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# Chemical composition and variability of the defensive secretion in *Nasutitermes* corniger (Motschulsky, 1885) in urban area in the Brazilian semiarid region

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### Abstract

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We analyzed the composition and chemical variability of the defensive secretion in Nasutitermes corniger in an urban area. We selected two environments with characteristics of Caatinga, and two surrounded by a semideciduous forest, in northeastern Brazil. Eighteen compounds were identified by gas chromatography-mass spectrometry. Cluster analysis classified the populations into two main groups: group I, seasonal forest, with  $\alpha$ -pinene,  $\beta$ -pinene, 2-hexanol and 3-hexanol as major compounds, whereas group II, Caatinga, showed a lower percentage for  $\alpha$ -pinene,  $\beta$ -pinene and limonene. It is suggested that climatic factors, geographical distance, and likely genetic differences between populations influence the chemical composition of the secretion of soldiers resulting in the possible formation of two ecotypes.

Additional key words: Brejo de altitude, chemical defense, monoterpenes, Nasutitermitinae, termite.

### Resumo

Mello AP, Azevedo NR, Barbosa-Silva AM, Bezerra-Gusmão MA. 2016. Composição e variabilidade química da secreção defensiva de *Nasutitermes corniger* (Motschulsky, 1885) em área urbana na região semi-árida brasileira. Entomotropica 31(11): 82-90.

Analisou-se a composição e a variabilidade química da secreção defensiva de *Nasutitermes corniger* em área urbana de dois ambientes com características de Caatinga, e dois ambientes circundados por uma Floresta estacional semidecidual no nordeste brasileiro. Dezoito compostos foram identificados por cromatografia gasosa acoplada a espectrometria de massas. A análise de agrupamento classificou as populações em dois grupos principais: grupo I, floresta estacional, com  $\alpha$ -pineno,  $\beta$ -pineno, 2-hexanol e 3-hexanol como substâncias majoritárias, enquanto que o grupo II, Caatinga, mostrou menor percentual para  $\alpha$ -pineno,  $\beta$ -pineno e limoneno. Sugere-se que os fatores climáticos, a distância geográfica, e as prováveis diferenças genéticas entre as populações influenciem na composição química da secreção dos soldados resultando na possível formação de dois ecótipos.

Palavras-chave adicionais: Brejo de altitude, cupins, defesa química, monoterpenos, Nasutitermitinae.

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### Introduction

Termites are among the most abundant arthropods in the tropical ecosystems (Martius 1994). Throughout their evolutionary history, they have developed techniques for collective defense, which become manifest in the elaborate constructions of their nests, in addition to the anatomical and behavioral adaptations for the defense of the colony (Deligne et al. 1981, Grasse 1982). Among the morphological and physiological adaptations of defense, the soldier caste shows a mandibular apparatus for mechanical defense, and a frontal gland, which produces various chemical compounds (Prestwich 1984, Quennedey 1984, Šobotník et al. 2010). These chemical defenses are found having efficient chemical weaponry, especially in Rhinotermitidae, Serritermitidae and Termitidae families (Quennedey 1984, Sobotník et al. 2010).

The diversity of chemical substances produced by the frontal gland of termites has been analized over the past four decades (Šobotník et al. 2010), and presents several synthesized substances of different chemical classes, including alcohols, mono-, di-, and sesquiterpenes, besides aromatic and heterocyclic compounds (Prestwich 1984, Šobotník et al. 2010, Krasulová et al. 2012). In soldiers with vestigial mandibles, this secretion is expelled by the nasute and has many functions, for example, a viscous and irritating solution for predators, blocking the movements of competitors, and as a repellent pheromone and alarm used to coordinate defensive activities (Prestwich et al. 1984, Roisin et al. 1990). However, based on the size of the soldier, there is a significant difference in the amount of secretion produced. Small soldiers (nasutoids) rely solely on the chemical weapons produced by their large front gland (reservoir), while large-jawed soldiers synthesize less defensive secretion (Prestwich et al. 1984, Roisin et al. 1990). In addition, this secretion seems to act as an inhibitor of the growth of fungi, probably

by the action of the monoterpenes  $\alpha$ -pinene and limonene (Rosengaus et al. 2000, Zhao et al. 2004, Fuller 2007).

The soldiers of the subfamily Nasutitermitinae (Termitidae) analyzed in this study differ from those of other subfamilies by their complete dependence on chemicals defense. Nasutitermes is one of the most diverse genera, with 74 species described only in the Neotropical region (Constantino 2002). In Brazil, Nasutitermes is represented by about 47 species, distributed in different biomes as tropical forests, Cerrado and Caatinga (Zorzenon e Potenza 1998, Constantino 1999). Chemical defense has been analised in 15 genera and 74 species of termites, 43 of them belonging to Nasutitermes (Gush et al. 1985, Valterova et al. 1993). Nasutitermes corniger (Motschulsky, 1885) is one of the main species of the genus and lives in nests constructed of chewed wood and other materials, such as cemented sand, saliva and feces. The nests are built on trees or inside houses (Fontes 1995), and the species is well known because of the economic damage it causes (Mello et al. 2014, Vasconcellos et al. 2002). The defensive secretion of N. corniger has been analyzed in populations from Costa Rica and Panama, where 19 monoterpenes and 12 diterpenes were detected (Gush et al. 1985). However, there is no record of the chemical composition of the secretion from populations of this species from urban areas of South America.

Studies discuss the variations between species and even inter-settlers in the composition of these substances, both in terms of chemical diversity such as percentage concentration, attributing these variations in some cases to oscillations of abiotic factors, such as temperature, precipitation and soil composition (Quintana et al. 2003, Azevedo et al. 2006, Perdereau et al. 2010). The characterization of the chemical profile and the factors that influence the variations in the composition of the defensive secretion of

termites provide an interesting tool for studies in taxonomy, biogeography, population ecology and in defensive strategies of these insects (Nelson et al. 2001).

The objective of this study was to analyze the chemical composition of the defensive secretion in soldiers of *N. corniger* located in urban environments in order to assess the existence of inter-colonial variation in the chemical profile of populations in two areas with different climatic characteristics.

### Material and Methods

### Study area

The study was conducted in two urban areas, one with typically semiarid Caatinga characteristics, and two environments surrounded by a semideciduous forest, known locally as "Brejo de altitude". Samples of 50 soldiers N. corniger were collected from five nests located in the four cities: Areia (lat 06° 57' 42" S, long 35° 41' 43" W) and Bananeiras (lat 06° 45' 00" S, long 35° 37' 58" O), with a distance of 45 km between them, and Campina Grande (lat 7° 13' 50" S, long 35° 52' 52" S) and Pocinhos (lat 07° 04' 37" S, long 36° 03' 39" O), separated by a distance of 35 km, for a total sample of 20 nests. The Areia and Bananeiras cities are located in a region of Brejo de altitude, with almost as wet characteristics as the areas of the forest zone, at an altitude between 650 - 1 000 m, annual average temperature between 18 °C to 28 °C and average annual rainfall of 1 194 mm. These regions are more humid than the other areas of the semiarid Brazilian region, due to the orographic effect on precipitation and in the reduction of thermal indices (Vivo 1997, Santos et al. 2007). The cities Pocinhos and Campina Grande are located in a region with semiarid characteristics, with high temperatures and a pattern of irregular rainfall, characterized as Caatinga environments, at an altitude between 500 - 640 meters, a temperature of 21 to 30 °C, and an annual precipitation between 382 and 802 mm (Sudene 1990).

## Collecting procedures and chemical analysis of the defensive secretions

The 50 soldiers of termites were kept under refrigeration (-18 °C) to the processing of chemical analysis, in order to minimize the loss of volatile components present in the defensive secretion. The samples were deposited in the Laboratory of Ecology of Termites, State University of Paraíba, Brazil.

The heads of each sample of soldiers were immersed in 0.5 ml of n-hexane (grade ultraresidue, BAKER, USA) and subjected to ultrasound (Bransonic, USA) for 15 seconds. Subsequently, this solution was filtered and analyzed on a gas chromatograph coupled to a quadruple mass spectrometer (GC-MS) QP5050A Shimadzu (Kyoto, Japan) in the following operating conditions: fused silica capillary column, model CBP-5 (30 m × 0.25 mm internal diameter × 0.25 mm thick film of a 5 % phenyl-methyl polysiloxane) (Shimadzu, Japan) a flow rate of 1 mL.min<sup>-1</sup> helium as the carrier gas; heating set temperature (60 °C with a gradient of 1 to 3 °C.min<sup>-1</sup> to 240 °C and then with a gradient of 10 °C.min<sup>-1</sup> to 270 °C, maintaining an isothermal 7 min with a total run time of 70 min). The ionization energy of the detector was 70 V, with the injection volume of the sample  $0.5~\mu l$  and a split ratio of 1:20injection. The temperature of the injector and detector were maintained at 220 °C and 240 °C, respectively. The analysis was conducted in the scan mode at a speed of 1.0 varredura.s<sup>-1</sup>, with a mass range 40-400 m/z. Quantitative analysis was obtained by integrating the total ion chromatogram (TIC) and expressed as mean values of two percentage replicates. The identification of the components was performed by automatic comparison and Manual mass spectra with those of the NIST / EPA / NIH (1998) libraries by comparison of mass spectra and retention indices (RI) with

those in the literature (Adams 1995, 2001) and co-injection with standards. Retention Indices were calculated by co-injection with a mixture of hydrocarbons, C8-C32 (Sigma, USA), and application of Dool and Kratz equation (Dool e Kratz 1963). The major constituents were also identified by carbon nuclear magnetic resonance (13C-NMR), recorded on a Varian Gemini spectrometer operating at 75 MHz using deuterated benzene as internal standard (128 ppm). The identification was done by comparing the signals of the <sup>13</sup>C NMR spectrum of the extract of the secretion of defense, with the literature (Kubeczka and Formáček 2002). The procedures were performed at the Institute of Chemistry, Federal University of Goiás, Brazil, in the laboratories of Gas Chromatography and Nuclear Magnetic Resonance.

### Edaphic-climatic factors

The climatic data's were obtained from the monthly agroclimatic journal, and from the Bulletin of the National Institute of Meteorology (Inmet 2012, 2013). Mixed samples of soil around the trees that sheltered the nests were obtained, each containing about 200 g, measuring up to 20 inches deep, and divided into three equidistant points of the host plant of the nest. The granulometric analyses were performed by the hydrometer method proposed by Bouyoucos (1951), and later modified by Forsyth (1975). The physical composition (granulometric), moisture, pH and percentage of organic matter in soil (OM) were all analyzed in the Laboratory of Soil Physics and Chemistry of the Federal University of Paraíba, Campus II, Areia, PB.

### Statistical analysis

In order to verify the similarity in chemical composition of the defensive secretion of the populations, a MDS ordination diagram (non-metric multidimensional scaling) was built based on Euclidean Distance. For comparison between the groups formed a nonparametric

permutation procedure the ANOSIM was made . These tests were performed using the Primer6 program.

Using a matrix of the chemicals, environmental data (temperature, humidity, precipitation) and soil composition (granulometric, humidity, pH and percentage of organic matter in soil), a canonical correlation analysis was performed (using the program R) to verify the influence of the environment on the chemical composition of the defensive secretion.

### Results and Discussion

Despite reports in the literature about the presence of diterpenes and sesquiterpenes in the defensive secretion of some Nasutitermitinae (Constrictotermes, Curvitermes, Syntermes, Subulitermes and Nasutitermes, viz. N. corniger, N. infuscatus, N. octopilis) (Prestwich 1984, Gush et al. 1985, Azevedo et al. 2006), these classes of terpenes were not observed in populations of *N. corniger* analyzed in this study. This could be a reflection of the spatial conditions of the urban environment in which the populations analyzed are inserted, such as availability and kind of offered food resources. It is known that the biosynthesis of these substances in the defensive secretion of endogenous origin termites is subject to interference from external environmental factors.

The composition of the defensive secretion in N. corniger had 18 volatile components, observing the qualitative and quantitative dissimilarity among the populations studied. Fourteen components were monoterpenes, with significant amounts of  $\alpha$ -pinene and  $\beta$ -pinene in the bulk composition of the secretions tested in both study areas (Table 1). The 2-hexanol and 3-hexanol were identified only for the populations of Brejo de altitude (Table 1).

The high percentage of  $\alpha$ -pinene observed in the defensive secretion of *N. corniger* populations in the study areas (Table 1) corroborates the study

**Table 1.** Average percentage composition (± standard deviation) of chemicals identified in the defensive secretion of soldiers of *Nasutitermes corniger* in urban areas of the Northeast of Brazil.

Substances	Brejo de altitude		Caatinga	
	Areia	Bananeiras	Pocinhos	Campina Grande
2-Methyl-3-heptanone -	1.60±1.15	1.08±1.50	0.11±0.07	0.10±0.11
α-Pinene*	30.36± 24.02	49.94±11.30	39.91±12.87	37.54±10.46
3-Hexanol-	21.13±11.30	6.18±8.30	-	-
2-Hexanol-	11.15±15.20	6.48±1.80	-	-
Unknown-	2.37±1.60	1.08±4.50	-	-
β-Pinene*	12.71±6.50	24.68±12.60	45.24±15.48	33.85±6.03
β –Myrcene*	$0.25 \pm 0.33$	$0.43 \pm 0.23$	0.84±0.26	$0.45 \pm 0.72$
Limonene*	3.18±1.66	6.12±2.17	8.38±1.23	6.49±1.81
Ocimene*	1.14±1.08	1.66±0.34	0.1±0.01	$0.1 \pm 0.0$
3-Carene*	$0.1 \pm 0.0$	0.1±0.26	0.1±0.50	$0.1 \pm 0.0$
3-Carene-2-ol*	0.1±1.90	0.1±0.75	0.1±0.24	0.1±0.03
Verbenone+	0.1±0.29	0.1±0.08	0.13±0.35	2.97±0.78
Canphene*	-	-	0.22±1.09	0.1±0.38
4-Carene*	-	-	0.1±0.06	0.1±1.05
Octatriene-3,7-dimethyl*	-	-	2.34±0.87	1.47±1.26
Terpinolene*	-	-	0.1±0.06	0.1±0.06
Pinocarveol*	-	-	0.1±0.65	1.93±1.12
L-carveol*	-	-	0.1±1.2	5.17±1.7

<sup>(\*)</sup>Monoterpenes; (+)Oxygenated monoterpene; (-) Other.

of Gush et al. (1985) and is common among the Termitidae (Roisin et al. 1990, Valterová et al. 1993, Azevedo et al. 2006). This monoterpene can be found both in many essential oils of plants such as conifers (Pinaceae) as well as in insects, such as termites and beetles (Geron et al. 2000). Large part of the analyzed nests (80 %) were fixed on trees of Terminalia catappa L., a species commonly used for urban forestry in the region. The representatives of the genus Terminalia are widely distributed in tropical areas of the world, and are known as a rich source of secondary metabolites such as triterpenoids and other aromatic compounds (Katerere et al. 2003). However, in this study it was not possible to compare the percentage of this compound, in the plant with defensive secretion of the termites analyzed, to determine if a relationship exists. In the ecology of termites, this compound

exhibits high toxicity for insect predators and competitors such as ants and beetles (Simões et al. 1999). Moreover, antifungal, antibacterial and antiseptic features, which act to reduce the imminent risk of disease within the colony, may also be expected (Rosengaus et al. 2000, Stow e Beattie 2008).

The MDS analysis showed a pattern of spatial separation in the distribution of the *N. corniger* populations in the areas of study (Figure 1): Group I, formed by populations of Brejo de altitude, perhaps by high amounts of  $\alpha$ -pinene (43.83 ± 18.4), $\beta$ -pinene (15.09 ± 9.8),2-hexanol (8.9 ± 12.21) and 3-hexanol (7.7 ± 10.31), whereas Group II, formed by the populations of the Caatinga, by high amounts of  $\alpha$ -pinene (39.34 ± 11.8),  $\beta$ -pinene (34.94 ± 11.1) and limonene (7.55 ± 1.82) (Table 1). The difference

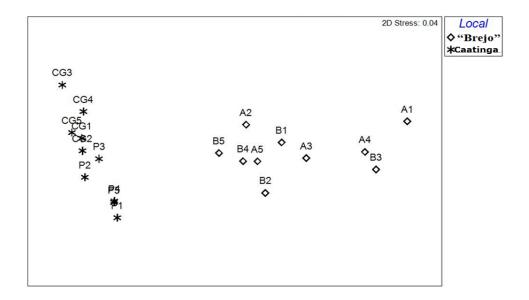


Figure 1. Non-metric multidimensional scaling (n-MDS) between the *Nasutitermes corniger* populations regarding their chemical composition of the defensive secretion in urban areas of Brejo de altitude (A1, A2, A3, A4, A5, B1, B2, B3, B4, and B5) and Caatinga (P1, P2, P3, P4, P5, CG1, CG2, CG3, CG4 and CG5) in a region of Northeast Brazil.

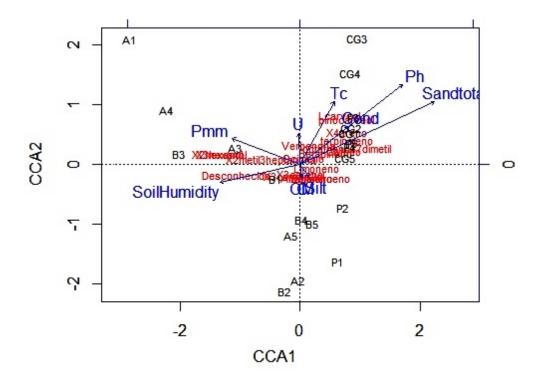


Figure 2. Canonical correlation between the principal volatile components of the defensive secretion in soldiers of populations of *Nasutitermes corniger* (Brejo de altitude: A1, A2, A3, A4, A5, B1, B2, B3, B4 and B5; Caatinga: P1, P2, P3, P4, P5, CG1, CG2, CG3, CG4 and CG5) and environmental variables (Temperature: T; Precipitation: Pmm; Humidity of the soil: Soil Humidity; Humidity of the air: U; Sand percentage: Sand total; Clay; Silt; Organic matter: OM; Carbon of soil: C; pH of soil: Ph) in a region of Northeast Brazil.

between the chemical composition of the defensive secretion of the studied populations was confirmed through the permutation test ANOSIM (R=0.941; p=0.1 %).

The clusters of populations occur possibly because of the distance between the collection points, suggesting the existence of a relation with the genetic expression of the populations in the analyzed areas. There may be the formation of two distinct ecotypes in the populations. Perhaps the populations that are located in geographically close regions and subjected to similar ecosystem conditions present a homogeneous pattern qualitatively and quantitatively in the chemical composition of the defensive secretion. This pattern corroborates Azevedo et al. (2006) who studied populations of C. cyphergaster (Termitidae) and Krasulová et al. (2012) who evaluated populations of Psammotermes hybostoma (Rhinotermitidae).

Still referring to the formation of population groups based on chemical composition, it was found that the main components responsible for clustering the population group I, 2-hexanol and 3-hexanol, in the first canonical variable dependent was positively correlated with climatic variable independent (rainfall, relative humidity, soil moisture and clay in the soil), while the  $\alpha$ -pinene showed an inverse correlation with these variables. But the main components of group II, the  $\alpha$ -pinene,  $\beta$ -pinene and limonene in the first dependent variable was correlated positively with climate independent variables (temperature, sand and silt percentage, electrical conductivity and soil pH). The first correlation accounted for 66 % of the cumulative variance (Figure 2).

One of the populations of Pocinhos (P1) had the highest percentage of  $\alpha$ -pinene observed (64.9 %), which can be attributed to the fact that this sampling point had lowest rainfall rate and highest thermal index among sampling sites. The opposite was observed for 2-hexanol and 3-hexanol, present only in populations

of Brejo de altitude, perhaps due to the occurrence of higher rainfall and low thermal indices. Azevedo et al. (2006) found a positive correlation between the 3-hexanol and climate variables to C. cyphergaster as  $\alpha$ -pinene was negatively correlated.

These factors may encourage populations of termites to regulate the concentration of each chemical, if not directly, than indirectly, because these factors significantly influence the availability of food resources, diet, frequency of foraging and biomass increment of termites in the study areas (Moura et al. 2006, Vasconcellos et al. 2007). This regulation could explain the occurrence of unique compounds in the populations of each ecoregion. This hypothesis is based on the fact that the areas studied have different environmental characteristics and variations in abiotic factors, resulting in different plant communities adapted to the limitations and heterogeneity of each environment. However, it is possible that these variations are the product of a seasonal effect or even the needs of the colony in relation to defense activities, recruitment or alarm pheromones (Vrkoč et al. 1978). The results of this study contrast with studies by Prestwich et al. (1984) and Gush et al. (1985), which concluded that environmental factors did not influence the chemical composition of the defense secretion of N. corniger, but corroborate Azevedo et al. (2006) and Krasulová et al. (2012).

### Conclusion

The present study demonstrated that the chemical composition of the defensive secretion of *N. corniger* varies depending on the characteristics of the ecosystem where the termites live. Populations located in areas that have similar ecosystems and are geographically close tend to have higher chemical similarity in terms of both concentration and composition of substances. As expected, the populations from urban areas of Brejo de Altitude presented

chemical differences compared to the areas of Caatinga, which is probably the result of environmental pressures and climatic factors that influence the proportions and chemical composition in each ecoregion. In addition, it is likely that genetic variations, due to the geographic isolation of populations combined with the low capacity of dispersion of the termites, promote variations in the phenotypic patterns of the defense secretion, which has resulted in the formation of two possible ecotypes.

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