



# INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA PROGRAMA DE

# PÓS-GRADUAÇÃO EM ECOLOGIA

# ALLANA ATAIDE NEGREIROS

# FITOQUÍMICA E ECOLOGIA DA INTERAÇÃO DE TRÊS ESPÉCIES (PLANTA, LEPIDOPTERA E PRIMATA) EM UMA FLORESTA DE IGAPÓ, AMAZONAS, BRASIL.

# MANAUS, AM

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Dissertação apresentada à Coordenação do Programa de Pós-Graduação em Ecologia, como parte dos requisitos para obtenção do título de Mestre em Biologia com ênfase em Ecologia.

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#### Sinopse

Estudou-se a seleção alimentar de primatas da espécie *Cacajao ouakary*, Pitheciidae por folhas de *Buchenavia ochroprumna*, Combretaceae, infestadas e não infestadas por lagartas e as respostas químicas *B. ochroprumna* em sua interação com herbívoros primatas e lagartas em uma floresta de igapó no entorno do Parque Nacional do Jaú, Novo Airão, Amazonas.

**Palavras-chave**: Seleção alimentar, compostos fenólicos, lagartas, macacos, folhas.

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#### **RESUMO**

Buchenavia ochroprumna, Combretaceae, também conhecida popularmente como Tanimbuca, é uma das quatro espécies de igapó mais comuns no Parque Nacional do Jaú. Sendo uma espécie decídua, durante sua renovação foliar anual que se inicia ao final do período da cheia e início da vazante dos rios da bacia do Rion Negro, suas folhas são amplamente ingeridas tanto por lagartas da família Nymphalidae quanto por macacos uacaris das costas douradas, Cacajao oucakary, Pitheciidae. Porém, os uacaris demonstram forte seleção (índice Ivlev + 0,84) por folhas de indivíduos de B. ochroprumna que não estão sendo predados por lagartas e extrema rejeição (índice de Ivlev - 0,64) por plantas com lagartas. Uma vez que muitos estudos demonstraram mudanças na qualidade foliar em plantas após ataques de herbívoros, o presente estudo propôs investigar se a infestação por lagartas induziu mudanças químicas nas folhas de B. ochroprumna que pudessem explicar a seletividade pelos primatas C. ouakary. Para a investigação e comparação de compostos fenólicos entre indivíduos, nós coletamos folhas de B. ochroprumna não-infestadas como controle e folhas infestadas como tratamento. Para a investigação de variação individual de compostos fenólicos como forma de averiguar os efeitos da herbivoria por lagartas no local do dano e por toda a planta, foram coletados galhos de plantas infestadas por lagartas em diferentes situações: (i) com lagartas presentes e (ii) sem lagartas presentes. Os resultados obtidos não mostraram valores significativos de p na quantidade de compostos fenólicos em plantas infestadas e não infestadas nem variação entre folhas de indivíduos infestados relacionada à herbivoria. Devido a isso, possíveis razões para a falta de efeito da herbivoria na quantidade de compostos fenólicos foram discutidas, incluindo a possível falta de tempo necessário para a planta responder ao ataque inicial por insetos herbívoros, outros compostos sendo responsáveis pela defesa química da espécie B. ochroprumna, a rejeição do macaco estar direcionada as lagartas propriamente ditas e principalmente, sugerimos que a quantidade de compostos fenólicos presente nas plantas estudadas pode estar sendo direcionada ao papel de defesa contra estresse abiótico e não aos herbívoros.

## Abstract

Buchenavia ochroprumna, Combretaceae, is a common species in Jaú National Park igapós, whose leaves have been target of both unidentified nymphalid caterpillars and monkeys Cacajao oucakary, Pitheciidae, during flooding season every year. However, C. ouakary shows strong selection for trees not being fed on by caterpillars and strong rejection of those with caterpillars feeding upon them. A variety of studies have found leaf quality in plants after herbivore attacks. Consequently, the current study proposed to investigate whether caterpillars infestation changed chemical aspects of B. ochroprumna in ways could explain C. ouakary's preference for those trees lacking caterpillars. To test whether the effects of caterpillars herbivory occurred only at the site of damage and throughout the whole plant, we sampled and compared the amount of phenolic compounds in leaves of the same trees but in different branch situations: (i) with caterpillars feeding upon them and (ii) with no caterpillars upon and no signals of damage, as well as (iii) between infested and uninfested plants. The results showed no significant difference in total phenols between herbivorized and unhebivorized branches on the same tree, as well as between infested and uninfested trees. Possible explanations for this lack of herbivory-induced effects on the amount of phenolic compounds include insufficient time-lag between damage and full response by trees, other compounds being responsible for defense in B. ochroprumna trees, monkeys rejection for caterpillars themselves and, probably most importantly, the role of phenolic compounds in B. ochoprumna trees in defense against abiotic stressors.

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# INTRODUÇÃO GERAL

Interações entre plantas e animais são diversas e comumente são mediadas por compostos químicos, como na polinização, onde a composição química dos aromas florais age como um dos principais canais de comunicação entre as plantas e seus polinizadores (Raguso, 2008), assim como também na dispersão onde as cores chamativas dos frutos maduros se originam de processos químicos e atraem animais para alimentar-se deles (Harborne, 2001). Os compostos químicos da planta também podem ser utilizados por insetos ovipositores no reconhecimento de plantas hospedeiras (Simmonds, 2001), na sinalização de plantas inapropriadas para oviposição (Rothschild et al., 1988) e na defesa das plantas em resposta ao dano causado por herbívoros, a qual pode atrair inimigos naturais desses herbívoros (Vet & Dicke, 1992) ou produzir compostos tóxicos que podem prejudicar tanto os herbívoros responsáveis pelo dano inicial quanto herbívoros subsequentes (Poelman & Kessler, 2016).

No contexto da folivoria, insetos e mamíferos provocam impactos nas plantas ao se alimentarem extensivamente delas e removerem seus tecidos vegetais, principalmente folhas (Coley & Barone, 1996), Muitos folívoros demonstram preferência por folhas jovens uma vez que estas são mais palatáveis, tem mais nutrientes e baixo conteúdo de fibras do que folhas maduras (Coley & Barone, 1996; Kursar & Coley, 2003). Uma vez que a planta recebe danos por herbívoros, ela pode ser induzida a produzir ou aumentar a quantidade de compostos químicos defensivos em seus tecidos (Wold & Marquis, 1997; Rodriguez-Saona et al., 2009; Mithöfer & Boland, 2012). Dependendo da severidade do ataque, tal resposta pode ocorrer somente no local do dano ou por toda a planta (Rossiter et al 1988, Zhou et al., 2016).

Muitos estudos demonstram que os compostos químicos das plantas são um fator relevante na seleção de itens alimentares por mamíferos, os quais muitas vezes se baseiam em estímulos pré-digestivos (sabor e odor) e pós-digestivos (baixa digestibilidade, nausea e tocixidade) na escolha do alimento (Foley et al., 1999; Iason & Villalba, 2006). Takemoto (2003) reportou que a quantidade de taninos nas folhas é um fator importante na escolha de alimentos por chimpanzés na África, onde as folhas consumidas apresentaram menores quantidades desse composto do que folhas não consumidas. Marks (1988) mostrou em um estudo com macacos da espécie *Macaca* 

*mualatta* que a escolha alimentar desses macacos esteve negativamente correlacionada com a quantidade de fenóis totais presentes em todos os itens alimentares analisados. De acordo com Wrangham & Waterman (1981), os macacos da espécie *Cercopithecus aethiops* selecionam partes de *Acacia xanthophloea* e *A. tortillis* – duas espécies de planta que compõem mais de 50% da dieta do macaco – que tiveram menor quantidade de fenóis totais.

Interações isoladas na natureza são raras, pois no geral estas são difusas e envolvem um amplo leque de indivíduos (Stam et al., 2014). Sendo assim, o dano causado por insetos herbívoros pode induzir respostas defensivas nas plantas capazes de afetar suas interações posteriores com outros indivíduos. Dessa forma, baseado em mediação química de compostos químicos entre plantas e outros indivíduos, Barnett et al. (2013) and Negreiros et al. (submetido) sugerem que uma interação química tritrófica pode estar ocorrendo entre a planta tanimbuca tree (*Buchenavia ochroprumna* Eichler, Combretaceae), lagartas de uma espécie não identificada de lagartas e um primate (*Cacajao ouakary* Pitheciidae) em uma floresta de igapó, no Parque Nacional do Jaú.

O uacari de costas douradas é um primata de tamanho médio endêmico das florestas de igapó na Bacia do Rio Negro, Amazonia, Brasil e Colômbia (Barnett, 2005). Sua alimentação consiste principalmente de frutos imaturos, possuindo dentes especializados na abertura de sementes (Barnett et al., 2005, 2013). Porém, nos meses em que seu item alimentar principal encontra-se pouco disponível, este primata incorpora em sua dieta itens como folhas (10,8%), flores (7,2%) e insetos (2,4%). Dentre dos itens foliares, as folhas jovens de tanimbuca constituem 10.9% da dieta do uacari (Barnett, 2010).

A tanimbuca é uma espécie comum nas margens do igapó. Cresce aproximadamente 15 metros de altura e possui copa em formato de cone invertido quando não está parcialmente imersa durante a inundação do rio. É uma espécie decídua que perde todas as folhas quando o rio atinge o pico de inundação no mês de junho e começa a lançar novas assim que inicia a vazante, levando por volta de 40 dias até sua expansão total e endurecimento, janela de tempo na qual a espécie é amplamente predada por larvas de Lepidopteros da família Nymphalidae (Barnett, 2010). As lagartas da família Nymphalidae encontradas na tanimbuca são marrom escuro cujas médias de peso e comprimento de 32 animais no último estágio larval foram de 295.15 mg (SD ±76.64, variação 186-416) e 22.67 mm (SD±2.0, variação 19.2-25.4), respectivamente. Possuem espinhos ramificados por toda a região abdominal superior, com quatro espinhos maiores localizados na cabeça e final do abdome. Sua coloração críptica as permitem se camuflar nas bases dos pecíolos e região abaxial das folhas.

## **OBJETIVO GERAL**

Investigar a seletividade de primatas da espécie *Cacajao ouakary* em relação a indivíduos de *Buchenavia ochroprumna* infestados e não infestados com larvas de Lepidoptera e analisar o efeito da herbivoria das larvas na composição química das folhas da planta.

# **OBJETIVOS ESPECÍFICOS**

- **a.** Investigar dados de observações de campo em relação à alimentação do primata *C. ouakary* e identificar se há seleção por indivíduos de *B. ochroprumna* correlacionada à presença ou ausência de larvas de uma espécie de Lepidoptera.
- **b.** Analisar e comparar a composição química de folhas de *B. ochroprumna* infestadas e não infestadas por larvas de uma espécie de Lepidoptera para verificar o efeito da herbivoria desse inseto na indução de defesas pela planta.

# The bitter end: primate avoidance of caterpillar-infested trees in a central Amazon flooded forest.

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#### **ABSTRACT**:

Animal-plant interactions are often mediated by chemical compounds. It has been widely reported that herbivore damage to plants induces chemical defenses which are able to affect their subsequent interactions with both invertebrates and vertebrates herbivores. The present study investigated the effects of the interaction between an unindentified larvae of Nymphalid butterfly and tanimbuca tree (*Buchenavia ochroprumna* Eichler, Combretaceae) on folivory by a primate, the golden-backed uacari (*Cacajao ouakary* (Spix) Pitheciidae). Primate feeding observations, records of the extent of Nymphalid/*Buchenavia* interactions and tree distribution occurred in Jaú National Park, Amazonas State, Brazil. Ivlev Electivity Index values showed that uacaris were strongly reject trees infested by caterpillars (- 0.68), while uninfested trees are highly selected by them (+ 0.84). Given this behavior, we suggest that uacari monkeys may be deterred by (i) caterpillars; (ii) change in leaf chemical composition induced by caterpillars; (iii) or a combination of both.

KEY WORDS: avoidance, *Buchenavia, Cacajao,* igapó, induction, Lepidopteran herbivory, secondary compounds

#### INTRODUCTION

Herbivorous animals can have both direct and indirect interactions with plant leaf metabolites (both primary and secondary) (Mithöfer & Boland, 2012). Direct interactions can include selective ingestion of leaves with high levels of primary metabolites such as protein or carbohydrates (Pérez-Harguindeguy et al., 2003), avoidance of leaves rich in secondary metabolites toxins (phytoalexins) (Narberhaus, Zintgraf & Dobler, 2005), flavonoids (Truetter, 2006), silica (McNaughton & Tarrants, 1983), terpenes and alkaloids present in latex exudates (Agrawal & Konno, 2009), irritant volatile compounds (Paré & Tumlinson, 1999), and mainly tannins (Bernays, Driver & Bilgener, 1989; Barbehenn & Constabel, 2011), as well as the use of extrafloral nectaries (Heil et al., 2000; Koptur, 1984), and the sequestration of plant defensive chemicals (Opitz & Müller, 2009). Indirect interactions may include interactions with other animals that have sequestered plant defensive chemicals (Dobler, 2001; Müller et al., 2006), the use of damage associated volatiles to locate potential prey (Amo et al., 2013), or interactions with plants whose defenses have been induced, either by previous incidences of herbivory that occured to the plant being fed upon (Green & Ryan, 1972; Karban & Myers, 1989; Frost et al., 2007), or to a neighbor (Kessler & Baldwin, 2001). Such induced changes may be short- (Clausen et al., 1989) or long-term (Kost & Heil, 2006).

In primates, most reported interactions are direct, with tannins/protein ratios influencing leaf choice by folivorous primates at the between-species level (Milton, 1979; Oates, Waterman & Choo, 1980; McKey *et al.*, 1981; Davies, Bennett & Waterman, 1988; Kool, 1992; Hanya *et al.*, 2007), though there have also been studies of leaf-choice between conspecific trees, and at the within-individual level (Kar-Gupta & Kumar, 1994; Silver *et al.*, 2000; Matsuda *et al.*, 2013; Ganzhorn *et al.*, 2017). Indirect

interactions are rarely reported and appear to be restricted to interactions between foraging individual primates and defensive actions of commensal ants maintained by the host plant's sugary exudates (McKay, 1974; Barnett *et al.* 2015), and the medicinal use of arthropods bearing sequestered phytoalexins (Overdorf, 1993; Birkenshaw, 1999; Zito, Evans & Weldon, 2003; Valderrama, 2000; Weldon *et al.*, 2003), or the ingestion of such species by specialist micro-carnivores (e.g., slender loris, *Loris tardigradus*: Nekaris, 2001; Nakaris & Rasmussen, 2003).

While there are many examples of induced chemical defences subsequently deterring invertebrates (Koul, 2008; Maffei, Mithöfer & Boland, 2007) or vertebrates (Lindroth & Batzli, 1984; Bryant & Clausen, 1992; Tixier *et al.*, 1997; Zimmerling & Zimmerling, 1996; Adams, 2014), such a system has never been reported between a primate species and its food plant. Here we report such a possible interaction, involving the tanimbuca tree (*Buchenavia ochroprumna* Eichler, Combretaceae), the larvae of an unidentified Nymphalid butterfly and the golden-backed uacari monkey (*Cacajao ouakary* (Spix), Pitheciidae) in seasonally-flooded forests in central Brazilian Amazonia.

Other studies at Jaú have found that (i) for some plant species, uacaris select seeds with insects inside them (Barnett, 2010), and that (ii) in the season of low fruit availability they eat insects, in addition to large volumes of leaves, and that (iii) these include several types of lepidopteran larvae (Barnett, 2010). Consequently, based on the hypothesis that uacaris are using leaves and insects as fall-back foods in the season of low fruit availability, we tested the following predictions: (1) Uacaris will visit *B. ochroprumna* trees with both new leaves and caterpillars, and will prefer such trees to those with no new leaves or trees with new leaves but without caterpillars; (2) At infested trees both the leaves and the caterpillars will be eaten more frequently than one

single item alone; and (3) The closest tree to a feeding tree will be the next feeding tree, but only if it bears caterpillars.

#### **METHODS**

### Study site

Field observations were made between Oct 2006 and April 2008 in Jaú National Park, (1°53'15.96"S 61°41'25.46"W), a 2.3 million ha protected area between the Unini and Carabinani rivers in Brazilian Amazonia, situated some 220 km west of the city of Manaus on the south bank of the Rio Negro, Amazonas State, Brazil (Fig. 1). Habitats include rainforest (80%+), igapó (12%), white sand vegetation types (2%), palm and aroid swamps (2%), and disturbed land (<1%: FVA-IBAMA, 1998). The peak rainy season is between November and April, the minima between June and September. The river level is at its peak in April-September and its nadir between October-January (Ferreira, 1999).



FIG. 1. Study site, showing Carabinani Research Base, Jaú National Park and its location in the Brazilian Amazon.

#### Species

## The plant

The tanimbuca tree (Buchenavia ochroprumna) occurs principally in western Amazonia (Rio Negro and western Orinoco river basins) (Marquete & Loiola, 2015). It is a common tree of seasonally flooded forests on the margins of blackwater rivers in the Amazon basin (igapó, sensu Prance, 1979). Such forests have a strong seasonal floodpulse that lasts for up to nine months of the year, during which waters may attain heights of 10 m above their lowest mark (Haugaasen & Peres, 2006). Many igapó species, including B. ochroprumna, are water-dispersed (Correa et al., 2007), and so peak-fruiting most commonly occurs at the time of highest inundation (Ferreira & Parolin, 2007; Hawes & Peres, 2016). Inundation also appears to be responsible for leaf-drop, an event that occurs simultaneously in many species of igapó tree (Parolin et al., 2002). Surviving protracted seasonal inundation requires a suite of specialized physiological adaptations, both in adult trees (Parolin, Ferriera & Wittmann, 2005), and in newly-germinated seedlings (Parolin, 2002; Parolin, Ferreira & Junk, 2003). Species vary in their capacity to survive prolongued inundation and, as a result, the species composition of the igapó plant assemblage is highly stratified, with distinct banding occuring across the area between the never-flooded (terra firme) forest and the open river that igapó occupies (Ferreira, 1997; Ferreira & Stohlgren, 1999). Such an area will generally be some 30-150m wide (Prance, 1979; Barnett, 2010). Buchenavia ochroprumna has medium tolerance to flooding and, as such, is found in the middle band of the igapó forest (Barnett, 2010).

*Buchenavia ochroprumna* grows to some 15 m, has a characteristic inverse-cone shape when fully emergent from floodwaters (the result of flood-time grazing by herbivorous fish) and spatulate (sometimes bluntly cunate) coreaceous leaves that occur in clusters of terminal whorls of five to nine leaves (A.A. Negreiros, A.A. Barnett, unpublished data) (Fig. 2a and b). These leaves are shed in June, with new flushing within two weeks (A. A. Barnett, unpublished data). Leaves take 30-40 days to reach full size (some 4.5cm) and hardness, during which time they are preyed upon intensively by the larvae of a Nymphalid lepidopteran (Barnett, 2010).



FIG. 2: *Buchenavia ochroprumna* showing (left) adult tree with characteristic inverted cone-shaped growth form, and (right) whorls of bluntly cunate new leaves in an uninfested tree.

#### The insect

The Nymphalid caterpillars of were dark mottled brown and smooth skinned, but with irregular stiff protruding spikes (Fig. 3). Mean weights and lengths of 32 animals in the final instar, were 295.15 mg, (SD  $\pm$ 76.64, range 186-416) and 22.67 mm (SD $\pm$ 2.0, range 19.2-25.4), respectively. A count of the number of caterpillars on 73 leaf whorls of *B. ochroprumna* found a mean of 3.08 caterpillars per whorl (SD  $\pm$ 1.73, range 1-7).



FIG. 3: Nymphallid caterpillar feeding on new leaves of *Buchenavia ochroprumna* (left) and (right) leaves of *Buchenavia ochroprumna* showing characteristic damage patterns from caterpillar feeding that allowed infestation assessment at a distance of several meters.

#### The primate

The golden-backed uacari (*Cacajao ouakary*: Pitheciinae) is a medium-sized primate (mean weight 3.5 kg; mean body length (mm) 389 (f), 414 (m): Hershkovitz, 1987) whose principal habitat is the blackwater flooded forests (*igapó*) of the Rio Negro Basin, Amazonia, South America (Barnett, 2005). The home range can exceed 2 km<sup>2</sup> (Bezerra, Souto & Jones, 2010). The diet is principally immature seeds (Barnett *et al.*, 2005, 2013). However, in those months of the year when, as a result of the highly-seasonal phenology of igapó, there is little fruit available, these primates become predominantly folivorous (Barnett, 2010). At this time, the leaves and buds of tanimbuca constitute 10.9% (118/1084) of all *C. ouakary* foliage-based feeding records (Barnett, 2010).

#### Field methods

As part of part of a broader study of the foraging ecology (see Bezerra *et al.*, 2010; Barnett & Shaw, 2014; Barnett *et al.*, 2012, 2013, 2016) golden-backed uacaris were followed through their black-water seasonally flooded forest (igapó, *sensu* Prance, 1979) habitat in a wooded canoe, and, following Altman (1974), feeding observations were made using both scan sampling and *ad libitum* observations.

Within igapó five 0.25 ha botanical study plots were set up, positioned to cover the range of inundation durations. In each, all woody plants above 10 cm DBH were tagged and identified. Phenology (leaf flush, flowering and fruiting) was recorded once-a-month from December 2006 to November 2008, with all incidences and extent of insectivory on leaves noted as a part of standardized field protocol, which included a classification of infestation intensity in 20% increments. Feeding was defined as an item being placed in the mouth and ingested. For plants species from which uacaris ate leaves, individual plants were always checked for leaf-damange that indicated recent prior herbivory. Both in and outside the study plots, trees on which uacaris fed, were marked with numbered tags.

Distances to nearest conspecific and to nearest known food tree of any species measured or each tree in which feeding was recorded. Incidences of trees being revisited were also recorded. For *B. ochroprumna* whether the fed-upon tree was being eaten by caterpillars was recorded, as was (for non-infested trees) the distance to the nearest infested tree, we also measured whether the infested tree was the closest living individual of *B. ochroprumna* to the tree on which leaf-feeding had been recorded, or whether closer trees existed. If they did, we measured the distance between then and the tree at which

the uacari had fed and the tree with caterpillars. Distances were measured with tape measures or a lazer rangefinder (Bushnell Yardage Pro).

Infested trees were recognised by reduced size of new leaves, characteristic patterns of leaf-margin reduction and by the abundant frass, both falling from the trees and collecting beneath them on the surface of the slow-moving (0.2 m/hr: Barnett & Shaw, 2014) igapó waters.

#### Statistical analyses

Both predictions 1 (uacaris will visit more frequently *B. ochroprumna* with both new leaves and caterpillars) and 2 (at infected trees, uacaris will eat leaves and caterpillars more frequently than single item alone) were tested by calculating Ivlev electivity index (Ivlev, 1961) for fed-upon trees (counting multiple visits as one). This index compares the relative frequency of used items in the diet with their relative frequency in the habitat. Ivelv's electivity index ranges from -1 to +1, with species used by the animal at parity to their relative frequency in the habitat having an Ivlev electivity index of 0. Items with a use frequency exceeding that in the habitat sample are considered to be positively selected (and so have positive value), while those used at less than parity will have a negative value. The index is calculated with the equation  $r^i - p^i / r^i + p^i$  where  $r^i$  is percentage of item i in the diet and  $p^i$  is its percent composition in the habitat.

We also compared the frequency of eaten infested trees with their frequency among the total sample of 94 tanimbuca trees present in the five 0.25 ha igapó phenology and botanical composition study plots. For that we summed the counts of each *B. ochroprumna* category (eaten uninfested, eaten infested, uneaten uninfested and uneaten infested) of the five plots, and compared if uacaris feed more frequently on infested trees than expected by chance using chi-squared test.

To analyse the effect of proximity on feeding behavior, Euclidian distances between *Buchenavia* trees in which uacaris fed and nearest infested and non-infested *Buchenavia* trees and Euclidean distances between not infested and infested caterpillar trees were measured in three plots. Given the unbalance sampling design the data sets were compared with bootstrap permutation tests (sampling with replacement) based on 999 replicates implemented for each sampling plot. In each run the two distances measures were randomized and a t-test test computed. We then compare the t-value of the 999 permutations with the observed value to calculate the probability that the observed value was different from chance. All analyses were done in R (R Core Team 2016).

#### RESULTS

Buchanavia ochroprumna comprised 6.6% of the 1,416 trees present in five 0.25 ha igapó botany inventory and phenology study plots (hereafter BIP plots: 94 individuals, the 4<sup>th</sup>. most common tree). During the study period 77 of these trees (81.9%) were infested with caterpillars. Infestation levels on individual trees varied from low ( $\leq$  20% of leaf whorls with signs of feeding) to extremely high ( $\geq$  80%). At the same time, *C. ouakary* individuals were recorded eating *B. ochroprunna* leaves on 118 occasions, making this species the 3<sup>rd</sup> most commonly eaten for leaves in the uacari diet. Feeding was observed at 43 separate trees, of these only 3 were infested and these very lightly (6.9%). Of the eaten trees, 19 were in the botanical inventory and phenology study plots (44.2%), including two eaten while lightly infested. No uninfested in these plots remained unvisited by uacaris. All other *B. ochroprumna* feeding records occurred outside the study plots. Two other igapó areas bounded (maximum convex polygon: and known Tanimbuca 1 and 2, hereafter T1 and T2) by an area 10 m beyond 10 fed-upon trees had an additional 63 (T1) and 72 (T2) individual *B. ochroprumna* trees, of which 61 (T1, 83.5%; feeding trees included, so N=73) and 54 (T2, 65.8%; feeding trees included, so N=82) were infested, respectively. T1 also had two uninfested trees on which uacaris were not seen to feed; one feeding tree was lightly infested. T2 also had twenty eight uninfested trees on which uacaris were not seen to feed at 18 (64.3%). Summing the three samples gave a mean infestation rate of 76.6% (SD±8.5). Uacari use rate of uninfested trees varied from 100% (botanical study plot) to 83.3% (T1) and 35.8% (T2). Comparing these summed proportions for all eaten uninfested and uneaten infested trees gives an combined Ivlev value of -0.68, a value indicating extreme rejection of infested plants; while the value for eaten uninfested and eaten infested trees is + 0.84, indicating strong selection of uninfested trees.

Though uacaris feeding on lightly infested plants were seen to select only leaves and do so from uninfested parts of the tree, Prediction 2 could not be tested due to the very small sample size available (N = 3). Though this, in itself, reinforces the selectivity of the uacaris against infested plants.

Uacaris did not feed in trees adjacent to infested trees. The closest records achieved were for all three study plots was 9.7 m from such trees. the closest records between (non-infested) feeding trees was 36.9 m and the mean was 46.28 m ( $\pm$ 7.43, N=10), to a non-infested uneaten tree was 55.91 m ( $\pm$ 21.79, N=10), and to an uneaten infested tree was 18.26 m ( $\pm$ 4.93, N=10). The mean between distance infested uneaten trees was 10.96 m ( $\pm$ 4.07, N=60), and the closest distance was 4.1 m. A summary of comparisons, and the result of statistical tests, are given in Table 1.

Table 1: Effect of existing caterpillar infestation on tanimbuca tree chosen by a	uacaris as
sites for leaf-feeding.	

Plot	Category	mean (± SD)	P-value	Interpretation
Plot 73	Distances between tanimbuca trees with caterpillars on and the next nearest infested tanimbuca tree Distances between tanimbuca	10.96 (±4.07) 55.91	p = 0.001	Non-infested trees are rare or widely dispersed, while infested
	trees not-infested and the nearest not-infested tanimbuca tree.	(±21.79)		tress are common and/or clumped.
Plot 82	Distances between tanimbuca trees with caterpillars on and the next nearest infested tanimbuca tree	28.03 (±20.91)	p = 0.001	
	Distances between tanimbuca trees not-infested and the nearest not-infested tanimbuca tree.	35.97 (±18.23)		
Plot 7	Distances between tanimbuca trees with caterpillars on and the next nearest infested tanimbuca tree	3.46 (±1.03)	NA	
	Distances between tanimbuca trees not-infested and the nearest not-infested tanimbuca tree.	All trees infested in this plot		
Plot 73	Distances between tanimbuca trees at which uacaris fed and the nearest tree caterpillar infested tanimbuca tree	18.26 (±4.93)	p = 0.001	Uacaris are travelling further to feed from uninfested
	Distances between tanimbuca trees at which uacaris fed and the next tanimbuca on which the uacaris fed (in this plot not- infested tree).	46.28 (±7.43)		trees, reinforcing the data from the Ivlev Electivity Index that they
Plot 82	Distances between tanimbuca trees at which uacaris fed and the nearest tree caterpillar infested tanimbuca tree	19.3 (±11.34)	p = 0.001	are avoiding infested trees.
	Distances between tanimbuca trees at which uacaris fed and the next tanimbuca on which the uacaris fed (in this plot not- infested tree).	56.68 (12.51)		
Plot 7	Distances between tanimbuca trees at which uacaris fed and	4.625 (0.86)	p = 0.001	

the nearest tree caterpillar		
infested tanimbuca tree		
Distances between tanimbuca	8.16 (±2.95)	
trees at which uacaris fed and		
the next tanimbuca on which		
the uacaris fed.		

#### DISCUSSION

Contrary to expectations based on dual-item diet optimization, *Cacajao ouakary* did not feed more commonly on *Buchenavia ochroprumna* trees that had both caterpillars and new leaves. Instead, they almost completely avoided such trees, paying just a few visits to lightly infested trees (6.9% of feeding trees) where they ate leaves from branches well-away from those on which the caterpillars were feeding.

Given this behavior, three explanations seem possible: uacaris were either deterred by the caterpillars, by some change in leaf chemical composition induced by caterpillar herbivory, or a combination of both.

Certainly, uacaris do not dislike caterpillars *per se.* Barnett *et al.* (2013) reported incidences of this species actively seeking the larvae of tortricid moths in still-unfolded young *Swartzia* leaves, and this primate also preferentially eats seeds infested with lepidopteran larvae, including members of the owlet moth (Noctuidae) and clear-wing moth (Sesiidea) families (Barnett, 2010). Additionally, members of the genus *Chiropotes*, closely-related to *Cacajao*, have been reported eating hairy and spiny caterpillars, despite the obvious discomfort caused by handling them (Veiga & Ferrari, 2006). However, uacaris have been observed to actively avoid social caterpillars of the saturniid *Arsenura armida* and aposomatic Dalceridae caterpillars, both of which are known to be highly toxic (Barnett, 2010). They also avoided Lasiocampidae and Lymantriidae caterpillars. Many of the former are both hairy and toxic, while for the

latter, the defence is both long barbed setae which are both irritant and toxic (Deml & Dettner, 1995).

In addition, while choice based on avoidance of toxic or noxious chemicals has not yet been undertaken in the genus *Cacajao*, there are indications that some species known to be toxic are avoided, including the seeds of Dipteryx cf. oppositifolia, Swartzia laevicarpa and S. polyphylla (seeds of these genera being known for their high concentrations of toxic coumarins: Venugopala, Rashmi & Odhav, 2013 and Dembitsky & Tolstikov, 2004 - respectively). In addition, there is the fact that, since golden-backed uacaris only eat leaves in any quality for three months of the year, they are very much part-time folivores. As such they might well lack some of the detoxifying enzymes and gut bacterial flora found in primates or whom leaves from a greater part of the diet (e.g. Lambert, 1998) (though they do have some anatomical specializations to the gut that suggested partial folivory before this was known from the diet: MacLarnon, Chivers & Martin, 1986). This may explain the fact that, apart from tanimbuca, the other group which predominated in the leaf diet of C. ouakary at Jaú was young Fabaceae leaves (Barnett, 2010). Such items are known for high levels of nitrogen, and low levels of toxins (McKey, 1994). Thus, C. ouakary may be acutely susceptible to induced changes in phytoalexin levels, such as might be facilitated by massed caterpillar attacks on B. ochroprumna, and which are known to produce robust responses from other plant species (Pinus spp.: Hodar, Zamora & Castro, 2002; Populus tremuloides: Hemming & Lindroth, 1995; *Quercus suber*: Staudt & Lhoutellier 2007).

In addition, there is the fact that uacaris avoided neighboring tanimbuca trees, even when they were not infested with caterpillars. This opens the possibility that such trees were also showing induced phytochemical responses, as a result of volatile compounds released by neighboring trees (see Baldwin *et al.*, 2006 and Heil & Karban, 2010 for topic reviews).

In-field attempts to raise caterpillars to the imago stage, and so facilitate species (or genus) identification, were forstalled by ants. Thus, it is currently not possible to distinguish between the three options cited above, even if uacari avoidance of proximate uninfested trees favors the phytochemical option. The situation can only be fully examined with future studies that (i) identify the species (or genus) of Nymphalidae involved, so allowing analysis of potential chemical defenses and toxicity, (ii) assay the larvae for the presence of toxic and/or noxious compounds, and (iii) chemically analyse leaves of *B. ochroprumna* from both infested and uninfested trees to test the possibility that chemical responses induced by the herbivory of the caterpillars had made the leaves unpalatable to these part-time folivores.

Further analysis of this system could be highly relevant to an enhanced understanding of multi-species interactions in igapó ecology. Indirect invertebrate/vertebate folivore interactions mediated by damage-induced phytochemical responses of the herbivorized plant, may not be uncommon in this habitat as heavy infestations of young *Eschweilera tenuifolia* (Lecythidaceae) leaves by Hesperiid caterpillars were ignored by uacaris at Jaú, even as the primates ate the emerging leaves of uninfested conspecifics.

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## **Running Header: flooded forest phytochemistry**

# Lack of herbivore-induced phytochemical responses in an Amazonian flooded forest tree: phenological and energetic responses to an extreme environment?

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## Abstract

Buchenavia ochroprumna, Combretaceae, is a common species in Jaú National Park igapós, whose leaves have been target of both unindetified nymphalid caterpillars and monkeys Cacajao oucakary, Pitheciidae, during flooding season every year. However, C. ouakary shows strong selection for non-grazed by caterpillars B. ochroprumna individuals and extreme rejection for those with caterpillars feeding upon them. Once many studies have revealed changes in leaf quality in plants after herbivore attacks, our study proposed to investigate if caterpillars infestation changed chemical aspects of B. ochroprumna both inter and intra-tree. We sampled non-infested trees as controls and infested ones as treatments and compared amounts of total phenols on them for intertree investigation. For intra-tree variation, we sampled and compared amounts in phenolic compounds in leaves of the same trees but in different branch situations: (i) with caterpillars feeding upon them and (ii) with no caterpillars upon and no signals of damage. The results showed no significant difference in total phenols neither between infested and uninfested trees, nor between same tree branches with caterpillars presence and absence. We suggested some possibilities for this lack of herbivore effects on the amount of phenolic compounds, which include non sufficient time-lag between damage and full response by trees, other compounds being responsible for defense in B. ochroprumna trees, monkeys rejection for caterpillars themselves and mostly, the role of phenolic compounds in *B. ochoprumna* trees in defense against abiotic stressors.

Key words: Induced defense – phenolic compounds – caterpillars – herbivores - monkeys

## INTRODUCTION

Plants can synthesize a great variety of chemical compounds using both primary and secondary metabolism. Primary metabolism compounds are involved in vital activities such as growth and development, whilst the products of secondary metabolism operate mainly as mediators of plant interactions (Croteau et al., 2000; Harborne, 2001; Iason, 2005; Iason & Villalba, 2006; Mithöfer and Boland, 2012) both negatively (allelopathy: Bais et al., 2003), and positively (inter-tree chemical warning of herbivore attacks: Baldwin and Schultz, 1983). With animals, such products facilitate pollination (Raguso, 2008), and seed dispersion (Harborne, 2001), and play key roles in the attraction or deterrence of ovipositing insects (Thompson & Pellmyr, 1991) and invertebrate herbivores (Coley & Barone, 1996).

In the context of folivory, insect and mammalian herbivores may exert great pressure over plants feeding extensively and removing expensive-to-make tissues from plants, mainly leaves (Coley & Barone, 1996). Many folivores show a marked preference for younger leaves as they are more palatable, higher in nutrients and palatability and have a lower content of fibrous and digestible tissues than do old ones (Coley & Barone, 1996; Kursar & Coley, 2003). To achieve either initial deterrence or to reduce the possibility of subsequent attacks, such feeding may induce the production or increase the content of chemical plant defenses (Wold & Marquis, 1997; Rodriguez-Saona et al., 2009; Mithöfer and Boland, 2012). Depending on the severity of the attack, such responses may occur locally at the site of damage or systemically over the entire plant (Rossiter et al., 1988, Zhou et al., 2016).

In response, herbivores have developed a great variety of strategies to deal with plant defenses, ranging from avoidance to detoxification (Foley et al., 1999; Harborne, 2001; Iason & Villalba, 2006). Insect herbivores may also use plant defense chemicals to their own advantage, by sequestering toxic chemicals from their food plants in order to protect themselves of bird predation (e.g. *Danaus plexippus*: Reichstein et al., 1968; *Junonia coenia*: Dyer & Deane-Bowers, 1996; *Althalia* sawflies: Opitz et al., 2010; see Opitz & Muller 2009 for review). Mammals in turn may learn to select their food based in association between pre-ingestive stimuli (taste, smell) and post-digestive stimuli (reduced digestibility, nausea and toxicity) (Foley et al., 1999; Iason & Villalba, 2006).

Among many other secondary metabolites known to protect plants against their biotic stressors, the class of phenolic compounds include many that may reduce digestibility, palatability and nutritional rates (Todd et al., 1971; Harborne, 2001), and are capable of deterring herbivory both by insects and mammals (Foley et al., 1999). Marks (1988) showed on a study that food choice of rhesus monkeys (*Macaca mulatta*) was significantly negatively correlated with total phenols in all samples of their food itens. Also, according to Wrangham & Waterman (1981), the vervet monkeys (*Cercopithecus aethiops*) select the parts of *Acacia xanthophloea* and *A. tortillis* – the major (50%) plant on vervet monkey diet – that were lower in total phenolics. Phenolics

such as salicin and narigin may add bitter taste to food in high concentrations leading *Saimiri sciureus*, *Ateles geoffroyi*, and *Macaca nemestrina* monkeys to refuse them (Laska, 2009).

It is rare that interactions in nature directly and simultaneously involve only two actors, the great majority are diffuse and involve a broad spectrum of species (Stam et al., 2014). In consequence, damage caused by insect herbivores may induce defensive responses in plants that affect all subsequent interactions of the plant (Poelman & Kessler, 2016). Thus, based on mediation of chemical compounds among plants and other organisms, Barnett *et al.* (2013) and Negreiros et al. (subtmitted) suggested that a tritrophic chemical interaction might be occurring between the tanimbuca tree (*Buchenavia ochroprumna* Combretaceae), caterpillars of an unidentified species of Nymphalidae butterfly, and a primate (*Cacajao ouakary* Pitheciidae) in igapó, a seasonally-flooded forest type occurring on the margin of blackwater-rivers in western Amazonia (Prance, 1979; Junk et al., 2015).

In the Jaú National Park, Brazil, *Buchenavia ochroprumna* is the 4<sup>th</sup> most common tree in igapó. There, of 229 *B. ochroprumna* individuals 132 were infested with caterpillars of an unidentified species of Nymphalidae butterflies. This infestation occurs annually on the new leaves that flush following leaf drop by all *B. ochroprumna* during the June flooding peak of the rivers in Rio Negro basin. The new leaves are produced in July as soon as the river starts to drop (Barnett, 2010; Barnett et al., 2013; Junk et al., 2015; Negreiros et al., submitted). Caterpillars appear soon after, with levels of infestation varying from low ( $\leq 20\%$  of leaf whorls with signs of feeding) to severe ( $\geq 80\%$ ) (Negreiros et al., submitted). Adult *B. ochroprumna* have only their crowns above the flood water during the inundation period that usually occurs for up to 8 months annually (Junk & Piedade, 2010).

Newly flushed leaves have higher nitrogen contents and lower proportions of lowdigestibility structural components than mature ones, so that they are generally preferred by herbivores (Lamarre et al., 2014). Accordingly, when B. ochroprumna flushes new leaves, besides being eaten by caterpillars, it is also the 3<sup>rd</sup> most commonly item among leaves in the diet of the C. ouakary. This primate feeds on this tree throughout the year, but uses different parts according to seasonal availability; from November to June it feeds mainly on *B. ochroprumna* fruits, whereas from July to October it feeds mostly on new leaves of this tree (Barnett, 2010; Barnett et al., 2013). Negreiros et al. (submitted) observed that, of the 229 Buchenavia ochroprumna trees present in their study plots, 132 were infested with caterpillars, while the uacaris fed on leaves from 29 trees (26 of them being uninfested with caterpillars and 3 just lightly infested). From these observations, it was made an index that compares relative frequency of used items in the diet with their relative frequency in the habitat, the Ivley index. The calculated Ivley Electivity Index value for uninfested trees was + 0.84, indicating their strong selection by uacaris. In contrast, the Ivlev value was -0.68 for infested trees, indicating extreme rejection.

Based on the extreme rejection of infested *B. ochroprumna* trees by uacari monkeys, this study proposed to investigate whether this rejection was due to an increase in defensive chemical compounds in the eaten young leaves. Assays of phenols were chosen as a general proxy for defensive compounds, since (i) they are one of the most extensively compound measured in the herbivory context, and (ii) have been widely implicated in herbivore forage selectivity. For example, ungulates in the Mediterran region select plants with lower phenolic (Massei et al., 2000); primates choose food items with low phenolic content in both Africa (*Cercopithecus aethiops*, Oates et al., 1977; *Colubus guereza*, Wrangham & Waterman, 1981), and Asia (*Presbytis johnii*, Oates et al., 1980), and changes in leaf quality are known to occur after herbivore attack (e.g.: *Quercus rubrum* after *Lymantria dispar* defoliation in north America: Schultz & Baldwin, 1982; *Betula pendula* after *Apocheima pilosaria* grazing in Europe: Hartley & Firn, 1989).

Given the importance and relevance of phenolic compounds in studies involving herbivores, the current study was therefore designed to test: a. If there is difference in the content of phenolic compounds between individual trees of *B. ochroprumna* that have, or have not, been eaten by caterpillars that may explain the primate's avoidance of those with caterpillars; b. If so, whether infested trees show intra-tree variation in the amount of phenols branches where caterpillars are feeding on and branches where caterpillars have yet to feed, in order to determine if caterpillars damage is severe enough to cause systemic induced defense or if induced defense occur only locally.

## MATERIAL AND METHODS

#### Study site

Field work was conducted in 2016 from the  $11^{th}$  to the  $16^{th}$  August in Jaú National Park, Amazonas, Brazil, from the IBAMA field base *Carabinani* (1°54'17.1"S 61°25'48.2"W), situated near the mouth of the Jaú River, a southern (right side) affluent of the Rio Negro, some 220 km upriver from Manaus, the Amazonas state capital (Figure 1). Fieldwork involved visits to unimpacted *igapó* forests on both margins of the Jaú river, at the beginning of the ebb when *B. ochroprumna* new leaves were open but still expanding and hardening.



Fig. 1. Study site, showing Carabinani Researh Base, Jaú National Park and its location in the Brazilian Amazon.

## Data collection

Leaf secondary compound content can vary daily depending on intrinsic (e.g. metabolic cycles) (Pichersky et al., 2006) and extrinsic (e.g. temperature) factors (Zobayed & Kozai, 2005). To minimize the influence of such variation, *B. ochroprumna* leaves were collected daily between 0700 - 1000. This is also the period when, probably to avoid thermal stress, primates and lepidopteran larvae forage most extensively (Fitzgerald et al., 1988; Campos & Fedigan, 2009).

During field studies 145 *B. ochroprumna* individuals were encountered, of which 10 individuals were sampled as a control group (uninfested trees) and 10 sampled as examples of infested individuals. The growth form of *B. ochroprumna* is that of an inverted cone, with branches first growing horizontally and then vertically. Leaves are flushed at the point of transition and the next season's shoot grows from this base; a growth-form is known rhythmic appositive (Ribeiro et al., 1999) (Fig. 2). The new years' shoots each bear a whorl of 4-7 leaves (mode, 5). Consequently, for the control group, sampling consisted of cutting a random branch with three leaf whorls (a total of 15 leaves) of 10 individual trees. For the infested group, sampling consisted of removing (i) a three whorl branch whose leaves were being directly eaten by caterpillars, and (ii) a three whorl branch with no caterpillars on. Samples were placed in a botanical press and air-dried in indirect sun (30°C), stored away from moisture and checked regularly for fungal or insect infestation.



Fig. 2. Growth form of Buchenavia ochroprumna trees.

## Experimental design

*Buchenavia ochroprumna* trees occur principally in the band of *igapó* that is inundated for intermediate durations (294-308 days: Barnett et al., 2015). The species is common (Negreiros et al., submitted), and occurs in a thin band (approx 10m wide) that is mediated by the inundation tolerance of the species (see Parolin, 2012; Parolin et al., 2014). Studies across a wide variety of plant taxa have shown that volatiles from herbivore-damaged leaves can incite increases in defensive chemicals in close-by conspecifics (Baldwin et al., 2006). Consequently, for the control group, only uninfested conspecifics at least 10 meters from any infested *B. ochroprumna* were sampled. When sampling infested trees individuals were chosen that, on visual inspection, appeared to have high infestation rates. This value was adjusted for canopy size so that small trees (canopy approximately 4m<sup>3</sup>) were considered viable when 2 or more caterpillars were detected, whilst for large trees (approximately 200m<sup>3</sup>) 50 caterpillars was the minimum.

## Phytochemical extraction method

Extractions of *B. ochroprumna* leaves were performed by leaving the dried leaves for 24 hours in 80 mL of a solution of methanol (75%) and chloroform (25%). After this, the extracts were dried in silica and weighed. Following drying, ethanol were added to the extracts (10~100 mg/mL according to weight of extracts). Then, aliquots (10~100 $\mu$ L) were added to DMSO (Dimethyl sulfoxide) to achieve 1mL.

#### Total phenols

Total phenols measure was made using  $10\mu$ L of the samples prepared above with an addition of  $50\mu$ L of Folin 10% (1mL of Folin for 9mL of distilled water). This assay was incubated for 8 min in a black box for 8min at room temperature (25 °C), after which 240µL of sodium carbonate of 0.4% (10mL of distilled water for 40mg of sodium carbonate) was added. After three minutes samples were placed in a Beckman Coulter spectrophotometer at a wavelength of 620nm in the Pharmacy Laboratory in Amazon Federal University. Results were expressed as micrograms of equivalent of gallic acid per mililiter (µg EAG/ml). This method for total phenols measurement followed Singleton & Rossi (1965) with modifications.

### Statistical analysis

Chemical analysis was made in triplicates, so the results of chemical analysis were expressed as the average of the triplicates  $\pm$  standard deviation. Hypotheses were tested using a t-test, with a minimum p-value of < 0.05, and run using the program R 3.3.1.

#### RESULTS

The results of total phenols show that there is no significant difference for this class of secondary compounds between plants that are being damaged by caterpillars and plants that are not (p = 0.9). Total phenols for the infested group varied from  $55 \pm 2.4$  to  $256 \pm 0.5 \ \mu\text{g}$  EAG/ml and for the control group results varied from  $70.9 \pm 1$  to  $270.3 \pm 1 \ \mu\text{g}$  EAG/ml (Fig. 3) which are not consider high values of total phenols when compared to plants with over 800  $\mu\text{g}$  EAG/ml (Bahorun et al., 2004). Accordingly, the selection by *C. ouakary* for non-damaged individuals of *B. ochroprumna* reported by Negreiros et al. (submitted), is not related to an induced raise in the amount of phenolic compounds due to caterpillars damage.

Total phenols



Fig. 3 – Comparison total phenols between infested and non infested *Buchenavia ochroprumna* trees.

In addition to lack of variation between plants, there was also a lack of withintree variation, with no variation being detected in levels of phenolic compounds between branches that had and did not have caterpillars and associated leaf damage (p = 0.9) (Fig. 4). This indicates that phenolic production in *B. ochroprumna* is nonsystemic, and that in-leaf concentrations are not related to caterpillar herbivory.



Fig. 4 – Comparison of total phenols between damaged and not damaged branches within the same individuals of *B*.

ochroprumna trees.

#### DISCUSSION

Studies measuring total phenols as defensive compounds against herbivores are many, and results depend greatly on the plant species. For example, Bixenmann et al. (2016) tested effects of damage for four species of Inga: Inga marginata, I. pezizifera, I. umbellifera (widely distributed throughout lowland tropical forests in Central and South America) and I. multijuga (distributed throughout Central America near water and in swamps). In two of them, Inga umbellifera and I. marginata, there was a significant increase of phenolics following damage by herbivores, whereas in I. multijuga and I. pezizifera, herbivory produced no effects, a result that mirrors the present study as does a study conducted by Kursar & Coley (2003) with 12 pairs of plant species (each pair with one species representing a fast-expanding young leaves type and the other representing a slow-expanding young leaves type) from families Sapindaceae, Rubiaceae, Chrysobalanaceae, Myrtaceae, Ochnaceae, Olacaceae, Clusiaceae, Piperaceae, Lecythidaceae, Annonaceae and Connaraceae, including species from humid and wet forests (Licania platypus, Eugenia oerstediana, Garcinia intermedia: Fern, 2014) and swampy ground (Prioria copaifera, Gustavia superba: Fern, 2014) where damage by herbivores was not correlated with in-leaf phenolic compounds concentrations. However, for *Betula pendula* an increase in phenolic levels was recorded for both artificial leaf damage and caterpillar grazing, with caterpillar grazing causing a larger, longer-lasting and systemic increase, whereas artificial damage increases occurred only locally on damaged leaves, not on the adjacent ones (Hartley & Firn, 1989).

How then to explain the notable avoidance of caterpillar-infested *B. ochroprumna* trees reported by Negreiros et al. (subtmitted)? Firstly, other secondary metabolites might be responsible by induced defenses in plants. One possibility is volatile terpenoids, which are able to function as olfactory cues to herbivores in food selection and in which the Combretaceae is known to be unusually rich (Eloff et al., 2008), or alkaloids that are either highly toxic and bitter (Iason & Villalba, 2006; Kimball et al., 2012; Mithöfer & Maffei, 2016), thus, if *Buchenavia ochroprumna* is chemically defended by other compounds, further chemical analysis are needed to detect them.

In addition, there is the aspect of response time-lag: plants respond very differently to either the amount of damage and type of elicitors, and after the initial damage plants may take from hours to days to fully respond to the attack (Heil & Baldwin, 2002; Stam et al., 2014, Bixenmann et al., 2016). In Rumex obtusifolius, host plant of the chrysomelid beetle Gastrophysa viridula, only 5% of leaf removal was enough to induce plant resistance, which began within 24h after attack and peaked on the third day after G. viridula feeding (Moore et al., 2003). Similarly, in Populus x euroamericana ramets, there was an increase in synthesis of phenolic compounds within 52 hours after having 7 percent of their leaves removed (Baldwin & Schultz, 1983). Both mechanical and herbivore damage (12.2% and 13.8% of lost leaf area, respectively) in Quercus alba also induced changes in leaf quality (measured by protein-biding capacity) in relation to less damaged plants (4.5% of lost leaf area) within a period of two months of census, which resulted in reduced subsequent damage by other herbivores (Wold & Marquis, 1997). The igapó system in which B. ochroprumna grows is very poor in nutrients and trees are physiologically stressed by inundation, thus many physiological responses tend to be slow (Parolin, 2008). Accordingly, it is possible that in the sampled *B. ochroprumna* plants the extent of leaf removal at the moment of sampling had not yet been sufficient to trigger a defensive response by the plants or that full response had not yet been achieved.

There is also the question of extent of insect activity: the current collections were made in 2016, when the population of caterpillars was far below of that recorded by A. A. Barnett (unpublished data) between 2006 to 2008 when individuals of *B. ochroprumna* with over 80% of damage caterpillars-induced damage were common. This may indicate a population fluctuation of the nymphalid caterpillars such as has been reported in many other Lepidoptera, including gypsy moths (*Lymantria dispar*), who reach occasional population outbreaks after many years of low densities (Liebhold

et al., 2000) and Pandora moths (*Coloradia pandora*) with also very variable betweenyear population sizes (Speer et al., 2001).

Besides their functions as herbivore deterrents, phenolic compounds also may function as an antioxidant system to facilitate plant tolerance and eliminate metabolic residuals resulting of abiotic stressors such as drought, UV radiation and flooding, especially when this environmental adversities occur concomitantly (Winkel-Shirley, 2002; Hernández et al., 2009; Pollastri and Tattini, 2011; Agati et al., 2012; Brunetti et al., 2013). With many months of uninterrupted inundation, low soil nutrient levels, and high levels of insolation, the *igapó* habitat is a very demanding one for plants (Parolin et al., 2004). This may mean that phenolic levels in individual B. ochroprumna trees reflect individual physiological stresses not related to herbivory, but to the more allpervasive abiotic environment. Consequently, observed inter and intra-tree variation of phenolic compounds on B. ochroprumna trees (Fig. 4) may be due to their individual response to differing levels of external stressors, including inundation duration, light intensity and soil nutrients, which may lead to different levels of phenolic antioxidants both in different individuals of the same species and within leaves and branches of one single tree, depending on their position on the plant, where leaves in sunny parts of the plant receiving higher insolation have higher phenolic levels because they need more protection from photodamage than leaves that are in shady parts (Close and McArthur, 2002).

A further notable aspect of the tritrophic interaction that is the focus of this study is that C. ouakary does not avoid only Buchenavia ochorprumna trees but also, apparently, the nymphalid caterpillars themselves. It was reported by Barnett et al. (2013) that C. ouakary also includes arthropods seasonally on its diet, when fruits and seeds are less available. However, several caterpillars were ignored, such as aposematic Dalceridae larvae feeding on the leaves of Securidaca sp. (Polygalaceae) and the social caterpillars of the saturniid moth, Arsenura armada. The former can biosynthesize toxins including cyanoglucosides (Niehuins et al., 2006); these chemicals can kill or severely disable mammals that ingest even small quantities of them (Roth & Eisner, 1962; Rothschild et al., 1970). The latter are lethally toxic to some vertebrate predators (e.g. chicks of the trogon bird: Costa et al., 2003). Uacaris also avoided Lasiocampidae and Lymantriidae caterpillars (Barnett 2010; Barnett et al., 2013). Many of the former are both hairy and toxic, while for the latter, the defence is both long barbed setae which are both irritant and toxic (Deml & Dettner, 1995). Many of these caterpillars avoided by uacaris were very abundant, which is also the case for the nymphalid caterpillars feeding on B. ochroprumna threes. They are cryptic dark mottled brown caterpillars and usually feed on abaxial face of leaves or on shoots near the leaves. Besides their cryptic colour, they have a conspicuous pattern of spines on the dorsal surface of their bodies, with four bigger branched spines over their thorax and at the end of the abdomen and smaller ones all over the body (Fig. 5). According to Murphy et al. (2009), spines are usually highly deterrent to generalist predators, being able to be very painful or toxic. Thus, it is possible that the avoidance by monkeys C. ouakary demonstrate its fear of touching potentially toxic caterpillars based on previous experience (Iason & Villalba, 2006), however it need to be tested on future research.



Fig. 5: Nymphalid caterpillar grazing on *B. ochroprumna* tree, showing the body covering of long strong spines.

In summary, there is a multiplicity of possibilities that may explain the lack of patterns in results obtained with phenolic compounds measurement as the action of other defensive secondary compounds, time-lag between grazing by herbivores and full response, and severity of damaged influenced by density of herbivore population. However, results different from what we expected are mostly pointing out to responses to abiotic stressors, as the values of phenolic compounds were seen to be different even in different parts of the same individual plant, which demonstrates the effects of external stressors according to the plant position. Moreover, evaluating total phenolics may be a very general measurement which could mask patterns in more specific phenolic compounds such as tannins, which are very important protein-binding substances usually associated with animal food selectivity and insect herbivore deterrence studies (Harborne, 2001; Moles et al., 2003; Bergvall & Leimar, 2005).

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# CONCLUSÃO

O presente estudo mostrou que, apesar de incluir em sua alimentação folhas e insetos nos períodos de baixa disponibilidade de seu principal item alimentar (frutos imaturos), o primata *C. ouakary* não otimiza a ingestão desses itens alimentares quando encontra indivíduos de *B. ochroprumna* infestados por larvas de lepidópteros como esperado. Ao contrário, o primata demonstra extrema rejeição por indivíduos da planta infestados e forte seleção por aquelas não infestadas e distantes de indivíduos infestados. Tal rejeição nos levou a sugerir que a planta *B. ochroprumna* seria induzida pelas lagartas a produzir compostos químicos defensivos tanto em folhas de árvores infestadas quanto em folhas de árvores próximas. Porém, ao comparar os compostos fenólicos de indivíduos infestados e não infestados, vimos que não houve diferença desses compostos em ambos os grupos de plantas. Portanto, a indução de defesas químicas, se houver, pode estar relacionada a outras classes de compostos químicos ou à possível toxicidade das lagartas.

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