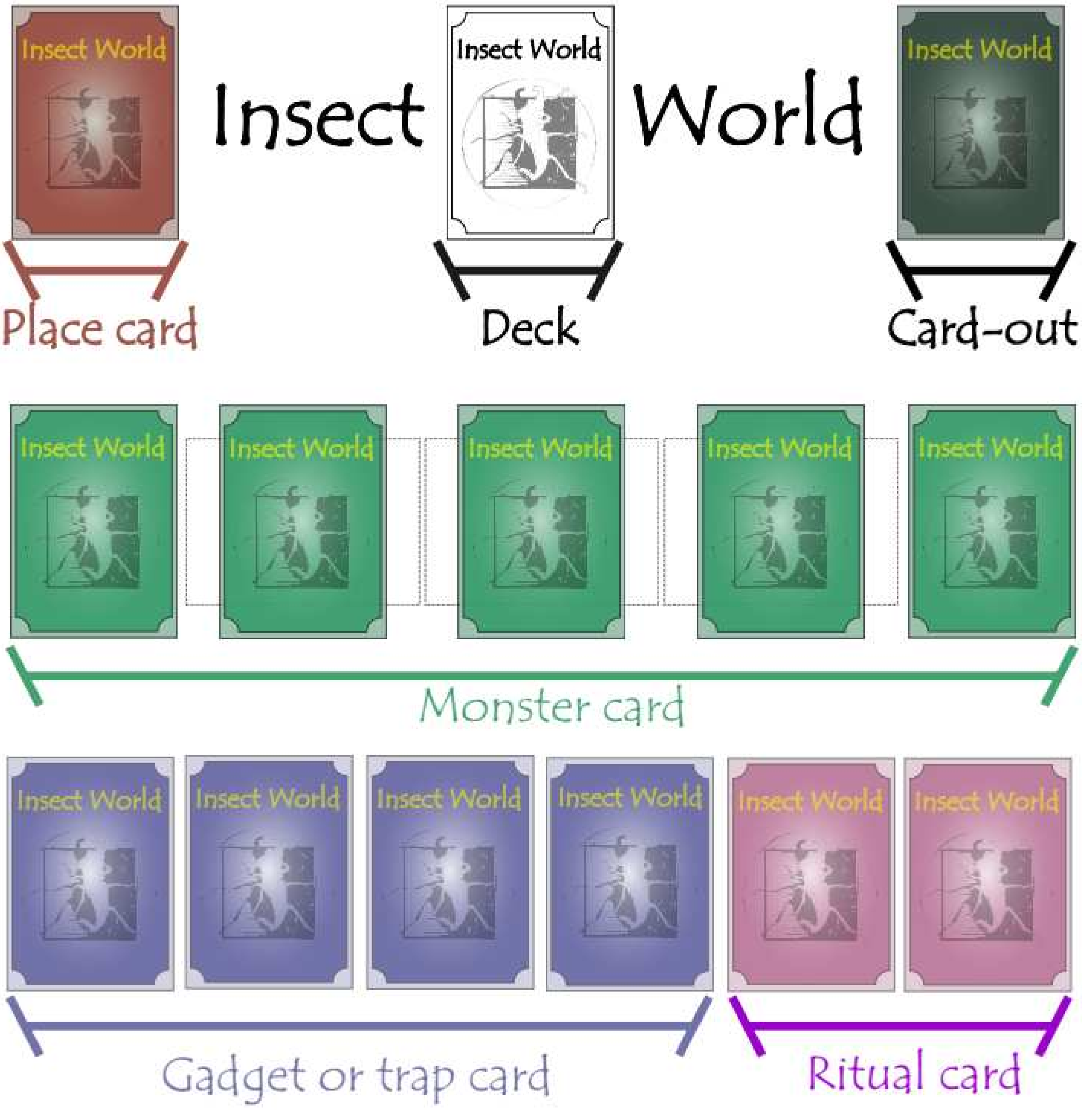
APÊNDICES

Rules of Insect World



Cardboard

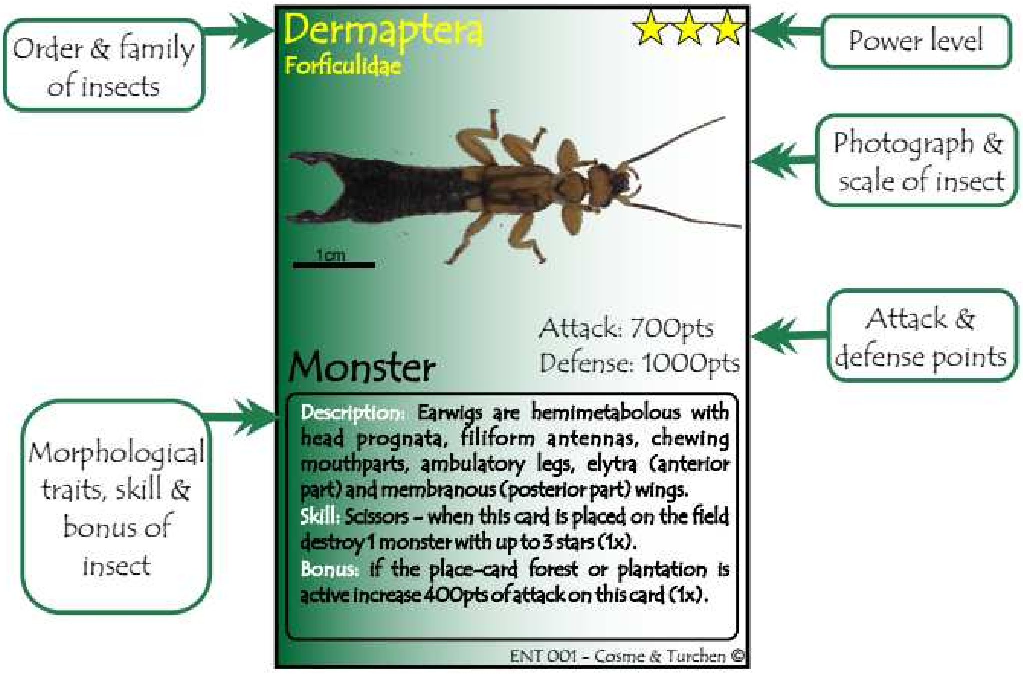
In cardboard each color indicates the position for each card-type, where: 1) red: place-card; 2) white: deck-cards; 3) black: eliminated card; 4) green: monster card (defense or attack); 5) blue: gadget or trap card; 6) purple: rituals-card (see illustration below).



2

Monster cards - green

The monster-cards exhibit a realistic photograph and describe the order and family of the insect, its morphological traits, skills, and power level (see illustration below). The power level of cards can range of one up to six stars.



Description: Earwigs are hemimetabolous with head prognata, filiform antennas, chewing mouthparts, ambulatory legs, elytra (anterior part) and membranous (posterior part) wings.
Skill: Scissors - when this card is placed on the field destroy 1 monster with up to 3 stars (1x).
Bonus: if the place-card forest or plantation is active increase 400pts of attack on this card (1x).

Instructions:

a) Only one monster-card can be inserted by turn, where the monsters with 1-3 stars can be inserted any moment; 4 stars always require a sacrifice of another monster within the board and; 5-6 stars require interaction with a specific ritual card.

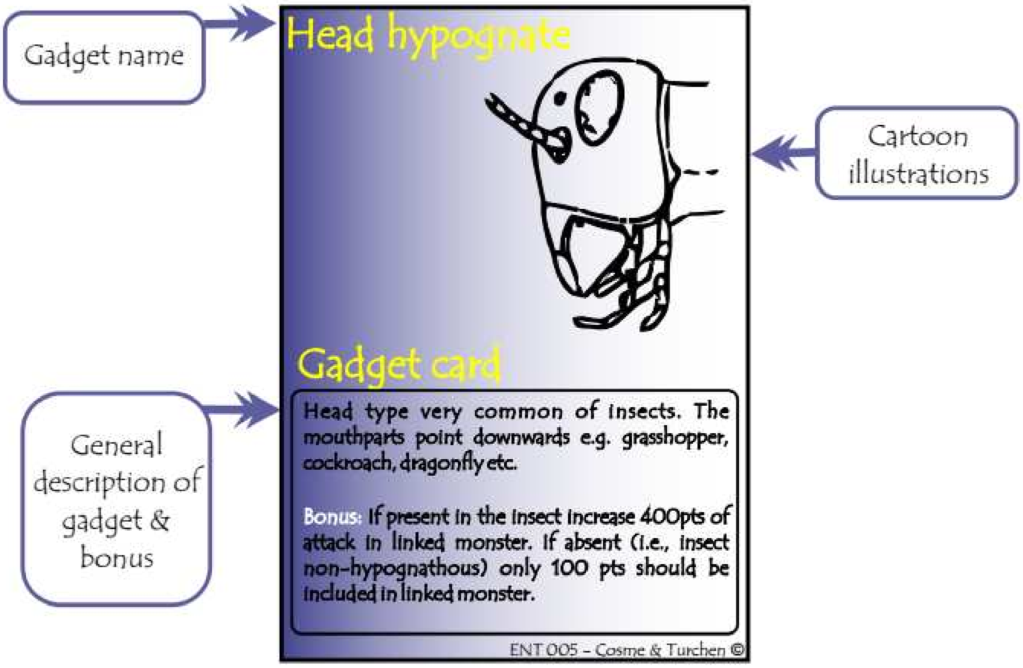
b)The monster card can be inserted in board as an active (front) or inactive (back) mode. Also, may change for vertical (attack) and horizontal (defense) position (see p. 8).

c) Skill and bonus only can be activated in your own turn.

3

Gadget cards - blue

The gadget-cards exhibit an external morphological trait of the insect (e.g., head, legs, wings, and antennae) and offer a supplement that improves given insect skills (i.e., monster-card).



Head type very common of insects. The mouthparts point downwards e.g. grasshopper, cockroach, dragonfly etc.

Bonus: If present in the insect increase 400pts of attack in linked monster. If absent (i.e., insect non-hypognathous) only 100 pts should be included in linked monster.

Instructions:

a) Only one gadget-card can be inserted by turn and linked in one monster. If monster linked is destroyed, the gadget-card is maintained in the board and can be linked in other monsters.

b) Similar morphological structures cannot be linked in the same monster-card.

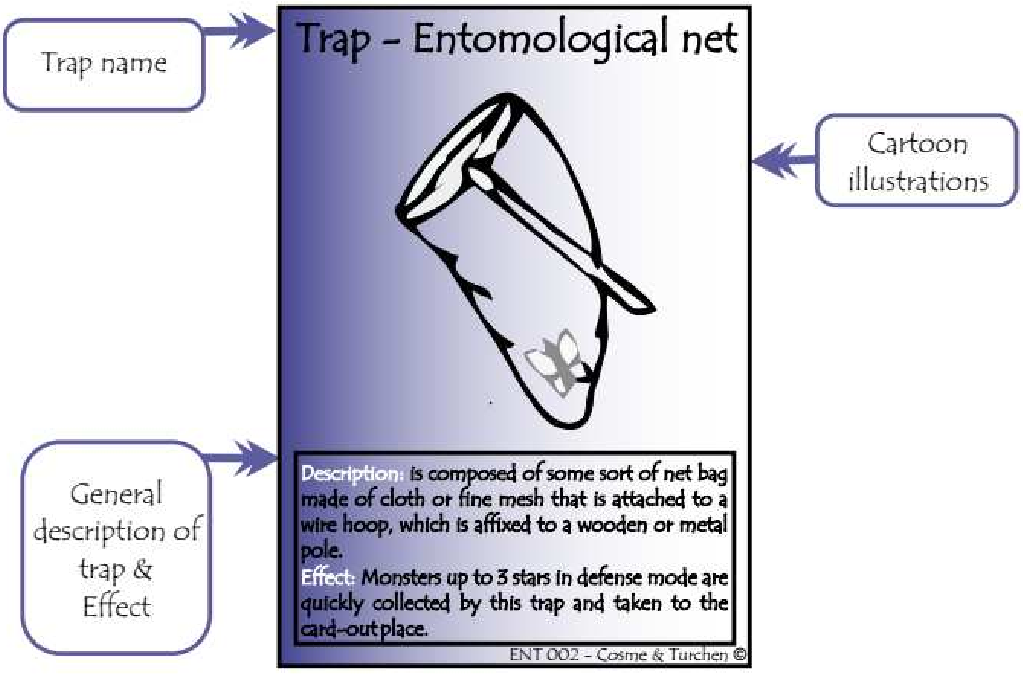
c) The gadget-card can be eliminated (i.e., card-out) at any time.

d) Attention: If the morphological structure will be present in the monster-card, it has more advantages. For instance, the gadget-card (head hypognate) linked with monster-card (grasshopper) plus 400pts of attack in the monster.

4

Trap cards - blue

The trap-cards are entomological instruments (e.g., entomological net, collecting jars) and can be used to destroy, cancel, steal, and block skills from the other cards.



Trap - Entomological net

Description: is composed of some sort of net bag made of cloth or fine mesh that is attached to a wire hoop, which is affixed to a wooden or metal pole.
Effect: Monsters up to 3 stars in defense mode are quickly collected by this trap and taken to the card-out place.

ENT 002 - Cosme & Turchen ©

Instructions:

a) Only one trap card can be inserted by turn. It can be inserted within the board as an active (front) or inactive (back) mode.

b) The card effect can be activated anytime, even throughout opponent turn (surprise effect).

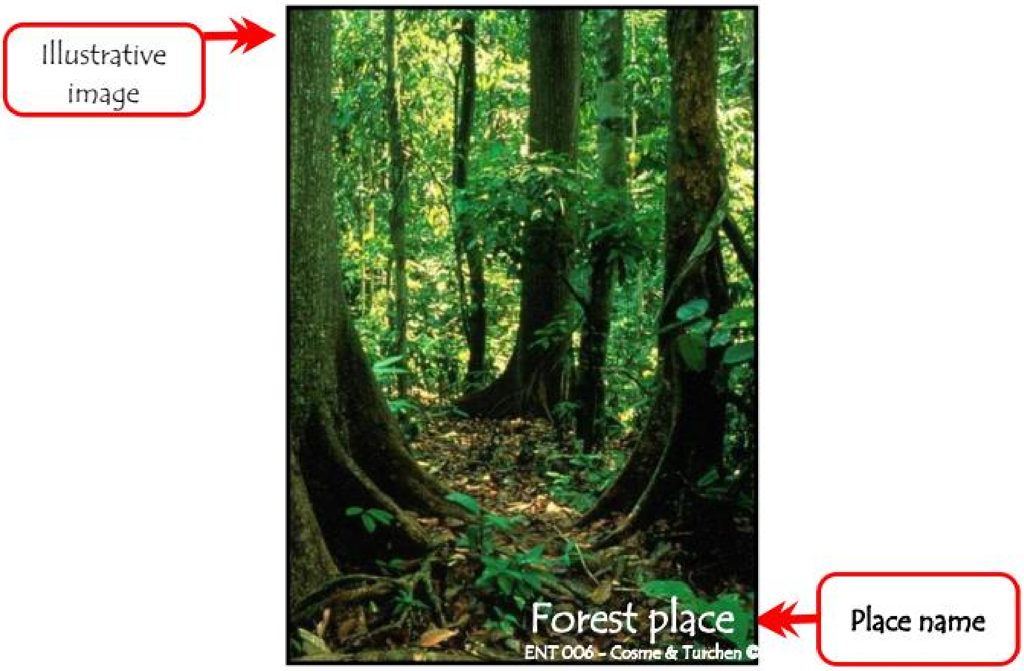
c) The trap card effect is used just one time, after that the card should be eliminated (i.e., card-out).

d)Attention: the player that activates the effect or the skill first has priority.

5

Place cards - red

In the place cards is exhibit the insect habitat (e.g., forest, desert, water, soil, crop-system, country, and others) it provides advantages in the insect skills.



Instructions:

a) Only one place card can be inserted by turn and should be as active (front) mode.

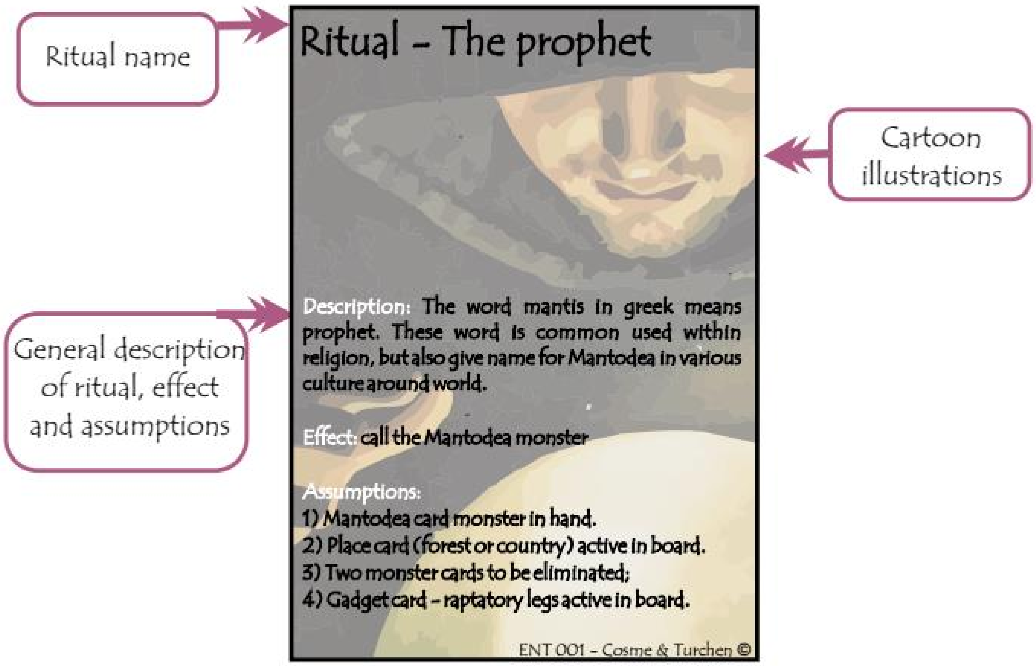
b) The card effect is available just for your own board.

c) The place card can be eliminated (i.e., card-out) anytime.

6

Ritual cards - purple

The ritual cards reports on physiological processes (e.g., respiration, insecticide resistance) that can increase the monster power (attack and defense), invite a new stronger monster, restore life-points or destroy another monster).



Ritual - The prophet

Description: The word mantis in greek means prophet. These word is common used within religion, but also give name for Mantodea in various culture around world.

Effect: call the Mantodea monster

Assumptions:
1) Mantodea card monster in hand.
2) Place card (forest or country) active in board.
3) Two monster cards to be eliminated;
4) Gadget card - raptatory legs active in board.

ENT 001 - Cosme & Turchen ©

Instructions:

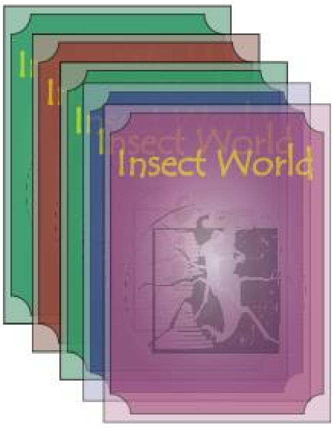
a) Only one ritual card can be inserted by turn. It can be inserted within the board as an active (front) or inactive (back) mode.

b)The ritual card can be eliminated (i.e., card-out) anytime.

c)The card effect only can be activated after the assumptions are attended.

d) Attention: the monsters with five or six stars only can be inserted in board through of specific ritual card.

Build a strategy



The deck (strategy) should contain 30-40 cards, independent of the card-type (i.e., monster, gadget, trap, ritual and, place cards). Each card can be repeated twice, excepted monster card, where a different family should be prefered.

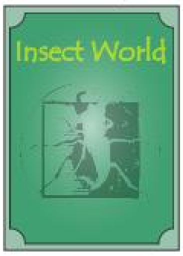
Starting the game

In the first moment, the head and tails are used to decide who will start the game and both players begin with 4.000 life-points. Subsequently, the players are able to purchase cards (5-cards/individual). **Attention:** In the first turn of each player the attack is non-allow, however some skills that reduce life-points can be applied.

Card positions

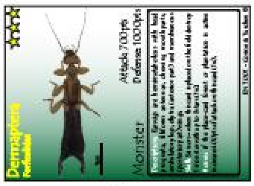
The cards number put down in the board should consider the previous rules, but the card can be inserted (or not) according to player strategy. Besides, the player should choose the card position (see below).

Attack & inaticve mode



Defense & inaticve mode

Attack & active mode



Defense & active mode

Attention: In the inactive mode any skill, bonus or effects are disabled.

The gameplay

The RPG-game require conversation among players, as the actions should be communicated for the opponent (excepted inactive mode) in high and good sound, included the reading of description general and components of cards (points, skills, bonus, effect, etc).

Instruction:

1) The read of cards description and components (active in the board), as well as the link among gadget with monster card specific, are mandatory.

2) In each turn, the player purchases one card on the top of the deck. Attention: the player can have a cards number unlimited in your hand.

3)The rules for put down cards in the board described previously, where each monster (1x), gadget (1x), trap (1x), place (1x) and ritual(1x) cards can be inserted in same turn and with different positions (see p.8).

4) The inactivated trap cards can be activated anytime. However, the player that activates the effect or the skill first has priority.

5) The card position can be changed in your own turn, but no-action (i.e. attack) should be conducted before.

6) The elimination of cards can be conducted in your own turn with different purposes, for instance, attended assumptions from rituals or only card-out.

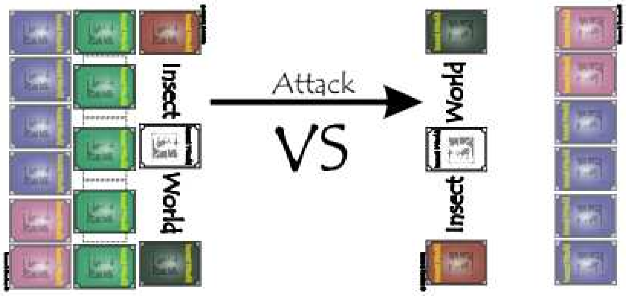
7) Attack (see p.10), skills, effect, and bonus can be used simultaneously in your turn.

8) In the last step, after finished plays, the player record your life-points and communicate for the opponent which finished your turn.

The gameplay - attack & defense

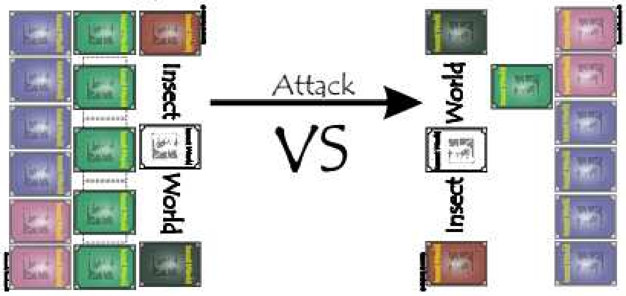
The attack can be conducted against the opponent by all monster present on board. However, the attack is paired, in other words, one monster attack another monster from your choice in the opponent board. There are three context to attack the opponent, see below:

1) the opponent without a monster on board:



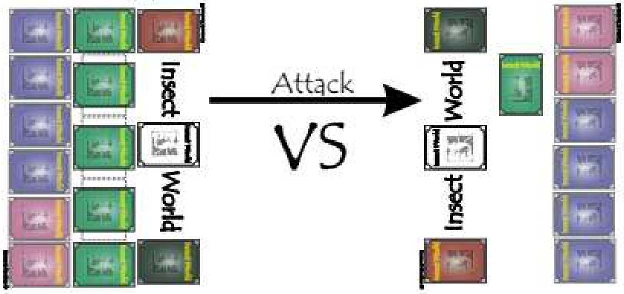
Here, the number of attack points from attacking monster is discounted in life-points of the opponent.

2) the opponent with a monster in attack position



Here, the attack points are subtracted among monster, where the card with low power is destroyed and overplus points are discounted in the life-points of the opponent. If the points are equal both monsters are destroyed.

3) the opponent with a monster in defense position



Here, the attack points are compared with defense points. If attack points higher than defense points - the defending monster is destroyed, but the opponent player do not loser life-points.

If attack points lower than defense points - the attacking monster is destroyed and player (on turn) loser life-points. If attack and defense points are equal, both monsters are destroyed and the players do not loser life-points.

The winner

The game winner will need to suppress all life-points of the opponent or finish with the opponent's cards.

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