SELEÇÃO DE RECURSOS ALIMENTARES EM UM CUPIM NASUTO: A IMPORTÂNCIA DA INFORMAÇÃO SOCIAL E O DILEMA ENTRE CRESCIMENTO E SOBREVIVÊNCIA DA COLÔNIA

por

ALINE DO NASCIMENTO FILGUEIRA SILVA

(Sob Orientação do Professor Paulo Fellipe Cristaldo - UFRPE)

RESUMO

A aquisição de recursos alimentares é um dos comportamentos mais importantes na história de vida dos organismos. Apesar de consistir em uma necessidade básica e aparentemente trivial, a seleção de recursos não é tão simples assim uma vez que os animais vivem em ambientes heterogêneos com recursos distribuídos de maneira irregular, variando em quantidade e qualidade ao longo do tempo. Além disso, a presença de espécies competidoras e predadoras pode impactar significativamente a utilização dos recursos. Sendo assim, os indivíduos precisam avaliar de forma eficiente os custos e benefícios envolvidos na exploração e seleção de um recurso. Para tomar decisões durante a seleção de recurso, os animais precisam adquirir informações de seu ambiente. Essas informações podem ser obtidas por meio de interações diretas com o meio ('*informações privadas*') ou mesmo observando outros indivíduos (da mesma espécie ou de espécies diferentes) e/ou suas pistas deixadas no meio ('*informações sociais*'). Os cupins (Blattodea: Isoptera) são insetos detritívoros que apresentam grande importância econômica e ecológica. No entanto, ainda não são totalmente conhecidos os fatores envolvidos na seleção de recursos por insetos. Com o intuito de preencher parte dessa lacuna, o objetivo dessa dissertação foi analisar o papel da informação social e da combinação de disponibilidade de recursos e sinais

de risco durante a seleção de recursos em *Nasutitermes corniger* (Motschulsky) (Termitidae: Nasutitermitinae). De um modo geral, nossos resultados indicam que a quantidade de recurso parece ser o fator determinante da seleção de recursos em *N. corniger*. O papel da informação social e dos sinais de risco parecem depender, em parte, da quantidade de recurso durante o processo de seleção de recursos. Assim, o presente trabalho contribui com novos conhecimentos a respeito da seleção de recursos em cupins e pode auxiliar no entendimento do uso do habitat por esse importante grupo de insetos.

PALAVRAS-CHAVE: Uso do habitat, forrageamento, cupins.

SELECTION OF FOOD RESOURCES IN A NASUTO TERMITE: THE IMPORTANCE OF

SOCIAL INFORMATION AND THE DILEMMA BETWEEN COLONY GROWTH AND

SURVIVAL

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ABSTRACT

The acquisition of food resources is one of the most important behaviors in the life history of organisms. Despite being a basic and seemingly trivial need, resource selection is not that simple since animals live in heterogeneous environments with unevenly distributed resources, varying in quantity and quality over time. Furthermore, the presence of competing and predatory species can significantly impact the resource use. Therefore, individuals need to efficiently assess the costs and benefits involved in exploring and selecting a resource. To make decisions during resource selection, animals need to acquire information from their environment. This information can be obtained through direct interactions with the environment (*i.e.,* private information) or even by observing other individuals (of the same or different species) and/or its cues left in the environment (*i.e.,* social information). Termites (Blattodea: Isoptera) are detritivores insects that have great economic and ecological importance. However, the factors involved in the selection of resources for these insects are not completely known. So, the objective of dissertation was to analyze the role of social information and the combination of resource quantity and risk signals during resource selection in *Nasutitermes corniger* (Motschulsky) (Termitidae: Nasutitermitinae). Our results indicate that resource quantity seems to be the determining factor in resource selection

in *N. corniger*. The role of social information and risk signals appears to depend, in part, on the quantity of resource during resource selection. This study contributes with new knowledge about the selection of resources in termites and can help to understand the habitat use by this important group of insects.

KEY WORDS: Habitat use, foraging, termites.

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Dissertação apresentada ao Programa de Pós-Graduação em Entomologia, da Universidade Federal Rural de Pernambuco, como parte dos requisitos para obtenção do grau de Mestre em Entomologia.

RECIFE - PE

Julho - 2021

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ALINE DO NASCIMENTO FILGUEIRA SILVA

Comitê de Orientação:

Paulo Fellipe Cristaldo – UFRPE

Ana Paula Albano Araújo - UFS

Carla Cristina Marques Arce - UniNe

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Banca Examinadora:
Paulo Fellipe Cristaldo - UFRPE
Ricardo Ildefonso de Campos - UFV
Fabiana Elaine Casarin dos Santos - UNIFESP

Aline Nascimento Filgueira Silva	Prof. Paulo F. Cristaldo – UFRPE
Mestre em Entomologia	Orientador

DEDICATÓRIA

A minha mãe, Célia Maria, por todo amor e dedicação durante minha formação. A minha avó Joana Firmino (*in memorian*) quem sempre sonhou o melhor para mim. A meus irmãos, Arcênia e Anderson por todo amor e incentivo durante essa caminhada. Ao meu namorado, Lucas Henrique, por todo amor, por acreditar em mim e me incentivar a nunca desistir.

AGRADECIMENTOS

A Deus por me permitir concluir mais uma etapa importante da minha vida, me proporcionando determinação, fé e resiliência por toda minha vida.

Agradeço a Universidade Federal Rural de Pernambuco (UFRPE) e ao Programa de Pós-graduação em Entomologia (PPGE) pela oportunidade concedida para realização do curso de mestrado e pelo suporte para o desenvolvimento do projeto.

A Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE), pela concessão da bolsa de estudo.

Ao Prof. Dr. Paulo F. Cristaldo, por sua orientação, atenção e dedicação para o meu crescimento acadêmico.

Aos membros da banca examinadora: Ricardo I. de Campos e Fabiana E. C. dos Santos, por aceitarem o convite, assim como pelas sugestões para o aperfeiçoamento desse trabalho.

Aos amigos do Laboratório de Insetos Sinantrópicos, em especial à Cátila e Renan, por toda amizade e auxílio na realização dos experimentos.

A minha família por acreditarem em mim, por todo incentivo e apoio prestado durante o curso de mestrado.

CAPÍTULO 1

INTRODUÇÃO GERAL

A aquisição de recursos alimentares é um dos comportamentos mais importantes na história de vida dos organismos. A taxa de entrada de energia para qualquer animal depende basicamente dos recursos alimentares selecionados durante o forrageamento (Boggs 1992; Boggs 2009); o que consequentemente irá afetar a energia alocada para a sobrevivência (crescimento, manutenção, defesa) e reprodução (Stearns 1989). Apesar de consistir em uma necessidade básica e aparentemente trivial, a seleção e aquisição de recursos não é tão simples assim uma vez que os animais vivem em ambientes heterogêneos, nos quais os recursos geralmente estão distribuídos de maneira irregular, variando em quantidade e qualidade ao longo do tempo. Além disso, a presença de espécies competidoras e predadoras pode impactar de maneira significativa a utilização dos recursos (Hassell & Southwood 1978). Todos esses fatores irão influenciar o balanço entre benefícios e custos durante a seleção de recursos alimentares pelos animais. Sendo assim, espera-se que os indivíduos capazes de discriminar a quantidade e/ou qualidade dos recursos bem como as informações sobre presença de competidores e/ou predadores possam ter vantagens por maximizarem sua sobrevivência e capacidade reprodutiva. Para isso, os indivíduos precisam avaliar de forma eficiente os custos e benefícios envolvidos na exploração e seleção de um recurso.

Para tomar essas decisões, os animais precisam adquirir informações acuradas do seu ambiente. Essas informações podem ser adquiridas diretamente ao interagir e avaliar o alimento ('*informação privada*') ou ainda através da interação com outros organismos presentes no ambiente ('*informação social*') (Valone 2007). A interação com outros indivíduos permite o recebimento de '*informações públicas*' sobre a adequabilidade do recurso através da observação

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da decisão comportamental e/ou de pistas deixadas no ambiente por outros indivíduos (Valone 1989; Valone & Templeton 2002; Danchin *et al.* 2004). O uso de informação social reduz os custos energéticos de avaliação prévia do recurso (Danchin *et al.* 2004). Dessa forma, os organismos ao perceber a informação deixada por outros indivíduos podem comparar com as informações prévias estimando a adequabilidade do recurso e, então, tomar decisão para seu uso (Valone 2007). Além disso, uma série de habilidades sensoriais e estratégias comportamentais permite que os animais minimize os custos envolvidos durante a seleção e aquisição de recursos alimentares (Alcock, 2011). Em insetos sociais (formigas, abelhas, vespas e cupins), por exemplo, uma gama de comportamentos sofisticados e um elaborado sistema de comunicação garante uma eficiente busca por recurso com uma redução nos custos e tempo envolvidos nesse processo (Krebs & Davies, 1993).

Os cupins (Blattodea: Isoptera) são considerados um dos mais importantes macrodetritívoros de florestas tropicais devido a sua participação ativa na decomposição e ciclagem de nutrientes, contribuindo assim para o fluxo de energia e matéria nos ecossistemas (DeSouza & Cancello, 2010, Jouquet *et al.*, 2011). Esses insetos também apresentam grande importância econômica devido aos danos causados em ambientes agrícolas e urbanos (Constantino 2002). Os cupins são insetos eusociais que vivem em colônias que abrigam um grande número de indivíduos estéreis que auxiliam os reprodutores (Costa-Leonardo 2002). Grande parte da importância ecológica e econômica desse grupo de insetos deve-se ao seu hábito alimentar. Esses insetos se alimentam de celulose em diferentes estágios de humificação (de madeira seca à solo) (Donovan *et al.* 2001). Apesar de ser um dos compostos orgânicos mais abundante no planeta Terra, poucos são os organismos capazes de utilizar matéria orgânica morta como fonte de alimento. A capacidade dos cupins em utilizar celulose deve-se à associação com microorganismos (protozoa e bactérias)

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(Costa-Leonardo 2002) ou pela produção própria de enzimas celulolíticas (Warnecke *et al.* 2007, Lima *et al.* 2014).

A busca por recursos em cupins é mediado pelos feromônios de trilha que são produzidos e liberados pela glândula esternal (Cristaldo 2018). O comportamento de forrageamento ocorre de maneira coletiva com a participação dos operários e, em algumas espécies, os soldados também participam ativamente nesse processo (ver Almeida *et al.* 2016, Casarin *et al.* 2008, Haifig *et al.* 2015, Traniello & Busher, 1985, Sacramento *et al.* 2020). Embora sejam comumente conhecidos pelo seu potencial de causar danos, os cupins são seletivos quanto ao recurso a ser utilizado. Estudos anteriores já demonstraram que cupins preferem itens em maior quantidade (DeSouza *et al.* 2009, Evans & Gleeson 2006), com maior qualidade nutricional (Araújo *et al.* 2011, Higashi *et al.* 1992), em alta densidade (Almeida *et al.* 2018) e que possua baixo risco de predação (DeSouza *et al.* 2009). No entanto, o papel da informação social bem como da combinação entre quantidade de recurso e sinais de sinais de risco durante seleção de recursos em cupins ainda não foram estudado.

Sendo assim, o objetivo dessa dissertação foi analisar o papel da informação social e da combinação de quantidade de recurso e sinais de risco durante a seleção de recursos em *Nasutitermes corniger* (Motschulsky) (Termitidae: Nasutitermitinae). A dissertação foi dividida em quatro capítulos. No capítulo 1 é apresentado a introdução geral, no qual fornecemos ao leitor uma abordagem geral sobre a temática e o objetivo geral do estudo. O capítulo 2 consiste no artigo "**Resource selection in nasute termite: the role of social information**" publicado na revista *Ethology,* no qual mostramos o papel da informação social durante a seleção de recursos em *N. corniger*. Já o capítulo 3, apresenta o artigo "**Food quantity is more important than levels of risk during resource selection in higher termite species**", que analisa o efeito da combinação de

quantidade de recurso e níveis de risco na seleção de recurso em *N. corniger*. Por fim, o capítulo 4

resume as considerações finais desse trabalho.

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CAPÍTULO 2

RESOURCE SELECTION IN NASUTE TERMITE: THE ROLE OF SOCIAL

INFORMATION[1]

ALINE NASCIMENTO FILGUEIRA SILVA[1,2], CÁTILA R. SILVA[1], RENAN E. C. SANTOS[1], CARLA C.

M. ARCE[3], ANA PAULA A. ARAÚJO[4] & PAULO FELLIPE CRISTALDO[1,2]

1. Synanthropic Insect Laboratory, Department of Agronomy, Federal Rural University of

Pernambuco, Recife, Brazil.

2. Post Graduate Program in Entomology, Department of Agronomy, Federal Rural University

of Pernambuco, Recife, Brazil.

3. Laboratory of Fundamental and Applied Research in Chemical Ecology, University of

Neuchâtel, Neuchâtel, Switzerland.

4. Ecological Interactions Laboratory, Post Graduate Program in Ecology and Conservation,

Federal University of Sergipe, São Cristóvão, Brazil.

ABSTRACT **-**. During foraging, organisms need to assess the costs and benefits related to resources wanted. An energy-efficient way of locating and decide among new food sources is to use cues left by other organisms in the environment (*i.e.*, social information). In the present study, manipulative bioassays were conducted to evaluate the role of intra- and interspecific chemical cues in the selection of food resource by *Nasutitermes corniger* (Termitidae: Nasutitermitinae). For this, we tested the hypothesis that individuals of *N. corniger* are able to perceive and use chemical cues by either colonies of the same species or from different species. Linear trail bioassays were conducted to verify whether *N. corniger* workers perceive and accept intra- and interspecific chemical cues. Then, resource bioassays were performed with a binary and multiple choices to evaluate whether the termite group's decision of food resources was based on intra-and/or interspecific chemical cues. In general, groups of *N. corniger* preferred baits with chemical cues of others intraspecific colonies or with undetectable cues (hexane solvent or interspecific cues) than those with their own chemical signals. These results suggest that intraspecific chemical cues seem to modulate the food resource selection in *N. corniger*, which may help researchers to better understand the use of habitat by termites.

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SELEÇÃO DE RECURSOS EM CUPIM NASUTO: O PAPEL DA INFORMAÇÃÕ SOCIAL

RESUMO – Durante o forrageamento, os organismos precisam avaliar os custos e benefícios relacionados aos recursos desejados. Uma forma eficiente, em termos de energia, de localizar e decidir entre novas fontes de alimentos é utilizar pistas deixadas por outros organismos no ambiente (*i.e.* informações sociais). No presente estudo, bioensaios manipulativos foram realizados para avaliar o papel de pistas químicas intra- e interespecíficas na seleção de recursos alimentares por *Nasutitermes corniger* (Termitidae: Nasutitermitinae). Para isso, testamos a hipótese de que indivíduos de *N. corniger* são capazes de perceber e utilizar pistas químicas de colônias da mesma espécie ou de espécies diferentes. Bioensaios de trilha linear foram conduzidos para verificar se os operários de *N. corniger* percebem e aceitam pistas químicas intra- e interespecíficas. Em seguida, bioensaios de seleção de recursos com escolha binária e múltipla foram realizados para avaliar se a decisão do grupo de cupins pelos recursos alimentares foi baseada nas pistas químicas intra- e/ou interespecíficas. Em geral, grupos de *N. corniger* preferiram iscas com pistas químicas de outras colônias intraespecíficas ou com pistas indetectáveis (solvente hexano ou pistas interespecíficas) do que aquelas com seus próprios sinais químicos. Esses resultados sugerem que pistas químicas intraespecíficas parecem modular a seleção de recursos alimentares em *N. corniger*, o que pode ajudar a compreender melhor o uso do habitat pelos cupins.

PALAVRAS-CHAVE: Comportamento, pistas químicas, forrageamento, uso de habitat, *Nasutitermes*

Introduction

The acquisition of resources is one of the main behaviors involved in the survival and reproduction success of organisms (Boggs 2009, van Noordwijk & de Jong 1986). However, animals live in heterogeneous environments in which resources are generally unevenly distributed, varying in size and quality over time. In addition, the presence of competing and natural enemy species can significantly impact the use of resources (Hassell & Southwood 1978). Faced with these variations in the spatiotemporal characteristics of resources (*e.g.* quantity, quality and suitability), individuals need to efficiently assess the costs and benefits involved in exploring and selecting a resource. To make these decisions, animals need to acquire relevant information from their environment, which can be obtained through their direct interactions with the environment (*i.e.* private information) or even by observing other individuals (from the same or different species) and/or their cues left in the environment (*i.e.* social information) (Leadbeater & Chittka 2009, Valone 2007).

Scouting for new food sources can take a long time or even not be well successfully. On the other hand, the use of social information (*e.g.* chemical cues left by other individuals) can be a more reliable and effective alternative to resource selection (Grüter & Leadbeater 2014). Despite looking like an economically energetic alternative, in some contexts the social information can either be dishonest or trigger conflict with individuals that already exploit the resource (Lee *et al.* 2016). Additionally, animals may also face situations during decision-making in which the social information obtained conflicts with their own private information (Kendal *et al.* 2005, Yfke *et al.* 2004).

Social insects are good models for studies on the use of social information during resource selection. Most of social insects are central-place foragers, which limits colonies from altering their foraging areas when local conditions are unfavorable. Therefore, being guided by cues or

signals left by other individuals can help reduce costs related to foraging. In the last years, a series of studies have been showed the use of social information during the exploitation and selection of resources in eusocial insects (Czaczkes *et al.* 2015), mainly in ants (*e.g.* Kolay *et al.* 2020, Stroeymeyt *et al.* 2017) and bees (*e.g.* Dunlap *et al.* 2016, Grüter & Farina 2009, Wray *et al.* 2011). For termites, however, studies on the use of social information during resource selection are still scarce.

Termites (Blattodea: Isoptera) are eusocial insects that feed on dead organic matter at different stages of humification (from dry wood to soil) (Davies 2002, Donovan *et al.* 2001). The foraging process in termites is mediated by trail-following pheromone produced and released by the sternal gland (Cristaldo 2018, Sillam-Dussès 2010). Although termites consume an abundant resource in the environment, they are selective about the use of the resource in the field (Araújo *et al.* 2017, DeSouza *et al.* 2009, Evans *et al.* 2005). Thus, the search for food resources is a costly process for these insects that need to invest in tunnels and galleries to explore the environment (for a comprehensive historical account, see Almeida *et al.* 2018, Araújo *et al.* 2011, Araújo *et al.* 2017, Cristaldo *et al.* 2016a). In addition, the perception and response to chemical cues in Nasutitermitinae species are preferably targeted to colonies that have the high availability of resources (Cristaldo *et al.* 2016a). In *Trinervitermes bettonianus*, choice tests have been demonstrated that workers were able to follow natural trails and glandular extract trails from different species with the same degree of motivation as their own trails (Ollo & McDowell 1982). Workers of *Inquilinitermes microcerus* were also reported to follow glandular extract trails from *Constrictotermes cyphergaster* (Cristaldo *et al.* 2014). Therefore, termites might use chemical cues left by other colonies and species to optimize their search for resources.

In the present study, we evaluated the use of social information during resource selection by *Nasutitermes corniger* (Motschulsky) (Termitidae, Nasutitermitinae). *Nasutitermes* are considered

one of the most derived genera within Termitoidea (Isoptera) (Legendre *et al.* 2008). Colonies of *Nasutitermes* species inhabit almost all type of vegetation and specialize in a wide range of resources (Boulogne *et al.* 2017). *N. corniger* is widely distributed in the Neotropical region (Boulogne *et al.* 2017, Mathews 1977). Specifically, we test the hypothesis that *N. corniger* foragers can perceive and use chemical cues by either colonies of the same species (intraspecific) or from different species (interspecific) during resource selection. Our results contribute to the understanding the mechanisms responsible for resource selection and habitat use by termites.

Material and Methods

Ethics Statement and Species Identification. Permits for termite collection were issued by ICMBio – IBAMA (#47652). *Nasutitermes corniger* and *Coptotermes gestroi* were individually identified and the voucher specimens are deposited in the collection of the Synanthropic Insects Laboratory at the Federal Rural University of Pernambuco (UFRPE).

Study Area and Maintenance of Termite Colonies. Termite colonies were collected at *campus* SEDE of UFRPE (8º04'03''S, 34º55'00''W), in Recife, Pernambuco, Brazil. The climate in the region is characterized as 'subtropical humid or sub-humid' (Aw) with average annual temperature of 25.8 ºC and average annual precipitation of 1804 mm (Climate, 2019).

Nests with active colonies of *N. corniger* were completely removed from tree and taken to the Laboratory of Synanthropic Insects at UFRPE to be used for the preparation of intraspecific chemical cues extracts and conduction of bioassays. In the laboratory, colonies were placed separately in plastic boxes with moistened cotton and sugarcane baits were offered as food resource. *N. corniger* colonies were maintained in the laboratory for at least 15 days prior the bioassays.

In order to have interspecific chemical cues extracts, three colonies of *C. gestroi* were collected in wood fragments and taken to the laboratory, where they were stored in plastic pots until the preparation of extracts. This species was chosen to be used as interspecific because in the sampling area it is common to find both *N. corniger* and *C. gestroi* foraging in the same food source.

Preparation of Intra- and Interspecific Chemical Cues Extracts. Intra- and interspecific chemical cues extracts were prepared following procedures described by Cristaldo *et al.* (2014) Cristaldo *et al.* (2016a). Briefly, each sample (individual colony) containing 100 workers were immersed in 1 mL of hexane and stored for 24 h. at 4 °C for the whole-body extracts. After this, the supernatant was collected and transferred to another vial. A second immersion was performed with approximately 100 µl of hexane at room temperature for 10 min. Both supernatants were combined. Subsequently, the volume of the extract was reduced, and it was calculated the equivalent *per* worker to be used in the bioassays (µl of extract equivalent to one worker). The final extracts were stored at -18 °C for further use in the bioassays. Intraspecific extracts were collected from *N. corniger* colonies (*N*=8) and interspecific cues from *C. gestroi* colonies (*N*=3). Each colony corresponds to one replicate.

Experimental design

Perception and Acceptance of Intra- and Interspecific Chemical Cues. Linear trail-following bioassays were conducted in order to check the perception and acceptance of intra- and interspecific chemical cues extracts by *N. corniger*. To this end, linear trails were drawn on filter paper according to Cristaldo *et al.* (2014) and Cristaldo *et al.* (2016a). For this, two 6-cm trails were laid down from opposite sides of a line. When meeting, such trails would overlap for 2 cm,

forming a 10-cm long trail (Fig. 1A). The first six centimeters consisted of the chemical signal of tested colony ("own signal") and the other six centimeters consisted of chemical cues of another colony (intra- or interspecific cues). Thus, there was an overlap of two tested odors (2 cm) in the central part of the trail (Fig. 1A). Control consisted of a 10 cm trail with only the chemical signal of the tested colony. After the evaporation of solvent for two min., a group of five individuals (four workers and one soldier) was placed in the release chamber at the base of the linear trail containing the own colony signal and the distance followed on this trail by the first worker was recorded. A total of three repetitions were performed for each combination of chemical cues (own colony signal [OC], intra- [DC] and interspecific cues [DS]) *x* colony (*N*= 6), totalizing 54 bioassays. Individuals were used only once in each repetition and they were randomly selected from each colony.

Selection of Resources with Intra- and Interspecific Cues. Choice bioassays (binary and multiple choices) were performed in order to check the effects of intra- and interspecific cues on selection of food resource by *N. corniger* termite groups.

Bioassays with binary choice were conducted in arenas consisted of a central plastic pot (250 ml) connected in a straight line with a transparent hole (7 mm diameter *x* 5 cm of length) to two other plastic pots (250 ml) at its end containing treatments (26 g of sugar cane with 10 µl of chemical cues extracts) (Fig. 1B). Bioassays with multiple choices were conducted in arenas consisted of a central plastic pot (250 ml) connected with a transparent hole (7 mm diameter *x* 5 cm of length) to four other plastic pots (250 ml) at its end containing treatments (26 g of sugar cane with 10 µl of chemical cues extracts) (Fig. 1C). Chemical extracts were dripped on top of baits using a microsyringe (Hamilton®). In each one of the pots, 22 g of the nest wall from colony tested were macerated and used as substrate. A group of 10 individuals (eight workers and two

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soldiers) was introduced in the central pot. Individuals were used only once in each repetition and they were randomly selected from each colony.

Bioassays with binary choice consisted of choosing termite groups for sugarcane baits with the following treatments (Fig. 1B): (1) blank control (bait without solvent [BC] x empty arena [EA]); (2) hexane solvent control (bait without solvent [BC] x bait with solvent [SC]); (3) signal control (bait with solvent [SC] x bait with chemical signal extracts of own tested colony [OC]); (4) intraspecific chemical cues (bait with solvent [SC] x bait with chemical cues extracts of different colony from same species [DC]); (5) interspecific chemical cues (bait with solvent [SC] x bait with chemical cues extracts from different species [DS]); (6) signal control x intraspecific chemical cues (bait with chemical signals extracts of tested colony [OC] x bait with chemical cues extracts of different colony from same species [DC]); (7) signal control x interspecific chemical cues (bait with chemical signals extracts of tested colony [OC] x bait with chemical cues extracts from different species [DS]); and (8) intraspecific chemical cues x interspecific chemical cues (bait with chemical cues extracts of different colony from same species [DC] x bait with chemical cues extracts from different species [DS]). A total of three repetitions were performed for each combination of treatments x colony ($N= 8$), totalizing 192 bioassays.

Bioassays with multiple choices consisted of choosing of termite groups for sugarcane baits with the following treatments (Fig. 1C): (1) solvent control (bait with solvent [SC]); (2) signal control (bait with chemical signals extracts of tested colony [OC]); (3) intraspecific chemical cues (bait with chemical cues extracts of different colony from same species [DC]); and (4) interspecific chemical cues (bait with chemical cues extracts from different species [DS]). Baits were placed in randomized order for each repetition. A total of three repetitions were performed for each colony ($N= 8$), totalizing 24 bioassays.

In both bioassays, the choice of the group for one of the treatments was recorded over 24 hours. In the first hour of bioassays, evaluations were performed every 10 min, then at intervals of 1 h until 6 h of bioassays, and finally at intervals of 6 h until 24 h. The choice of the group for one of the treatments was considered when five or more individuals were present in one of the arms containing baits.

Data analysis. All statistical analyses were carried out in R statistical software (R Development Core Team 2019) using Generalized Linear Models (GLMs) followed by residual analysis to check model assumption and the suitability of error distribution used in the models. In all models, the average value of each tested colony in each treatment was used as true repetition.

To check whether the distance followed in the linear trail (*y-var*) was affected by intra- and interspecific chemical cues (*x-var*), data were submitted to Analysis of Deviance (ANODEV, a maximum likelihood equivalent of ANOVA) with Poisson's error distribution corrected for overdispersion. Contrast Analysis was performed to check difference among treatments.

To test whether intra- and interspecific cues in the sugarcane bait (*x-var*) affect the resource selection by *N. corniger* groups (*y-var*) in the bioassays with binary and multiple chance of choice, data were submitted to ANODEV with Binomial error distribution corrected for overdispersion. The response variable (*y*) was considered as the choice of the group / total number of repetitions. An independent analysis was performed for each combination (bioassays).

Results

Perception and acceptance of intra- and interspecific chemical cues. The distance followed by *N. corniger* workers in the linear trails was affected by the origin of chemical cues (ANODEV, $F_{2,15}=4.80$, $P=0.02$; Fig. 2). *N. corniger* workers followed a greater distance on trails with signals

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from their own colony (OC; control) and on trails with intraspecific chemical cues (DC) compared to trails with interspecific chemical cues (DS) (*P*= 0.01) (Fig. 2).

Selection of resources with intra- and interspecific chemical cues. In general, the resource selection response by *N. corniger* groups was significantly affected by the origin of chemical cues tested (Tab. 01).

In the binary choice experiments, *N. corniger* groups perceived the resource choosing more sugarcane baits (BC; blank control) than empty arenas (EA) (GLM, *P*= 0.003; Tab. 01; Fig. 3A). Termites did not differ in their choice between sugarcane baits with (SC; solvent control) and without solvent (BC; blank control) (GLM, *P*= 0.78; Table 1; Fig. 3A). When *N. corniger* groups were exposed to treatments *versus* solvent control (SC), *N. corniger* groups avoided the signals from their own colony (OC) in relation to baits with only hexane (SC; solvent control) (GLM, *P* < 0.0001; Table 1; Fig. 3A), but they did not avoid sugarcane baits with intra- (DC) and interspecific chemical cues (DS) (Fig. 3A). Termite groups preferred sugarcane with intraspecific chemical cues (DC) than sugarcane with only hexane (SC; solvent control) (GLM, *P*= 0.01; Table 1; Fig. 3A). *N. corniger* groups exposed to interspecific chemical cues (DS) did not differ in their choice between baits containing interspecific chemical cues (DS) or baits with only hexane (SC; solvent control) (GLM, *p*= 0.51; Tab. 01; Fig. 3A).

In the binary choice experiments between treatments (OC *x* DC; OC *x* DS and DC *x* DS), there were no differences in the choice of termite groups between sugarcane baits with chemical signals from their own colony (OC) and those with intraspecific chemical cues (DC) (GLM, *P*= 0.53; Table 1; Fig. 3B). However, they preferred baits with interspecific chemical cues (DS) compared to baits with chemical signals from their own colony (OC) (GLM, *P* = 0.01; Table 1;

Fig. 3B). *N. corniger* groups were also more attracted to sugar-cane baits with intraspecific cues (DC) compared to interspecific cues (DS) (GLM, *P*= .0001; Table 1; Fig. 3B).

In the multiple choices experiments, *N. corniger* groups preferred sugarcane baits with intraspecific chemical cues (DC), followed by DS (interspecific cues) and SC (solvent control) (GLM, *P*= 0.03; Fig. 4). The baits with the own chemical signals (OC) showed the lower preferred resource by termites' groups.

Discussion

In general, our results indicate that social information has an important role in the selection of food resource by *N. corniger*. Although *N. corniger* workers followed a longer distance on intraspecific cues (from the same or different colonies) compared to interspecific ones (Fig. 2), termite groups were more likely to choose resources with or without chemical cues than resources with chemical signals from their own colonies (Fig. 3 and 4).

A range of studies have been shown the ability of animals to use social information to obtain benefits, such as access to food resources or information about local risk (*i.e.* presence of predators and competitors) (reviewed by Valone, 2007). For termites, the use of social information through chemical cues to obtain benefits has already been shown for inquiline species that coexist with host colonies (Cristaldo *et al.* 2014, 2016b), among neighboring colonies from the same species (Cristaldo *et al.* 2016a) and to have access to the presence of competitors (Evans *et al.* 2009; Ferreira *et al.* 2018a). However, to the best of our knowledge, no study had investigated the use of social information during decision-making in the resource selection by termite species.

Nasutitermes corniger were able to perceive and follow chemical cues of different intraspecific colonies (DC) as their own chemicals (OC); however, chemical cues from colonies

of different species (DS) were not perceived or identify as danger-free cues (Fig. 2). In fact, in the binary and multiple-choice bioassays, *C. gestroi* chemical cues (DS) did not differ from the solvent control (SC) (Fig. 3a and Fig. 4). Thus, it can be assumed that this interspecific chemical cue is undetectable (but further investigation is necessary to confirm it) or that the *N. corniger* group choice is not triggered by this chemical cue. This result indicates that *N. corniger* could use the social information by exploring the chemical cues of neighbor intraspecific colonies to access the food sources in the environment. Cristaldo *et al.* (2016a) have shown that *N.* aff *coxipoensis* also follow chemical cues of intraspecific colonies and the acceptance of them depends on the resource availability. In general, *N.* aff. *coxipoensis* individuals prefer to follow chemical cues from colonies that have access to greater abundance of resource. In the present study, all colonies were maintained for at least 15 days with the same resource (sugarcane baits) *ad libitum*. So, both genetic and environmental factors might have contributed to a higher acceptance of chemical cues among *N. corniger* groups from the same species. Thus, there would be no reason to avoid intraspecific colonies or prefer between resources with chemical cues from the same or different colonies (DC=OC, see Fig 3b). During foraging process, the use of social information can be an energy-saving strategy compared with the use of private information (Grüter & Leadbeater 2014). Obtaining information through private sources may involve prolonged searches, sampling of trial and error or high risks of encounters with predators and competitors (Kendal *et al.* 2005). However, in natural situations such perception of chemical cues could result in facilitation or competition depending on ecological context. Ferreira *et al.* (2018b) showed that individuals from colonies of *N.* aff. *coxipoensis* that use similar food resources increased alertness via a greater number of vibration than individuals who consumed different food resources. Additionally, the aggressiveness between neighbors of intraspecific colonies is more intense under low resource offer situation (Ferreira *et al.* 2018a).

Interestingly, termites preferred to choose for any food sources without their own signals, whether those resources containing detectable (from intraspecific [DC]) or undetectable (from solvent control [SC] and different species [DS]) chemical cues. Resources with undetected cues represent an opportunity to explore a new source while resources with intraspecific cues from other colonies can represent a reliable source of resources. On other hand, resources with own signals can indicate an already discovered resource by the colony; therefore, individuals have opted by discovery and evaluation of new food sources. A recent study showed that *N. corniger* in fact can explore the environment through scan the available food sources. In this species the soldiers promote a faster exploration which allows to encounter more food sources simultaneously and to make a better decision during foraging (Sacramento *et al.* 2020). Such result indicates that termite, as other eusocial insects, use an efficient strategy to select food source, especially in dynamic environments. According to Grüter and Leadbeater (2014), species able to combine exploitation of food resources with exploration of new food sources should benefit over species that use only one of these strategies. Although the mechanisms that determine foraging decisions in termites are still poorly studied, there is evidence that resource availability plays a key role in foraging modulation (see Araújo *et al.* 2007, 2017, Cristaldo *et al.* 2018, Cristaldo *et al.* 2016a, Ferreira *et al.* 2018a, Ferreira *et al.* 2018b). In addition, some studies have shown that the role of social information depends on many factors, including the quantity and distribution of the resource, the changes rate of the environment, the strategies of competitors, and also the predators' pressure (Lee *et al.* 2016). Therefore, it is possible to hypothesize that the avoidance of food resources with intra- and interspecific chemical cues would occur only when the availability of resources was intermediate (or even low). Future studies should evaluate the response of *N. corniger* termite colonies to intra- and interspecific chemical cues of colonies under different availability of resources in order to confirm this hypothesis.

In conclusion, the results obtained in the present study highlight the importance of social information during the selection of resources in termites. Such mechanism can influence the patterns of termite species coexistence on a local scale. The explanatory mechanisms for the pattern presented here maybe include the factors involved in the acceptance and use intra- and interspecific chemical cues. In addition, the ability to identify and respond to chemical cues may represent an important strategy for termites to cope with competing colonies in their natural habitats.

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Table 01. Effect of intra- and interspecific chemical cues on selection of food baits by *Nasutitermes corniger* termite groups.

	d.f	Desviance	Resid. d.f	Resid. Desv.	P	
A) EA x BC (blank control)						
Null model			15	20.71		
Treatments	1	12.55	14	8.15	0.0003	***
B) BC x SC (solvent control)						
Null model			15	20.03		
Treatments	1	0.08	14	19.94	0.78	*n.s*
C) SC x OC (control)						
Null model			15	45.04		
Treatment	1	31.80	14	13.23	0.0001	***
D) SC x DC (intraspecific chemical cues)						
Null model			15	25.57		
Treatment	1	12.19	14	13.38	0.01	***
E) SC x DS (interspecific chemical cues)						
Null model			15	33.21		
Treatment	1	0.78	14	32.43	0.51	*n.s*
F) OC x DC (control x intraspecific chemical cues)						
Null model			15	34.51		
Treatment	1	1.20	14	32.68	0.53	*n.s*
G) OC x DS (control x interspecific chemical cues)						
Null model			15	0.34		
Treatment	1	**0.13**	14	0.21	0.01	***
H) DC x DS (intraspecific chemical cues x interspecific chemical cues)						
Null model			15	1.29		
Treatment	1	**0.39**	14	0.89	0.001	***

EA= Empty arena; BC= sugarcane bait without solvent (blank control); SC= sugarcane bait with hexane (solvent; solvent control); OC= sugarcane bait with chemical signals of own colony tested (control); DC= sugarcane bait with intraspecific chemical cues (different colonies from same species); DS= sugarcane bait with interspecific chemical cues (colonies

from different species). Generalized Linear Modelling under Binomial error distribution corrected, when necessary, for overdispersion with quasiBinomial.
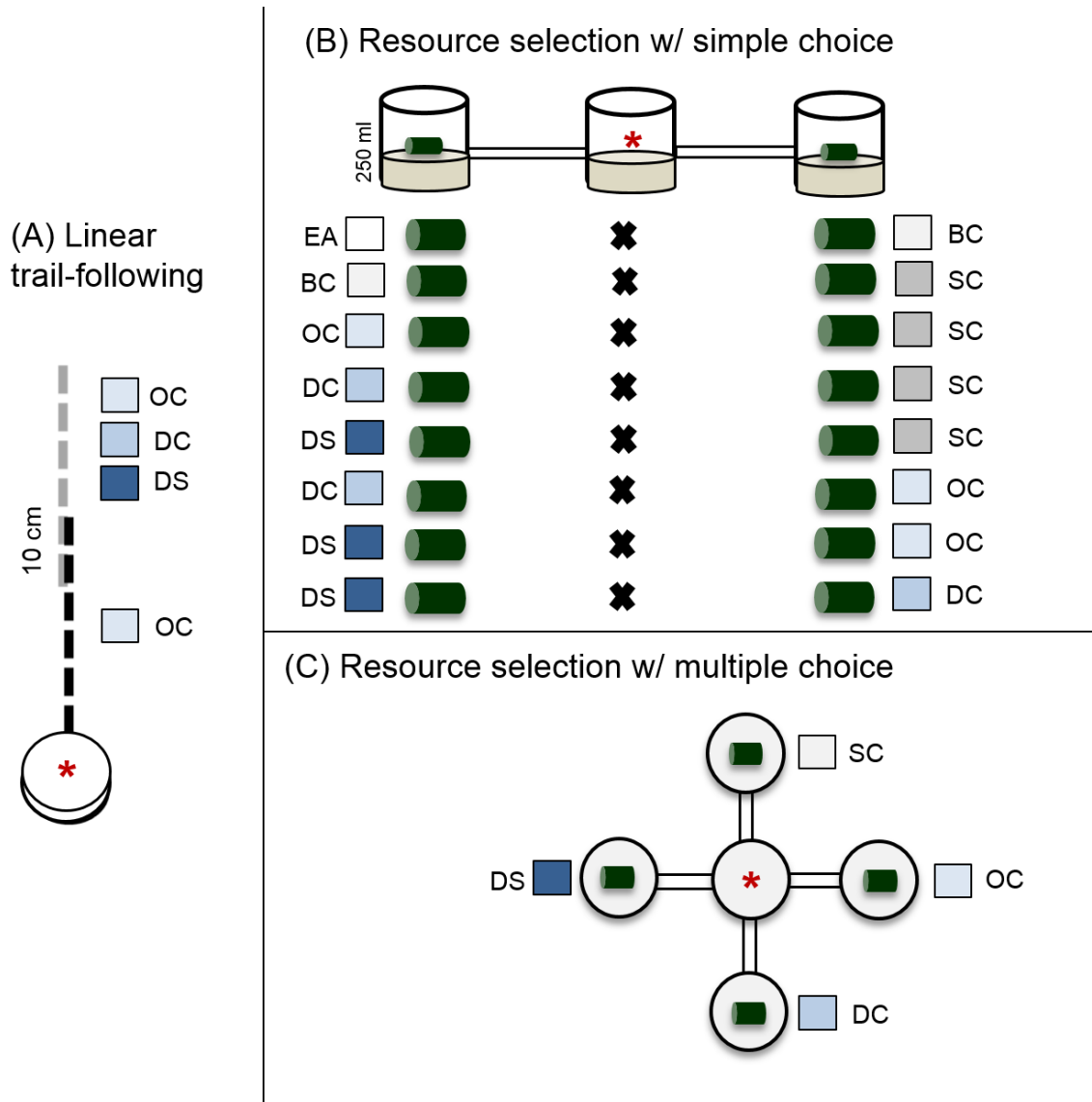
Fig. 01. Schematic design of experimental bioassays. EA= Empty arena; BC= sugarcane bait without solvent (blank control); SC= sugarcane bait with hexane (solvent control); OC= sugarcane bait with chemical signals of own colony tested (control); DC= sugarcane bait with intraspecific chemical cues (different colonies from same species); DS= sugarcane bait with interspecific chemical cues (colonies from different species).
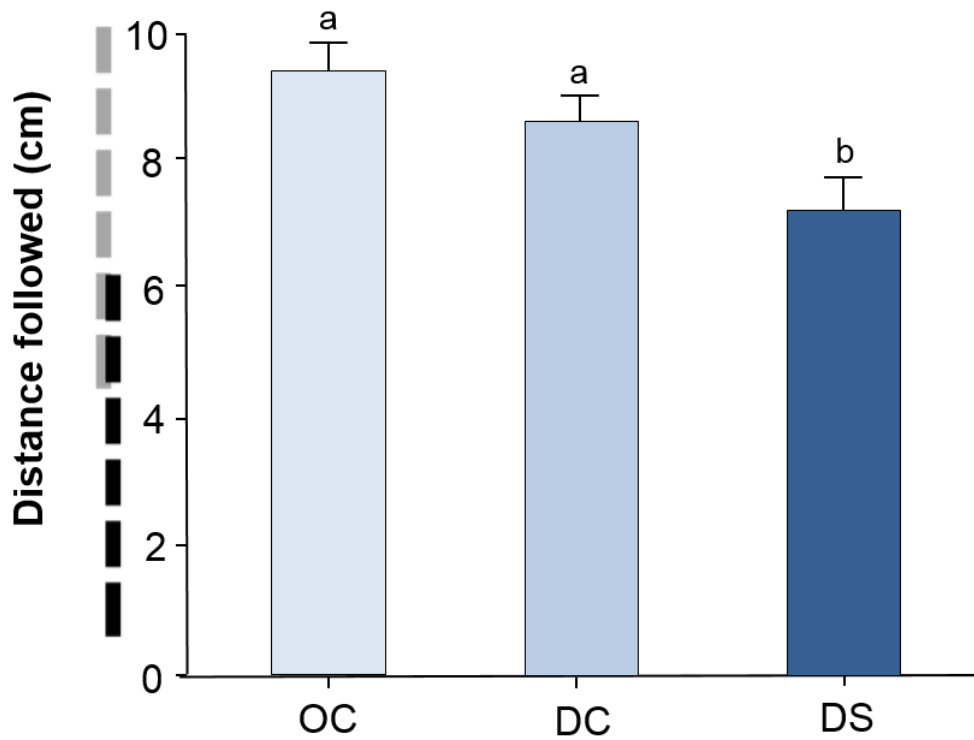
27

Fig. 02. Perception of chemical cues by *N. corniger* workers. Distance followed by *N. corniger* workers on odors from the same colony (OC; control), different colonies of the same species (DC) and colonies of different species (DS). Each bar represents the average followed by workers ± standard error. Similar letters mean no significant differences by Generalized Linear Models under quasi-Poisson distribution followed by Contrast Analysis.
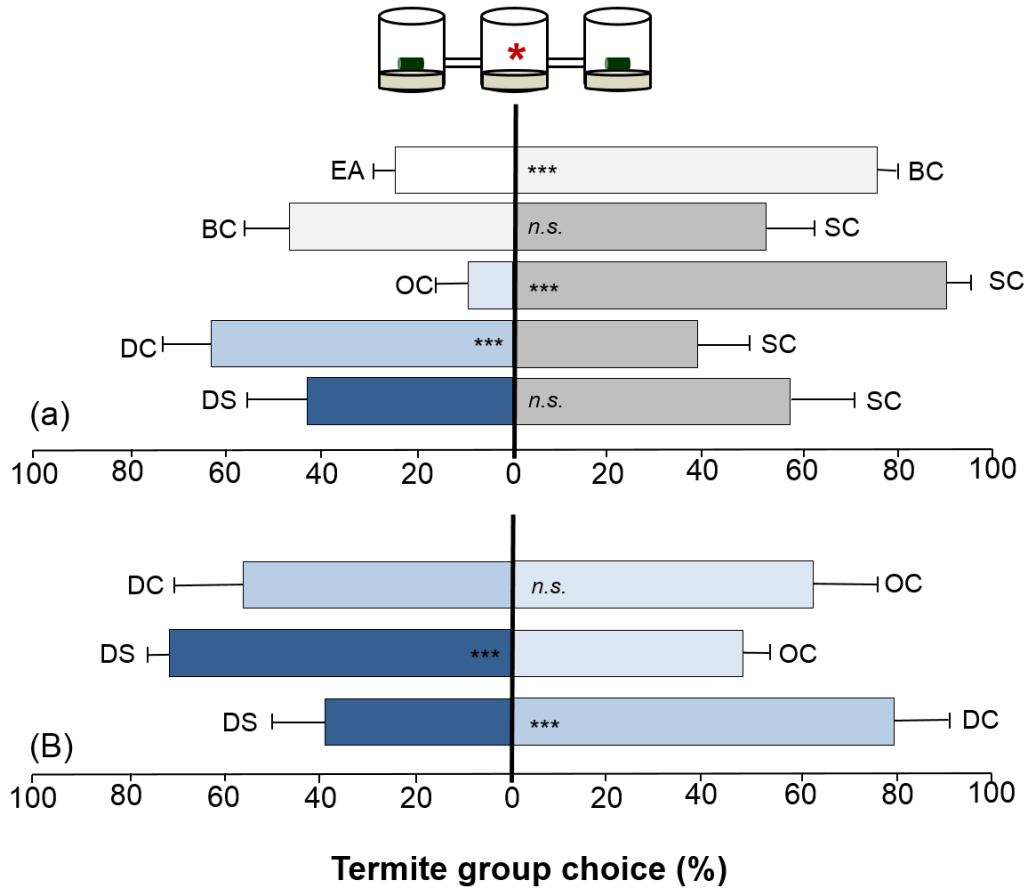
Fig. 03. Effect of intra- and interspecific chemical cues on resource selection (sugarcane baits) by groups of workers and soldiers of *Nasutitermes corniger* in binary choice bioassays. (A) Group choice (%) of termite groups for sugarcane baits with treatments *vs.* control. (B) Group choice (%) of termite groups for sugarcane baits with treatments. EA= Empty arena; BC= sugarcane bait without solvent (blank control); SC= sugarcane bait with hexane (solvent; solvent control); OC= sugarcane bait with chemical signals of own colony tested (control); DC= sugarcane bait with intraspecific chemical cues (different colonies from same species); DS= sugarcane bait with interspecific chemical cues (colonies from different species). Each bar represents the average choice of group (choose/total) ± standard error. Generalized Linear Models under Binomial distribution. *** mean *P < 0.001*; *n.s.* mean *P > 0.05*.
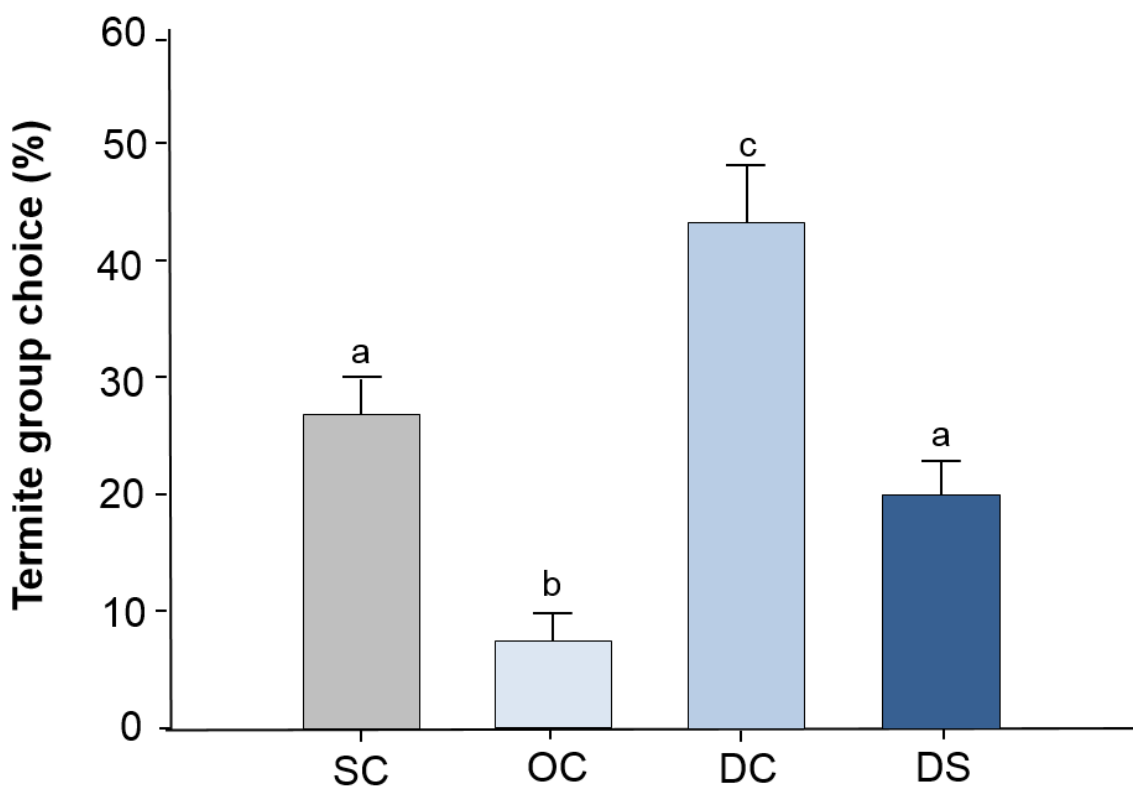
Fig. 04. Effect of intra- and interspecific chemical cues on resource selection (sugarcane baits) by groups of workers and soldiers of *Nasutitermes corniger* in multiple choice bioassays. Group choice (%) of termite groups for sugarcane baits with treatments in a multiple-choice bioassay. SC= sugarcane bait with hexane (solvent; solvent control); OC= sugarcane bait with chemical signals of own colony tested (control); DC= sugarcane bait with intraspecific chemical cues (different colonies from same species); DS= sugarcane bait with interspecific chemical cues (colonies from different species). Each bar represents the average choice of group (choose/ total) ± standard error. Similar letters mean no significant differences by Generalized Linear Models under Binomial distribution followed by Contrast Analysis.

CAPÍTULO 3

FOOD RESOURCE QUANTITY IS MORE IMPORTANT THAN LEVELS OF RISK SIGNALS DURING RESOURCE SELECTION IN HIGHER TERMITE SPECIES [1]

ALINE NASCIMENTO FILGUEIRA SILVA[1,2], CÁTILA R. SILVA[1], RENAN E. C. SANTOS[1], CARLA C. M. ARCE[3], ANA PAULA A. ARAÚJO[4] & PAULO FELLIPE CRISTALDO[1,2]

1. Synanthropic Insect Laboratory, Department of Agronomy, Federal Rural University of Pernambuco, Recife, Brazil.

2. Post Graduate Program in Entomology, Department of Agronomy, Federal Rural University of Pernambuco, Recife, Brazil.

3. Laboratory of Fundamental and Applied Research in Chemical Ecology, University of Neuchâtel, Neuchâtel, Switzerland.

4. Ecological Interactions Laboratory, Post Graduate Program in Ecology and Conservation, Federal University of Sergipe, São Cristóvão, Brazil.

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[1] Silva, A.N.F., Silva, C.R., Santos, R.E.C., Arce, C.C.M., Araújo, A.P.A. & Cristaldo, P.F.C. Food quantity is more important than risk levels during resource selection in higher termite species. To be submitted to Animal Behavior

ABSTRACT **-** Decrease the risk of being killed and increase the food intake is an important feature of the foraging behavior of most animals. In eusocial insects, foragers are vulnerable and the ability to trade-off benefits (food quantity) against costs (risk of being killed) may provide a considerable ecological advantage for colonies. Despite the increasing number of studies, the effects of both factors in combination on resource selection in eusocial insect is not well understood. Here, we investigated the combination of distinct levels of food quantity and risk signals on resource selection of a higher Neotropical termite, *Nasutitermes corniger* (Termitidae: Nasutitermitinae). Manipulative choice bioassays (binary and multiple choices) were conducted over time to check the preference of termite groups between the resources containing both signals (different levels of food quantity and risk). Overall, the quantity of food resource was more important than the risk signals during the resource selection by *N. corniger* termite groups. This work contributes to a better understanding of habitat use by termite species. Furthermore, it shows for the first time the combined effects of food quantity and risk signals on resource selection of an important ecological and economic termite species.

KEY WORDS: Foraging behavior, *Nasutitermes corniger*, habitat use, mortality risk

A QUANTIDADE DE RECURSO É MAIS IMPORTANTE QUE OS NÍVEIS DE SINAIS DE RISCO DURANTE A SELEÇÃO DE RECURSOS EM CUPIM

RESUMO – Diminuir os riscos de morte e aumentar a ingestão de alimentos é uma característica importante no comportamento de forrageamento da maioria dos animais. Em insetos eussociais, os forrageadores são vulneráveis e a capacidade de balancear os benefícios (quantidade de alimento) contra os custos (riscos de ser morto) pode garantir um considerável sucesso ecológico para as colônias. Apesar do número crescente de estudos, os efeitos da combinação de ambos os fatores na seleção de recursos em insetos eussociais ainda não são bem compreendidos. Aqui, investigamos a combinação de níveis distintos de quantidade de alimento e sinais de risco na seleção de recursos de *Nasutitermes corniger* (Termitidae: Nasutitermitinae). Bioensaios manipulativa de escolha (escolha binária e múltipla) foram conduzidos em laboratório ao longo do tempo para verificar a preferência de grupos de cupins por arenas contendo combinação entre os tratamentos (quantidade de alimento e sinais de risco). Em geral, a quantidade de recursos alimentares foi mais importante do que os níveis de sinal de risco durante a seleção de recursos por grupos de cupins *N. corniger*. Este trabalho contribui para uma melhor compreensão do uso do habitat por espécies de cupins. Além disso, mostra pela primeira vez os efeitos combinados da quantidade de alimentos e do sinal de perigo na seleção de recursos de uma importante espécie de cupim em termo ecológico e econômico.

PALAVRAS-CHAVE: Forrageamento, *Nasutitermes corniger*, uso do habitat, risco de mortalidade

Introduction

Animals that are efficient food gatherers may have a considerable ecological advantage which can leads to an increasing of fitness (Pyke 1984, Davies *et al.* 2012). Optimal foraging theory (OFT) predicts that animals face simple decisions choose food items that maximizes net energy returns (Stephens & Krebs 1986). In nature, however, animals face complex situations whose solutions must demand more than simply find and collect the food. For instance, during foraging, individuals are frequently confronted with dilemmas of balancing the value of a food source and its potential risk of be killed. Therefore, animals must be able to trade-off benefits and costs to make an efficient decision. Several studies demonstrate, for instance, that individuals among solitary animals can evaluate benefits (value of a food source) and costs (potential risk of be killed) and adjust their behavior accordingly (Valone & Lima 1987, Lima & Dill 1990, Kotler 1992, Abrams 1993, Vásquez 1994, Meyhöfer & Klug 2002, Lima 2009). Despite the increasing number of studies in eusocial hymenopteran insects (ants, bees, and wasp), the effects of interaction between food value and risk on behavior during resource selection in eusocial insects are still unclear with no manipulative studies with termites.

Unlike solitary animals, the foraging in termites (and others eusocial insects) is a collective behavior involving thousands of individuals (Bordereau & Pasteels 2011, Haifig *et al.* 2015, Cristaldo 2018). The food acquired during foraging is crucial to termite colony success because it is shared with all members of the colony by trophallaxis. When termite foragers leave the nest, they must not only find a food source but survive to return it to the colony and give it to the nestmates. However, it is not such an easy task since termite's forage occurs in a complex and heterogeneous environment. First, food is unpredicted in time and space (Araújo *et al.* 2017, Almeida *et al.* 2018) and in addition to that, several food sources can be available, and foragers must decide whether exploit simultaneously different sources or use only the most profitable (see

Sacramento *et al.* 2020). Second, individuals can be attacked by natural enemies and/or competitors, which will increase the risk of be killed and therefore, alter their foraging behavior. Thus, the most ecological successful colonies should have foragers able to maximize the energy intake and minimize losses of conspecifics. However, to the best of our knowledge, no study has evaluated the resource selection in termites testing both food quantity and risk signals simultaneously.

In the last years, plenty of studies have been shown that termites are consistent of maximize energy during foraging. The nasute termite *Nasutitermes* aff. *coxipoensis*, for instance, adjust their foraging area according to the density of resources in natural environment (Almeida *et al.* 2018). Artificial laboratory experiments have shown similar pattern in several termite species (Hedlund & Henderson 1999, Arab & Costa-Leonardo 2005, Gallagher & Jones 2005, Grace & Campora 2005, Evans *et al.* 2007, Inta *et al.* 2007, Lee & Su 2010, Araújo *et al.* 2011, Ferreira *et al.* 2018, Souza *et al.* 2018, Oberst *et al.* 2019). There are also evidences that termites change their foraging in response to risk of being killed (*e.g.* competitors or predator) (Traniello 1981, Gonçalves *et al.* 2005, Evans *et al.* 2009, Ferreira *et al.* 2018, Oberst *et al.* 2017, Silva *et al.* 2021). Simulated predations can result in cessation of foraging in termites (Korb & Linsenmair 2002). In a field experiment, baits with ants (major predator of termites) were occupied by termites latter than baits without ants (DeSouza *et al.* 2009).

Termites have an elaborate signaling system to communicate about complex information of either food (quantity or quality) and risk (presence of competitors and/or natural enemies). Several studies have demonstrated the nature and effectiveness of chemical signals in termites (for review, see Mitaka & Akino 2021). In Nasutitermitinae species, it is known that perception and response to chemical signals of foraging are modulated by resource availability (Cristaldo *et al.* 2016). Information about risk is transmitted to termite individuals through alarm pheromones produced in

the frontal gland of soldiers (Šobotník *et al.* 2010). Under imminent threats (*e.g.* nest disturbance, presence of enemies), alarm pheromones are released to inform nestmates about the risk. Recently, it has been showed that termites have a complex behavioral response to alarm signals (Delattre *et al.* 2015, Cristaldo *et al.* 2015). In the subfamily Nasutitermitinae, these responses are dose-dependent in which individuals modulate the response according to the levels of risk (Cristaldo *et al.* 2015). This potent and efficient alarm communication observed in Nasutitermitinae species might be used as different alarm levels to better respond to different threats (Cristaldo *et al.* 2015).

In this study, we aim to investigate the combination between resource quantity and levels of risk signals during the resource selection by the termite *Nasutitermes corniger* (Motschulsky) (Termitidae: Nasutitermitinae). Specifically, we hypothesize that resource selection by termite groups will be affected by the balance between benefits (quantity of resource) and costs (risk signals). Therefore, we predict that termite groups will prefer to select resources that will guarantee high benefit (high resource quantity) with low costs (low risk signal). Our results contribute to understand the mechanisms responsible for resource selection and the habitat use by this important ecological and economic group of insects, including the regulation of termite community structure at local scale.

Material and Methods

Termites. *Nasutitermes corniger* is a common, abundant, and dominant tree-nesting termite. This species has been chosen as a model species because many details of its behavior during resource selection and foraging have been recently clarified (Levings & Adams 1984, Sacramento *et al.* 2020, Silva *et al.* 2021). Additionally, soldiers have chemical defense, which can be easily manipulated to access the levels of risk.

Colonies of *N. corniger* were collected at the Federal Rural University of Pernambuco – campus SEDE (8°04'03" S, 34°55'00" W), in Recife, Pernambuco, Northeastern, Brazil. The climate in the region is characterized as "subtropical humid or sub-humid" (Aw) with average annual temperature of 25.8 ºC and average annual precipitation of 1804 mm (Climate, 2021). Permits for termite collection were issued by ICMBio-IBAMA (#47652). *Nasutitermes corniger* were individually identified and the voucher specimens are deposited in the collection of the Synanthropic Insects Laboratory at UFRPE.

Nests with active colonies of *N. corniger* were completely removed from tree and taken to the Laboratory of Synanthropic Insects at UFRPE. In the laboratory, colonies were placed separately in plastic boxes with moistened cotton and kept under controlled conditions (25 ºC, 70% r.h., darkness). All collected colonies were maintained in the laboratory for, at least, 24 hours prior the bioassays.

Experimental design. To test whether the effects of both of food resource quantity and risk signals effect on resource selection by *N. corniger*, we conducted binary and multiple choices bioassays. For this, the proportion of termites colonizing arenas with distinct levels of food quantity (mg of sugarcane baits; see later) and risk signals (µl of alarm extract; see later) over time were evaluated.

Manipulation of food resource quantity and risk levels. Fresh sugarcane baits were used as food resource in the bioassays. The choice of this food resource was based taking into account previous studies with *Nasutitermes* species that used sugarcane as bait in both laboratory and field experiments (see Cristaldo *et al.* 2016, Cristaldo *et al.* 2018, Ferreira *et al.* 2018a, Ferreira *et al.* 2018b, Almeida *et al.* 2018, Sacramento *et al.* 2020, Silva *et al.* 2021). Two quantity of food

resource were used in the behavioral bioassays: *(i)* 5 g of sugarcane [low resource; LR] and *(ii)* 25 g of sugarcane [high resource; HR]).

Alarm pheromone signal present in the frontal gland situated in the soldier's heads was used as a risk signal. The pheromone extraction was performed as described in Cristaldo *et al.* (2015). Briefly, each sample (individual colony) containing 50 soldiers head (nasus cut off) were immersed in hexane (10 µl per head) and stored for 24 hs at 4ºC. After this, the supernatant was collected and transferred to another clean vial. A second immersion was performed with approximately 100 µl of hexane at room temperature for 10 min. At the end, both supernatants were combined. Subsequently, the volume of the extract was reduced, and it was calculated the equivalent per soldier to be used in the bioassays (µl of extract equivalent to one soldier's head). The final extracts were stored at −20 ºC for further use in the bioassays. Two levels of risk signals were used in the behavioral bioassays: *(i)* 2 frontal gland equivalents (gl./Eq.) (low risk; LD) and *(ii)* 10 frontal gl./Eq. (high risk; HD). The gl./Eq. for each treatment was established based on results from Cristaldo *et al.* (2015) which showed that low doses of alarm indicate alertness whereas high doses of alarm triggers panic increasing the running activity.

Treatments in the behavioral bioassays were established combining food quantity and risk signals. Thus, a total of four combination of treatments were used as following: (1) low food resource quantity (5 g of sugarcane) with low level of risk signal (2 gl.Eq. of alarm signal) [LR+LD]; (2) low food resource quantity (5 g of sugarcane) with high level of risk signal (10 gl.Eq. of alarm signal) [LR+HD]; (3) high food resource quantity (25 g of sugarcane) with low level of risk signal (2 gl.Eq. of alarm signal) [HR+LD]; and (4) high food resource quantity (25 g of sugarcane) with high level of risk signal (10 gl.Eq. of alarm signal) [HR+HD]. Extracts of risk signals were applied on top of the baits using a microsyringe (Hamilton®) every 3 h; this time to reapplied the extract of risk signals was choose by an experimental test (data not shown).

Behavioral bioassays. Binary and multiple choice bioassays were conducted in experimental arenas under laboratory conditions following procedures described in Silva *et al.* (2021) with modifications. Experimental arenas consisted in plastic pot (250 mL) with internal bottom surface lined with filter paper.

In the binary choice bioassays, arenas consisted of a central plastic pot connected in a straight line with a transparent hole (7 mm diameter x 5 cm of length) to two other plastic pots at its end containing the treatments. In this bioassay, *N. corniger* groups were given access to different combination of all treatments determined by a complete factorial as previously detailed above (see section "Manipulation of food resource quantity and risk levels"). For each combination of treatments (*N*= 6), three repetition per nest (*N*= 8) were performed, totalizing 144 arenas. Control of binary choice bioassays consisted of choice of termites between treatments *versus* control solvent (sugarcane baits with only hexane). For each treatment (*N*= 4), three repetition per nest (*N*= 8) were performed, totalizing 69 control arenas. In addition, to test whether *N. corniger* groups may be able to distingue arenas with and without resources, positive control was conducted with arenas containing sugarcane baits (with low and high quantity) *versus* empty arenas. For each treatment (*N*= 2), three repetition per nest (*N*= 8) were performed, totalizing 48 positive control arenas. A total of 261 arenas were conducted in the binary choice bioassays.

Bioassays with multiple choices were conducted in arenas consisted of a central plastic pot connected with a transparent hole (7 mm diameter x 5 cm of length) to four other plastic pots at its end containing the treatments tested. Therefore, bioassays aimed to check choice of termite groups for all treatments tested: (1) low food resource quantity (5 g of sugarcane) with low level of risk signal (2 gl.Eq. of alarm signal), (2) low food resource quantity (5 g of sugarcane) with high level of risk signal (10 gl.Eq. of alarm signal); (3) high food resource quantity (25 g of sugarcane) with

low level of risk signal (2 gl.Eq. of alarm signal); and (4) high food resource quantity (25 g of sugarcane) with high level of risk signal (10 gl.Eq. of alarm signal). Three repetition per nest ($N=$ 8) were performed, totalizing 24 bioassays.

Termite groups (four soldiers and 16 workers) were confined into central plastic pot of experimental arenas (in both binary and multiple choice bioassays) and the number of termites in each one of the treatments was recorded over 48 h. Individuals were used only once in each repetition, and they were randomly selected from each colony. All bioassays were conducted in laboratory under controlled conditions (25 ºC, 70% r.h.).

Statistical analysis. The differences in the proportion of termites colonizing baits (*y-var)* were assessed using generalized linear mixed models (GLMMs) with Binomial error distribution followed by Analysis of Deviance and FDR-corrected post hoc test. Models were validated by inspecting residuals and testing for overdispersion. We tested the *y-var* as a function of time (*x-var₁*), treatments (*x-var₂*) and the interaction of these two factors. Time, repetition, and colony identity were included in the model as random factor. Tests for each bioassay were done separately.

Data analysis were carried out in R statistical software (R Development Core Team 2019) using the packages "*lme4*", "*car*", and "*lsmeans*".

<div align="center">

Results

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In general, resource quantity in arenas was more important than levels of risk signals during the resource selection by *N. corniger* termite groups. To test whether *N. corniger* groups may be able to distinguish between arenas with and without resources, positive control experiments was conducted with arenas containing sugarcane baits *versus* empty arenas. *N. corniger* groups

consistently choose the arenas with resources regardless the quantity (low and high quantity) than empty arenas in all timepoints studied (Fig. SM01). When *N. corniger* groups were exposed to treatments (combination of resource quantity and risk signals) *versus* solvent control (SC), termite groups avoided sugarcane baits containing risk signals regardless the levels (low and high) compared to baits with only hexane solvent (SC, solvent control) in both low and high resource quantity and in all timepoint studied (Table 1, Fig. 1). However, in the binary choice experiments between treatments (combination of resource quantity and danger signals), there were no differences in the choice of *N. corniger* groups between low and high levels of risk signals (Fig. 2). Interestingly, the choice of termite groups was only affected by resource quantity in the arenas. Therefore, *N. corniger* groups exposed to same resource quantity did not differ in their choice between baits containing low or high levels of risk signals (Table 1, Fig. 2). However, when termite groups were exposed to sugarcane baits with different resource quantity, *N. corniger* groups always preferred baits with high resource quantity compared with low resource quantity, independently of risk levels (Table 1, Fig. 2).

In the multiple-choice bioassays, the proportion of termites colonizing the baits was significantly affected by treatment (Tab. 2). *N. corniger* groups preferred arenas containing sugarcane baits in high resource quantity compared to those containing low quantity (Fig. 3). Surprisingly, the levels of risk signals did not affect the preference of termite groups between sugarcane baits (Fig. 3).

Discussion

Foraging is a crucial behavior which have significant impact on their life history characteristics (Bloom 2012). The rate of energy input to any animal will basically depend on the resources selected during foraging (Boggs 1992, Boggs 2009), which consequently affect the energy

allocated to survival (growth, maintenance, defense) and reproduction (Stearns 1989). Therefore, it is expected that animals optimize their fitness selecting food resources during foraging that will provide high energy input with low costs. In the present study, we conducted behavioral bioassays to explore the relevance of the combination of distinct food quantity (benefits) and risk signals (costs) on resource selection in a Neotropical termite species. Our results showed that, in general, food quantity seems to be more crucial than risk signals in the selection of resource by *N. corniger*. The levels of risk signal affected the proportion of nasute termites selecting resources only when groups had choice between sugarcane baits with and without these signals, independently of food quantity (Fig. 1). In almost all experiments, the proportion of termites selecting resources was affected only by quantity of food in the arenas (Fig. 2 and 3).

The significant effect of resource quantity during resource selection and foraging behavior in termites is well known. A plenty of termite species showed to be sensitive to resource quantity and/or quality (Hedlund & Henderson 1999, Arab & Costa-Leonardo 2005, Gallagher & Jones 2005, Grace & Campora 2005, Evans *et al.* 2007, Inta *et al.* 2007, Lee & Su 2010, Araújo *et al.* 2011; Araújo *et al.* 2017, Ferreira *et al.* 2018, Souza *et al.* 2018, Oberst *et al.* 2019) and consequently show to be consistent with a hypothesis of maximizing net rate of energy intake proposed by optimal foraging theory (OFT). In an unusual study under field conditions, for example, Almeida *et al.* (2018) found that foraging behavior in *N.* aff. *coxipoensis* is optimized according to resource density. That is, colonies under low resource density increase their search efforts through the establishment of more and longer trails. However, foraging effort seems to be minimized in sites with more profitable resources (with intermediate and high resource density) by the initial establishment of trails which are then converted into galleries. The results found in the present study reinforce the key role of resource in the foraging behavior of termites. Besides that,

our results showed that role of food quantity is already important in the initial phase of foraging, that is, during resource selection (see Fig. 2 and 3).

The ability to cooperatively exploit their habitat is key a factor in why termite and other eusocial insects, as a group, are so successful ecologically. The capacity of perceive the food resource quantity and/or quality allows termite colonies to track their environment and, therefore, effectively allocate the work force to the collection process in more profitable sites. In fact, previous study has been shown that perception and response to chemical signals of foraging in nasute termite is affected by resource density (Cristaldo *et al.* 2016). In addition, a recent study conducted with *N. corniger* demonstrated that nasute termites are efficient in perceiving changes in food resource quantity by redirecting individuals to explore more profitable sources (Sacramento *et al.* 2020). An open question now is why food resource is so import to termite colonies? The answer to this must be related to energy allocation to colony growth (number of individuals) and caste function. Empirical studies have been shown that number of individuals in the colony and the production of castes in both lower and higher termites' species are affected by resource quantity (Lenz 1994, Korb 2004, Korb & Katrantzis 2004, Korb & Schmidinger 2004, Cristaldo *et al.* 2018). Same pattern was already observed in ants (Aron *et al.* 2009, Wills *et al.* 2015) and bees (Carvell *et al.* 2008, Smith *et al.* 2012). Thus, differences in the food resource quantity can have important effects in the success of the colonies, and, for that reason, it may be so important during selection of resource of foraging process.

The levels of risk signals, on the other hand, did not significantly affect the resource selection in *N. corniger* (Fig. 2 and Fig. 3). Therefore, at least for the species studied here, risk signals seem not be a determinant factor in the resource selection. This result was a quite surprise since it was expected that the increase of cost (*e.g.* risk signal) would affect the proportion of termite selecting the sugarcane baits, as already observed in other eusocial insects (see Nonacs & Dill 1991, Detrain

et al. 1999). Two possible mechanisms could explain this result: (1) the inherent defensive abilities present in Nasuitermitinae species (*e.g.* high investment in the proportion of soldier caste); and/or (2) the state of hunger of colonies used in the bioassays.

In the derived subfamily Nasuitermitinae, soldiers can represent up to 30% of all individuals in the colonies (Haverty 1977) and they have a greater effectiveness of defense through a powerful chemical arsenal (Prestwich 1984, Šobotník *et al.* 2010). This high investment in defensive abilities observed in derived species seems to be an adaptation to complete separation between nest site and food source (for details, see Almeida *et al.* 2016; Almeida *et al.* 2018; Sacramento *et al.* 2020). In fact, soldiers in *Nasutitermes* species actively participate in the foraging not only as a protective caste but also in the initial phase of food searching (Traniello 1981, Almeida *et al.* 2016). Thus, *Nasuitermes* species must be less sensitives to risk signals. Such explanation for our results gains support from a field studied conducted with Neotropical termite community. Gonçalves & DeSouza (2009) verified that termite species richness responds differently to resource availability and predation at local scale. According to authors, this different response must be related with defensive abilities of each group. In Apicotermitinae species (soldierless group), both resource availability and predation affect termite species richness. On the other hand, in non-Apicotermitinae species (soldier group) neither resource availability nor predation affect termite species richness. Future studies should focus on this hypothesis by testing the response of soldier and soldierless termite species to risk signals during resource selection.

Foraging behavior is modulated not only by benefits and costs but also by internal factors such as starvation (Bell 1990). An alternative explanation to absence of response to risk signals during resource selection is the state of hunger of colonies used in the present study. Termite colonies were collected in the same site at least 24 hours of the bioassays. Thus, it is possible that individuals from colonies used in the behavioral bioassays were in the same state of hunger, which

make them braver to risk when there was no other choice; and, under this situation, only the quantity of food was important to selection of resources. This hypothesis is plausible once termite groups avoided baits with risk signals in control bioassays (see Fig. 1). However, futures studies evaluating the response of colonies under different resources offer to risk signals should be conducted to confirm this hypothesis.

In summary, our results suggest that, at least for *N. corniger*, food quantity was more important during resource selection than risk signals. This result indicates that benefits (quantity of food resource) present a crucial role in the resource selection during foraging in nasute termites. Such mechanism can help to understand the use habitat by termites including the regulation of termite community structure at local scale.

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Table 1. Summary of Analysis of Deviance (Type II Wald chisquare tests) of binary choice bioassays.

	Chisq	d.f.	P	
response: termites/total				
A) Low resource + Low risk (LR + LD) *x* Solvent Control (SC)				
time	1509.93	12	< 0.001	***
treatment	471.75	1	< 0.001	***
time:treatment	86.52	12	< 0.001	***
B) Low resource + High risk (LR + HD) *x* Solvent Control (SC)				
time	1667.98	12	< 0.0001	***
treatment	745.23	1	< 0.0001	***
time:treatment	101.17	12	< 0.001	***
C) High resource + Low risk (HR + LD) *x* Solvent Control (SC)				
time	1478.48	12	< 0.0001	***
treatment	961.02	1	< 0.0001	***
time:treatment	34.44	12	0.0001	***
D) High resource + High risk (HR + HD) *x* Solvent Control (SC)				
time	1429.88	12	< 0.001	***
treatment	2209.83	1	< 0.001	***
time:treatment	110.63	12	< 0.001	***
E) LR + LD *x* LR + HD				
time	1431.93	12	< 0.0001	***
treatment	1.10	1	0.41	*n.s.*
time:treatment	1.85	12	0.08	*n.s.*
F) LR + LD *x* HR + LD				
Time	1342.57	12	< 0.0001	***
Treatment	1.11	1	0.29	*n.s.*
time:treatment	78.78	12	0.0001	***
G) LR + LD *x* HR + HD				
time	1417.08	12	< 0.0001	***

	Chisq	*d.f.*	P	
treatment	265.48	1	< 0.0001	***
time:treatment	93.31	12	< 0.001	***
H) LR + HD *x* HR + LD				
time	1555.62	12	< 0.0001	***
treatment	556.71	1	< 0.0001	***
time:treatment	77.04	12	< 0.001	***
I) LR + HD *x* HR + HD				
time	1557.65	12	< 0.0001	***
treatment	916.71	1	< 0.0001	***
time:treatment	123.80	12	< 0.0001	***
J) HR + LD *x* HR + HD				
time	1523.66	12	< 0.0001	***
treatment	1.43	1	0.68	*n.s.*
time:treatment	1.45	12	0.27	*n.s.*

Generalized Linear Mixed Modelling under Binomial error distribution corrected, when necessary, for overdispersion with quasiBinomial.

Table 2. Summary of Analysis of Deviance (Type II Wald chisquare tests) of multiple choice bioassays.

	Chisq	*d.f.*	*P*	
response: termites/total				
time	1392.03	3	< 0.001	***
treatment	917.97	12	< 0.001	***
time:treatment	133.33	36	< 0.001	***

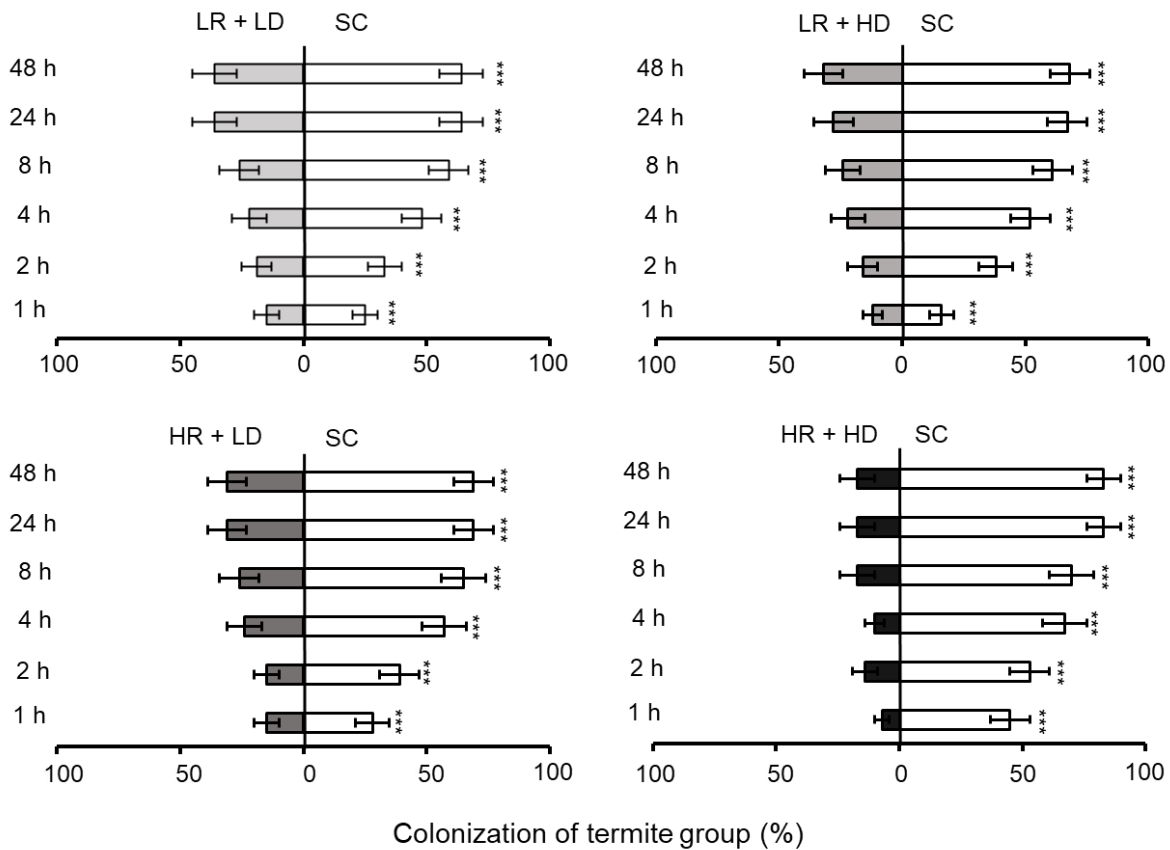Generalized Linear Mixed Modelling under Binomial error distribution.

Figure 1. Temporal dynamic of foraging by *Nasutitermes corniger* groups in binary choice bioassays with treatments (combination of resource quantity and levels of risk signals) *versus* baits with only solvent (control). LR + LD= low resource quantity and low level of risk; LR + HD= low resource quantity and high level of risk; HR + LD= high resource quantity and low level of risk; HR + HD= high resource quantity and high level of risk; SC= baits with only solvent (solvent control). Asterisks indicate significant differences between arenas (*** P <0.001).
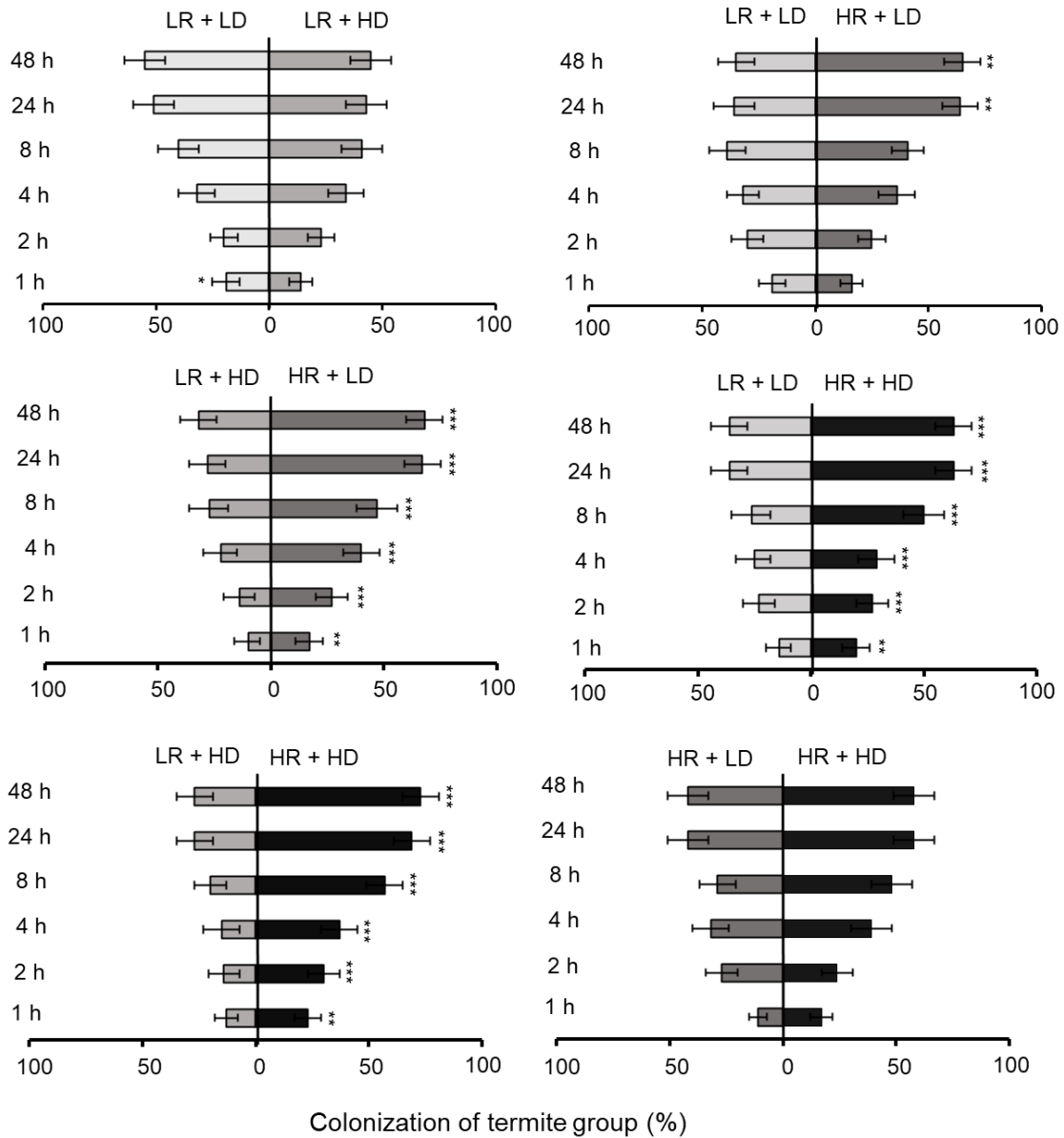
Figure 2. Temporal dynamic of foraging by *Nasutitermes corniger* groups in binary choice bioassays with treatments (combination of resource quantity and levels of risk signals). LR + LD= low resource quantity and low risk; LR + HD= low resource quantity and high risk; HR + LD= high resource quantity and low level of danger; HR + HD= high resource quantity and high level of risk. Asterisks indicate significant differences between arenas (** P< 0.01, *** P <0.001).
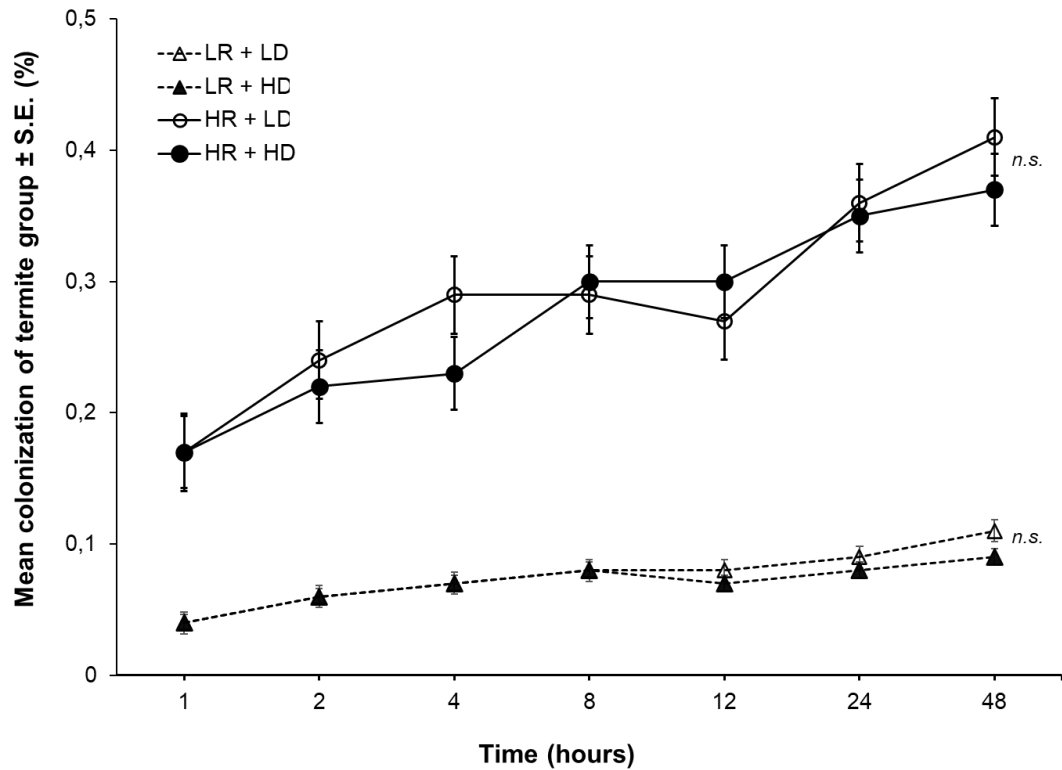
Figure 03. Temporal dynamic of foraging by *Nasutitermes corniger* groups in multiple choice bioassays. LR + LD= low resource quantity and low level of risk; LR + HD= low resource quantity and high level of risk; HR + LD= high resource quantity and low level of risk; HR + HDR= high resource quantity and high level of risk; The difference between treatments is given per time point using *lsmeans* function.
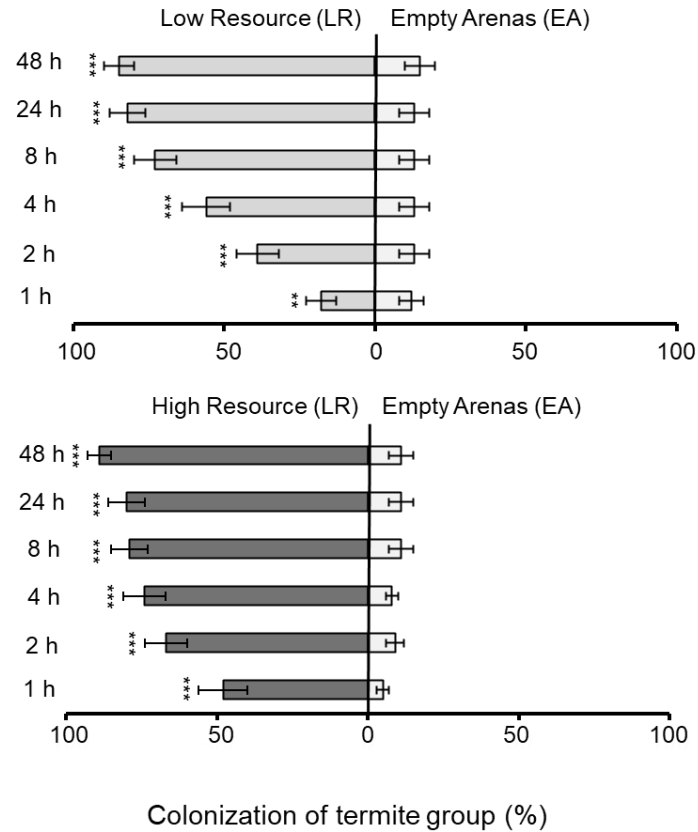
Fig. SM01. Temporal dynamic of foraging by *Nasutitermes corniger* groups in binary choice experiments with arenas containing sugarcane baits (low (LR) and high (HR) resource quantity) *versus* empty arenas. Asterisks indicate significant differences between treatments (**P <0.01; *** P <0.001).

CAPÍTULO 4

CONSIDERAÇÕES FINAIS

Os resultados dessa dissertação sugerem, de forma conjunta, que a quantidade de recurso parece ser o fator determinante da seleção de recursos em *Nasutitermes corniger*. A informação social parece ter um importante papel nesse processo uma vez que os resultados obtidos no capítulo 2 indicam que pistas químicas de forrageio intraespecíficas são capazes de modular a seleção de recursos em grupos de *N. corniger*. Porém, novos estudos devem ser realizados com colônias sob diferentes disponibilidades de recurso a fim de avaliar a resposta de seleção de recurso de *N. corniger* às pistas intra- e interespecíficas. Além disso, os resultados obtidos no capítulo 3 indicam que o efeito dos sinais de risco parecem ser menos determinante durante a seleção de recurso em *N. corniger*. Isso deve estar relacionado com o alto investimento em defesa presente nessa espécie e/ou ao nível de saciedade das colônias utilizadas no experimento.

Por fim, o presente trabalho contribui com novos conhecimentos a respeito da seleção de recursos em cupins e pode auxiliar na compreensão do uso do habitat por esse importante grupo de insetos.