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Effect of Leaf Quality on Herbivory of Three Atlantic Forest Species

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ABSTRACT

The present study evaluated the relationship between herbivory and leaf quality of three abundant tree species (*Cupania oblongifolia* Mart., *Siparuna guianensis* Aubl. and *Xylopia sericea* St. Hill.) in the understory of a eucalyptus plantation and an adjacent Atlantic Rainforest fragment. Herbivory differed among species, since *X. sericea* was less attacked, while *C. oblongifolia* and *S. guianensis* were more. The species showed similar herbivory patterns between the investigated areas (eucalyptus plantation *versus* forest), although *X. sericea* showed higher herbivory rates in the forest fragment. Fiber values (NDF, CEL and LIG) varied significantly among the species, being lower in *S. guianensis*. *X. sericea*, the species with the highest C/N ratio, higher concentrations of total phenols, smaller leaf area and higher trichome density, showed the lowest leaf herbivory rates, corroborating the hypothesis that herbivory is lower in species with lower nutritional quality and a greater set of leaf defense features.

Keywords: defenses, insect-plant interactions, leaf nutrients.

1. INTRODUCTION AND OBJECTIVES

Herbivores and plants are connected by one of the most important ecological relationships in nature, herbivory (Strong et al., 1984; Wilson, 2001). Existing herbivores, which include arthropods, mollusks, nematodes and vertebrates, consume about 15% of the annual plant biomass production, both in temperate and tropical forests. This makes herbivory a fundamental process for the energy flow between food webs (Agrawal, 2011; Cyr & Pace, 1993). In addition, herbivore pressure has allowed the development of different types of plant defenses, which have influenced their palatability to insects (Marquis, 2012).

Although herbivory may positively affect plants, most often it causes negative effects, slowing their development and their ability to reproduce and survive in natural communities (Boege & Marquis, 2005; Coley & Barone, 1996). Different plant characteristics can both directly or indirectly influence leaf damage rates by insects, such as leaf age and size, light, seasonality, spatial distribution, abundance, and certain particular characteristics of each species (Ballaré, 2014; Engelkes et al., 2016; Gonçalves-Alvim et al., 2010; Karolewski et al., 2013; Lowman, 1985; Silva & Neves, 2014).

Plants with lower nutritional value, for example, low in nutrient concentrations and high in C (carbon) concentrations, are less palatable to herbivorous insects (Coley & Barone, 1996; Kurokawa & Nakashizuka, 2008; Mundim et al., 2009). Plants also contain a variety of special chemical compounds and morphological traits that can influence their attractiveness to herbivores (Agrawal & Fishbein, 2006; Marquis, 2012; Trigo et al., 2012) such as cellulose and hemicellulose, and special compounds, like tannins (Kitajima et al., 2012; Linton & Greenaway, 2007).

Certain morphological traits may also affect the palatability of plants to herbivores, such as thorns (Cooper & Ginnet, 1998), leaf toughness (Kitajima et al., 2012; Lucas et al., 2000) and glandular and non-glandular trichomes (Barônio, 2012; Glas et al., 2012), among others. Glandular trichomes generally produce sticky substances and/or toxic exudates, thus becoming a chemical barrier, as well as mechanical, against herbivorous insects. The major classes of metabolites produced in trichomes include terpenoids, flavonoids and phenylpropenes, methyl ketones and sugar acyl defensive proteins (Glas et al., 2012).

In this context, the objectives of the present study were to: (1) evaluate herbivory percentages of chewing insects and leaf traits (nutrients, fibers and total phenols) of *Cupania oblongifolia* Mart., *Siparuna guianensis* Aubl. and *Xylopia sericea* St. Hill. individuals in the regeneration of the understory of an abandoned eucalyptus plantation and an Atlantic forest fragment; and (2) test the hypothesis that leaf herbivory is lower in species with lower nutritional quality and higher chemical and morphological defenses.

2. MATERIALS AND METHODS

2.1. Study site and species

The present study was conducted at the União Biological Reserve, located in the northern region of Rio de Janeiro (22°27'30" S and 42°02'15" W). Two sites were investigated: a former eucalyptus stand 39a (*Corymbia citriodora* (Hook) L.A, Jonhoson; K.D. Hill) with approximately 11.5 ha and which is 44 years old, abandoned for 16 years, suffering no silvicultural treatments (Ibama, 2008); and a fragment of Lowland Atlantic Forest, classified as a Tropical Rainforest (Carvalho et al., 2008; Ibama, 2008). The forest fragment of this biological reserve is one of the most preserved fragments of the São João River Basin (Carvalho et al., 2008; Carvalho et al., 2016) and is part of the Serra do Mar Ecological Corridor, thus considered an area of great ecological and conservationist relevance, since it shelters the largest continuous stretch of Atlantic Forest (Ayers & Marigo, 2005).

The climate of the study site is characterized as tropical humid, with annual average temperature of 24 °C, and annual rainfall around 2,200 mm/year. Much of the rainfall is concentrated between October and April (Nascimento et al., 2011).

The selected species, C*upania oblongifolia* Mart. (Sapindaceae), *Siparuna guianensis* Aubl. (Siparunaceae) and *Xylopia sericea* St. Hil (Anonnaceae), are considered the main and most abundant tree species in the understory of eucalyptus plantation areas (Evaristo et al., 2011) and Atlantic Forest fragments in the state of Rio de Janeiro (Carvalho et al., 2006, 2007; Carvalho et al., 2008). However, they are still not widely used in ecological studies, especially those focused on interactions between plants and animals (Costa et al., 2007; Nascimento et al., 2011).

2.2. Leaf samplings

A total of 15 young individuals from each species, with heights ranging from 1.50 m to 3.0 m, were randomly collected at each of the studied areas. Twelve mature leaves were removed from three branches per plant. Mature leaves were considered as being dark green and arranged below the fourth leaf, counting from the apex to the base branch (Nascimento & Proctor, 2001; Nascimento et al., 2011). In the case of compound leaves in *C. oblongifolia*, the leaflets were considered leaves. The leaves were first sampled in April 2009 (end of the rainy season), followed by August 2009 (end of the dry season). Only samples from the first sampling were used for chemical composition analyses and investigations of morphological/structural defenses. Bulked samples of undamaged leaves from five individuals among the 15 trees (n = 3 per area) from each species were used to obtain the mass (g) required for the chemical and morphological/structural analyses.

2.3. Leaf area (LA) and herbivory determinations

The sampled leaves were photographed (resolution: 1,200 × 1,600) using a digital camera (Samsung Digimax D53) on a white board with the aid of a tripod at a standard distance of 30 cm, according to the criteria used by Paul et al. (2012). The ImageJ software package was used for the leaf area and herbivory analyses (Rasband, 2003), since this method is considered very precise (Paul et al., 2012). Herbivory damage was defined as the percentage damage at one moment in time. The percentage of herbivory for each leaf was determined by dividing the area consumed by the total leaf area (cm^2) (Aide, 1993; Paul et al., 2012). Holes and missing leaf parts were considered to be due to herbivory. In cases of damage by herbivores at the end of the leaf tissues, leaf contours were redrawn using ImageJ drawing tools.

2.4. Chemical analyses

After being photographed, the leaves collected in April 2009 were washed with both tap water and deionized water and left in a circulation oven at 60 °C for 72 hours. After drying, they were ground in a knife mill. The total carbon (C) and nitrogen (N) determinations were performed on a CHNS/O auto-analyzer (Perkin Elmer 2,400) using 2 mg to 4 mg aliquots of leaf sub-samples.

The determinations of neutral detergent fibers (NDF), cellulose (CEL) and lignin (LIG) were performed at the Animal Science and Nutrition Laboratory (LZNA), in CCTA/UENF, according to the method reported by van Soest et al. (1991). The dry and pulverized material (0.5 g) was used for the sequential analyses.

LIG content was determined by the 72% sulphuric acid method, followed by incineration of the residue at 550 °C for 3 h. Cellulose content was determined by calculating the difference between ADF (acid-detergent fibers) and LIG. NDF include cellulose, hemicellulose and lignin as main components, in addition to heat-damaged proteins, cell wall proteins and minerals (ashes). In contrast, ADF consist primarily of cellulose and lignin (lignocellulose) and heat-damaged proteins, with small amounts of cell wall proteins and insoluble minerals (ashes).

For the determination of total phenols (TP), the hydroalcoholic leaf extracts (EtOH) were prepared using 0.2 g of the ground material, according to the method reported by Phillips & Henshaw (1977), modified. Total phenol concentrations were obtained through a standard curve of gallic acid at concentrations ranging from 50 mg/mL to 175 mg/mL, with interpolation of the sample absorbance (760 nm) from the calibration curve. All leaf analyses were conducted in replicate in cases of value disagreements.

2.5. Leaf trichomes

The trichome densities on the adaxial (AD) and abaxial (AB) leaf surfaces from five leafs per species were determined using a stereomicroscope

(40×) in four fields taken at random (Gilardón et al., 2001). Visual estimates of the leaf areas occupied by the trichomes were considered, categorized into four classes of trichome leaf coverage percentage: glabra (0%), rare (1-20%), abundant (21-50%), and dense (> 50%).

2.6. Data analyses

The variables of the residues analysis that showed normality and homoscedasticity issues were transformed by arcsines (herbivory) and square root (LA and phenols) for data standardization (Zar, 1984). Herbivory values, even after this transformation, did not meet normality and homoscedasticity criteria. Thus, for intraspecific leaf herbivory comparisons between the two study sites, the Mann-Whitney *U* test was applied and interspecific comparisons of this variable in each environment were performed by a Kruskal-Wallis test, followed by Dunn's comparison test. A factorial Anova and post-hoc Tukey test were used for variable comparisons (NDF, CEL, LIG, TP, LA, C, N and C/N), where study site (forest and eucalyptus) and species (*C. oblongifolia, S. guianensis* and *X. sericea*) were tested simultaneously. All variables were subjected to a Principal Component Analysis (PCA). Leaf areas between species for 15 individuals were compared, using the leaves from the first sampling. All data analyses were conducted using the Statistica software package (version 7.0).

3. RESULTS

3.1. Herbivory and leaf area

Herbivory damage varied from 1% to 13% among species, with significant differences within the study sites observed for leaves from both sampling (Kruskal-Wallis, $p \leq 0.01$) (Figure 1). *X. sericea* showed the lowest percentage of herbivory, significantly differing from *C. oblongifolia* from the forest (Kruskal-Wallis test, $p = 0.005$) and the eucalyptus stand (Kruskal-Wallis, $p \leq 0.0001$); differing, however, from *S. guianensis* in the first sampling only at the eucalyptus stand (Kruskal-Wallis test, $p = 0.003$) (Figure 1). Intraspecific loss of leaf area due to herbivory was similar between sites, considering leaves from both the first and the second samplings. Only *X. sericea* differed significantly (Mann-Whitney test, $p = 0.01$) between the sites, with higher percentages of leaf herbivory in the forest (4.6 % ± 5.1 %) compared to the eucalyptus stand (1.23 % ± 1.13 %), although for the first sampling only (Figure 1).

Interspecific differences were observed regarding LA, with *C. oblongifolia* presenting higher mean leaf areas, followed by *S. guianensis* and *X. sericea* (factorial Anova, $p \leq 0.05$) (Figure 2). Within the species, only *X. sericea* showed differences in mean leaf area when comparing the sites, with the highest value observed in individuals from the eucalyptus stand (Mann-Whitney test, $p \leq 0.001$) (Figure 2).

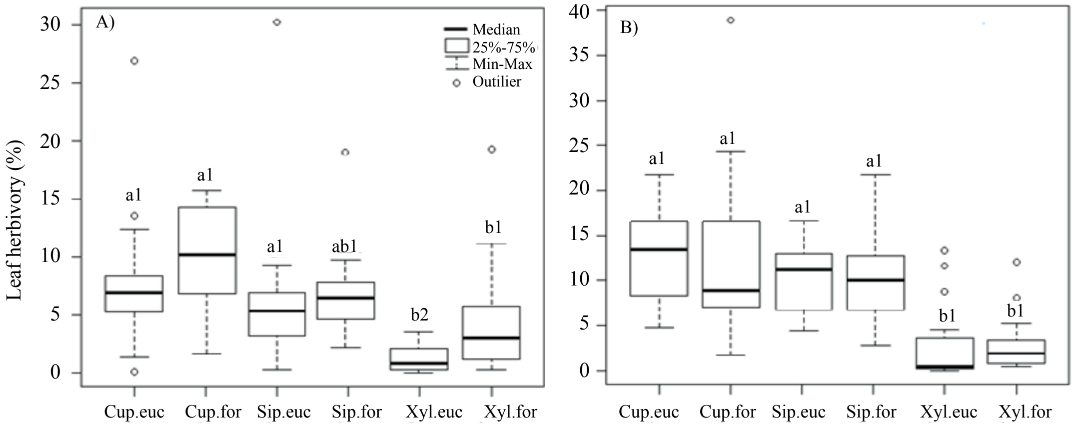


Figure 1. Leaf herbivory values (%) for *Cupania oblongifolia* (Cup), *Siparuna guianensis* (Sip) and *Xylopia sericea* (Xyl) sampled from the forest (for) and the eucalyptus stand (euc) for the first (A) and second (B) leaf samplings. The *boxplots* represent the median values, 1st and 3rd quartiles, extreme values and outlier. Different letters represent significant differences between species within each site (Kruskal-Wallis, $p \leq 0.05$) and different numbers represent significant differences within each species between sites (Mann-Whitney test, $p \leq 0.05$, n = 15).

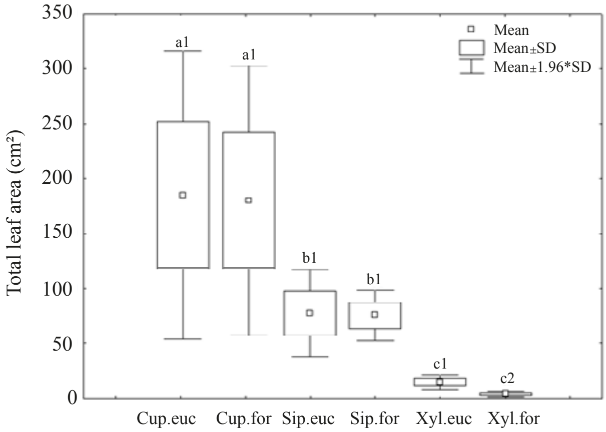
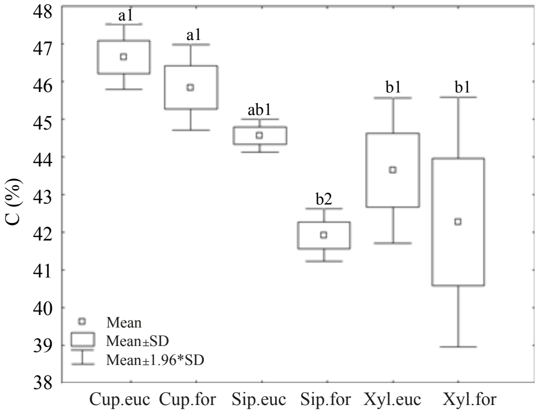
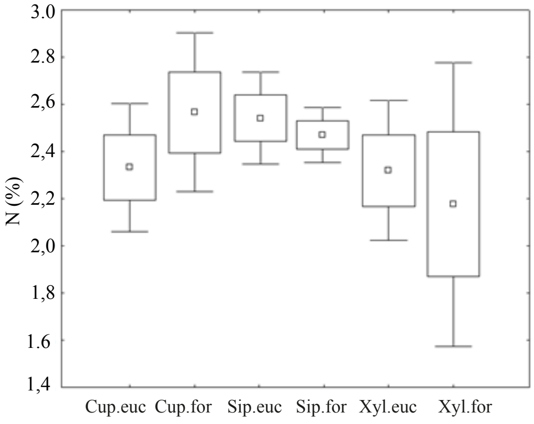


Figure 2. Total leaf area (means and standard deviation) for *Cupania oblongifolia* (Cup), *Siparuna guianensis* (Sip) and *Xylopia sericea* (Xyl) sampled from the forest (for) and the eucalyptus stand (euc) regarding leaves from the first sampling.Different letters represent significant differences between species within each site and different numbers represent significant differences within each species between sites (factorial Anova, $p \leq 0.05$, n = 15).

3.2. Foliar nutrient concentrations

Interspecific comparisons revealed that *C. oblongifolia* presented the highest C values, differing significantly from *S. guianensis* (factorial Anova, $p = 0.00002$) and *X. sericea* in the forest fragment (factorial Anova, $p = 0.0005$), with no significant difference between *S. guianensis* and X. *sericea* in this same area ($p > 0.05$). In the eucalyptus stand, the only significant difference was observed for C values between *X. sericea* and *C. oblongifolia* (factorial Anova, $p = 0.003$), with *C. oblongifolia* presenting the highest values (Figure 3). *X. sericea* showed higher C/N ratios, significantly differing from *S. guianensis* (factorial Anova, $p = 0.0001$) and *C. oblongifolia* (factorial Anova, $p = 0.0005$), with no significant difference for C/N values between *S. guianensis* and *C. oblongifolia* ($p > 0.05$) in the forest area. No significant differences in the C/N ratios between species were observed for the eucalyptus stand ($p > 0.05$) (Figure 3). The intraspecific analysis of C and N foliar concentrations between the forest and eucalyptus sites indicated that only C concentrations for *S. guianensis* were significantly higher in the eucalyptus stand individuals (factorial Anova, $p = 0.011$), while *X. sericea* showed higher C/N ratios in forest individuals (factorial Anova, $p = 0.008$). Regarding N, no significant differences were observed within species between the forest and eucalyptus areas, or between the investigated species within areas (factorial Anova, $p > 0.05$) (Figure 3).





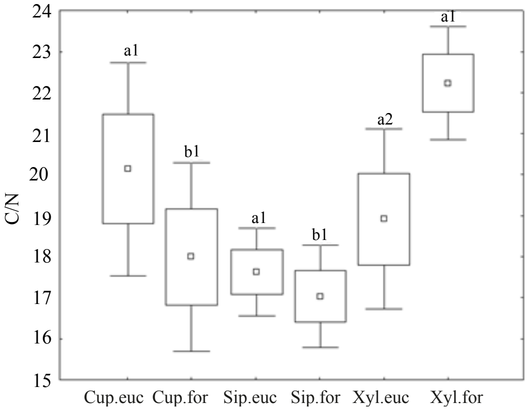


Figure 3. Carbon (C) and nitrogen (N) percentages and C/N ratios (means ± standard deviation) for *Cupania oblongifolia* (Cup), *Siparuna guianensis* (Sip) and *Xylopia sericea* (Xyl) sampled from the forest (for) and the eucalyptus sites (euc). Different letters represent significant differences between species within site and different numbers represent significant differences within species between sites (factorial Anova, $p \leq 0.05$, n = 3).

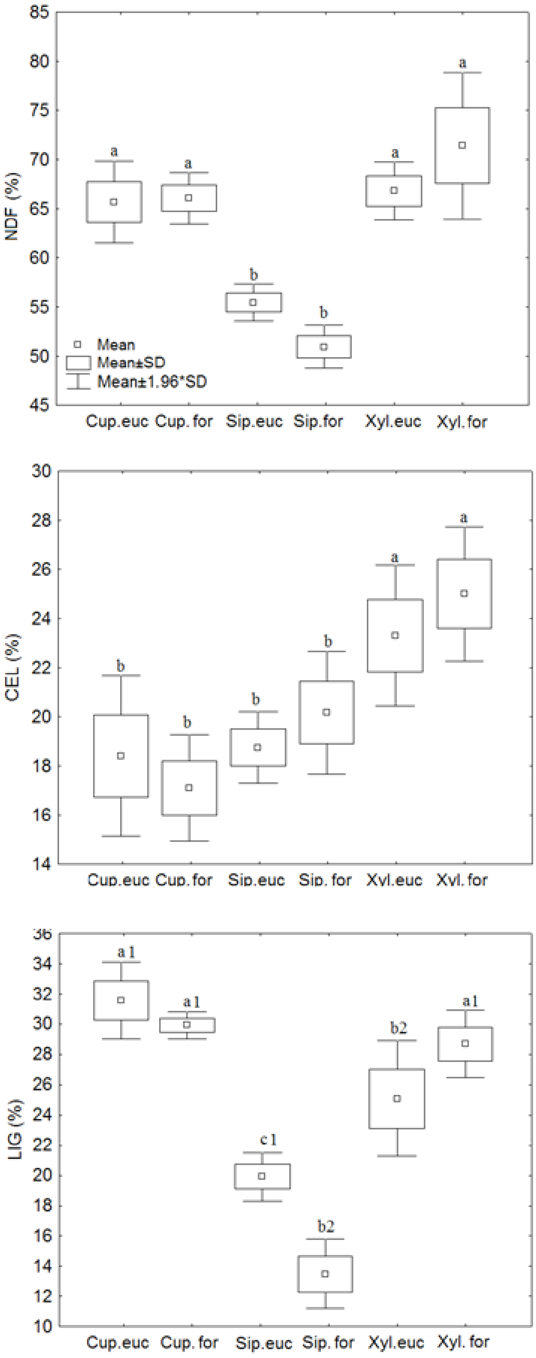
3.3. Structural and chemical defenses

The fiber analyses indicated no patterns between the three investigated species for NDF, CEL or LIG. Regarding interspecific variations of NDF values in the forest fragment, *S. guianensis* showed significantly lower values than *C. oblongifolia* and *X. sericea* (factorial Anova, $p = 0{,}0001$). The same pattern was observed for these species in the eucalyptus stand (factorial Anova, $p \leq 0.05$); however, no significant difference was observed for intraspecific NDF concentrations (factorial Anova, $p > 0.05$) between the sites (Figure 4).

Regarding cellulose concentrations, interspecific variations – both in the forest fragment and the eucalyptus stand – were observed, with *X. sericea* always showing the highest mean cellulose values (factorial Anova, $p \leq 0.05$). However, no intraspecific difference was observed when comparing sites (factorial Anova, $p > 0.05$) (Figure 4).

Interspecies comparisons revealed that *S. guianensis* presented significantly lower LIG values ($p < 0.05$) at both sites. On the other hand, *S. guianensis* and *X. sericea* showed significant variations in LIG concentrations between sites, with *S. guianensis* showing higher values in the eucalyptus stand (factorial Anova, $p = 0.004$), whereas *X. sericea* showed higher LIG values at the forest site (factorial Anova, $p = 0.03$) (Figure 4).

Interspecies variations in total phenol were observed, with *X. sericea* presenting higher total phenol concentrations at both studied sites ($p < 0.05$) (Figure 4). These concentrations also differed between environments (forest and eucalyptus) for *X. sericea* (factorial Anova, $p = 0.006$) and *S. guianensis* (factorial Anova, $p = 0.05$), being higher in forest individuals (Figure 4).



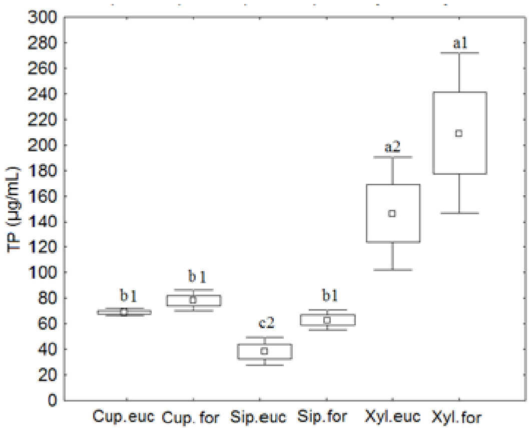


Figure 4. Fiber concentrations (%) and total phenol (µg/mL) (means ± standard deviation) for *Cupania oblongifolia* (Cup), *Siparuna guianensis* (Sip) and *Xylopia sericea* (Xyl) sampled from the forest (for) and the eucalyptus stand (euc). Fiber components: neutral detergent fiber (NDF), cellulose (CEL) and lignin (LIG). TP: total phenol. Different letters represent significant differences between species within each site and different numbers represent significant differences within species between sites (factorial Anova, $p < 0.05$, n = 3).

The three species also presented leaf trichomes, although differing in location (abaxial or adaxial) and/or density. *C. oblongifolia* and *S. guianensis* presented scarce (< 20% of leaf area covered with trichomes) and small trichomes on both sides, while *X. sericea* presented trichomes only on the abaxial surface, but more densely packed (> 70% of leaf area covered with trichomes) than the other species.

3.4. Relationship between leaf traits of the three investigated species

The PCA ordination diagram (Figure 5a and 5b), regarding the species data, indicates that the first axis represented 58.9 % of the variance, followed by the second axis at 34.9 %. Both accounted for 93.8 % of the total data variance. The eigenvalues produced by the PCA (axis 1 = 4.71, axis 2 = 2.79) were higher than the ones for the third and fourth axis (all less than 0.3), which were, thus, considered unlikely to have any meaning. This indicates that the species are distributed along gradients. The three species formed two groups, indicating interspecific discrimination, with *X. sericea* positioned on the negative end of axis 1 and *C. oblongifolia* and *S. guianensis* on the positive end of it (Figure 5a). However, no intraspecific difference was observed between the study sites. In addition, *C. oblongifolia* was positioned at the negative end of axis 2, while the other two species were positioned on the positive end of the same axis. Nitrogen (r = 0.93) showed a positive and significant correlation with axis 1, while TP (r = −0.97), CEL (r = −0.91) and C/N (r = −0.87) were negatively correlated (Figure 5b; Table 1). For axis 2, LIG (r = −0.91) and C (r = −0.87) showed the highest significant correlations (Figure 5b; Table 1).

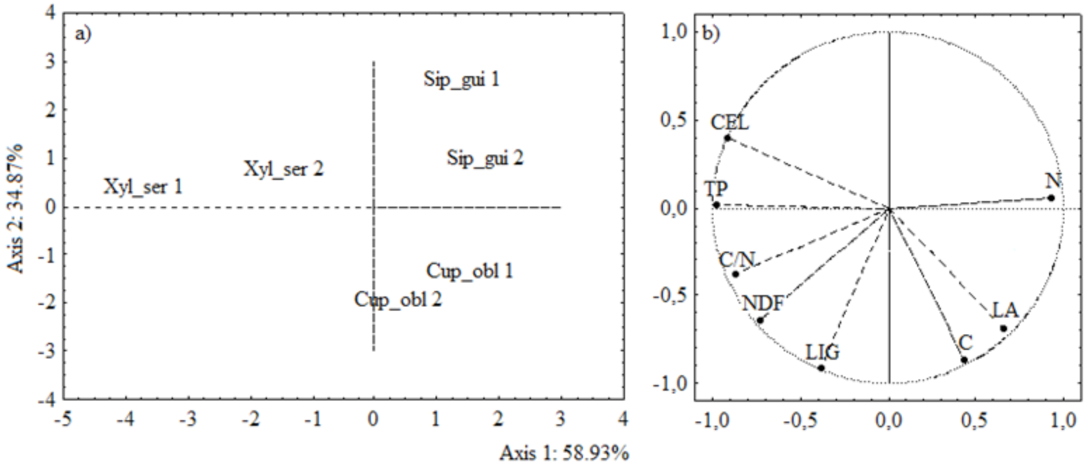


Figure 5. Biplot of the principal component analysis (PCA) for Si*paruna guianensis* (Sip_gui), *Cupania oblongifolia* (Cup_obl) and *Xylopia sericea* (Xyl_ser) sampled from the forest fragment (1) and eucalyptus stand (2). Projection of species (a) and variables (b) in relation to axes 1 and 2. NDF: neutral detergent fiber; CEL: cellulose; LIG: lignin; C: carbon; N: nitrogen; TP: total phenols; LA: leaf area.

Table 1. Correlation coefficients between the investigated variables and eigenvectors of the principal component analysis (PCA) regarding the species sampled from the forest fragment and eucalyptus stand.

Variables	Correlation coefficients of the axes	
	1	2
NDF	−0.73	−0.64
CEL	−0.91**	0.39
LIG	−0.38	−0.92**
C	0.43	−0.87*
N	0.93**	0.06
C/N	−0.87*	−0.38
TP	−0.97***	0.01
LA	0.66	−0.69
Total % of explained variance	59%	35%

NDF: neutral detergent fiber; CEL: cellulose; LIG: lignin; C: carbon; N: nitrogen; TP: total phenols; LA: leaf area.
Transformation of leaf traits. *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$.

4. DISCUSSION

The mean values of leaf herbivory found for the three investigated species (1 %-13 %) are near or within the range found for tropical rainforest species (0.09 % to 30 %) using the point method (Cárdenas et al., 2014; Peñuelas et al., 2013).

Non-glandular trichomes generally show negative relationships with herbivory (Barônio, 2012; Rodrigues et al., 2012), since trichomes obstruct herbivorous arthropod movements on leaf surfaces or prevent these predators from reaching the leaf surface with their mouthparts (Fürstenberg-Hägg et al., 2013; Glas et al., 2012; Marquis, 2012). It is worth noting that *X. sericea* leaves are densely covered by non-glandular trichomes on the abaxial surface of the leaves, which are much larger and denser than those in *C. oblongifolia* and *S. guianensis*. This may have contributed to the lower herbivory values observed for *X. sericea*, suggesting that the mechanical strength of trichomes in this species is probably more efficient against herbivory. In contrast to the results observed for *X. sericea*, the highest leaf herbivory percentages found for *C. oblongifolia* and *S. guianensis* (> 8%) appear to be related to the low leaf trichome density in these species, facilitating access to the leaves and their intake by herbivorous insects. Furthermore, *C. oblongifolia* has larger and wider leaves, followed by *S. guianensis*, whereas *X. sericea* shows the lowest leaf area. Insects belonging to the order Lepidoptera, such as some species of butterflies, can be influenced by leaf size, as they prefer wider leaves for oviposition (Godfray, 1986). In addition, some studies have found higher insect species richness in plants with wider leaves compared to narrower ones (Kennedy & Southwood, 1984; Moran & Southwood, 1982), suggesting that larger leaves may attract more insects and thus, increase herbivory percentages (Garibaldi et al., 2011; Gonçalves-Alvim et al., 2010).

The total phenolic concentrations found in *X. sericea* were about two to three times higher than those found for *C. oblongifolia* and *S. guianensis*, respectively, at both sites. Phenolic compounds are highly diverse in the plant kingdom, including about 10,000 different compounds. They are known to act in plant protection against herbivory, repelling and intoxicating several types of insects (Ballaré, 2014; Fürstenberg-Hägg et al., 2013; Karolewski et al., 2013; Mazid et al., 2011).

However, the lowest percentage of leaf herbivory found in *X. sericea* seems to be related not only to the high phenol concentrations in this species leaves, but also the highest leaf trichome density, smaller leaf area and lower nutritional quality (> C/N ratio and fibers). Such foliar traits appear to jointly contribute to increased *X. sericea* leaf resistance to herbivory, since they reduce leaf access or digestibility (Fürstenberg-Hägg et al., 2013; Marquis, 2012; Poorter et al., 2004), making this species less susceptible to herbivory.

Herbivory values did not differ between the forest fragment (richer in tree species) and the eucalyptus stand (poorer in tree species), for two (*C. oblongifolia* and *S. guianensis*) of the three species, and were higher for *X. sericea* in the forest fragment, as observed by Nascimento et al. (2011). Thus, the results observed herein did not corroborate the results of Brown & Ewel (1987) and Andow (1991), that observed higher herbivory in plant communities with lower species richness.

The principal component analysis did not indicate species separation between the forest and eucalyptus stand with regard to leaf quality (nutrients, total phenols and fibers), suggesting that these leaf traits are not considerably altered in these two areas, which may have contributed to the herbivory patterns observed, mainly for *C. oblongifolia* and *S. guianensis.*

5. CONCLUSIONS

The species that suffered the lowest percentage of leaf herbivory (*X. sericea*) showed the lowest nutritional quality and a better set of defensive leaf traits, corroborating the tested hypothesis.

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