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Leaf traits and herbivory on deciduous and evergreen trees in a tropical dry forest

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Abstract

Deciduous and evergreen trees coexist in tropical dry forests, but exhibit distinct leaf syndromes for resource-use efficiency and defenses against herbivores. Moreover, these functional groups may have contrasting patterns of temporal variation in leaf traits and herbivory along leaf ontogeny. We tested these predictions by comparing a set of leaf traits related to water stress and defense, and herbivory levels between young and mature leaves from evergreen leaf-exchanger and deciduous species. We evaluated deciduous and evergreen trees within the same habitat type: a tropical dry forest with 90–100% of leaf deciduousness during the dry season. We sampled 10 individuals of three deciduous and three evergreen species. Evergreen plants had greater leaf thickness and concentration of phenolics and tannins. On the other hand, deciduous plants had higher leaf nitrogen content and specific leaf area. Deciduous plants lost twice as much leaf area by herbivory as evergreen plants (6.48% versus 3.20%), and leaf damage was positively related to both phenolic compounds and nitrogen content. Mature leaves from both phenological groups had higher levels of tannins and phenolic compounds, and lower levels of nitrogen and herbivory increment than young leaves. Our results suggest an adaptive convergence on leaf traits primarily related to water stress for different species within each phenological group. Some of these traits also play a role in leaf palatability, although the fitness consequences of the absolute difference in leaf damage (approximately 3%) between evergreen and deciduous species remain unclear.

Zusammenfassung

Laubwerfende und immergrüne Baumarten koexistieren in tropischen Trockenwäldern, aber sie zeigen unterschiedliche Blattsyndrome hinsichtlich der Effizienz der Ressourcennutzung und der Verteidigung gegen Herbivore. Darüberhinaus können diese funktionellen Gruppen unterschiedliche Muster der zeitlichen Variation bei den Blattmerkmalen und des Herbivorenfraßes im Laufe der Blattentwicklung aufweisen. Wir testeten diese Vorhersagen durch den Vergleich einer Anzahl von Blatteigenschaften mit Bezug zu Wasserstress und Verteidigung und des Herbivorenfraßes bei jungen und voll entwickelten Blättern von immergrünen Blattwechslern und laubwerfenden Arten. Wir untersuchten solche Bäume im selben Habitattyp, einem tropischen Trockenwald mit 90 bis 100% Blattwurf während der Trockenzeit. Wir beproben jeweils zehn Bäume von drei laubwerfenden und drei immergrünen Arten. Immergrüne besaßen dickere Blätter und höhere Konzentrationen von Phenolen und Tanninen.

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Laubwerfende Bäume wiesen einen höheren Stickstoffgehalt der Blätter und eine höhere spezifische Blattfläche auf. Blattwerfende Bäume verloren doppelt so viel Blattfläche durch Herbivorenfraß wie die Immergrünen (6.48% gegenüber 3.20%), und die Schäden an den Blättern waren positiv mit dem Gehalt an phenolischen Komponenten und Stickstoff verbunden. Reife Blätter von beiden phänologischen Gruppen wiesen höhere Gehalte an Tanninen und phenolischen Verbindungen und geringere Stickstoffgehalte sowie geringeren Herbivorenfraß auf als junge Blätter. Unsere Ergebnisse legen nahe, dass bei unterschiedlichen Arten innerhalb der beiden phänologischen Gruppen eine adaptive Konvergenz bei den mit Wasserstress verbundenen Blattmerkmalen besteht. Einige dieser Merkmale spielen auch eine Rolle in Hinblick auf die Genießbarkeit, obwohl die Auswirkungen auf die Fitness der absoluten Differenz zwischen immergrünen und laubwerfenden Arten bei den Blattschäden (ca. 3%) unklar bleiben.

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Keywords: Evolutionary convergence; Phenological groups; Leaf herbivory; Deciduous forest; Leaf-trait syndromes

Introduction

Adaptive convergence in defensive leaf traits among different phenological groups has been a well-documented subject in ecological studies in the last decades (Chaturvedi, Raghubanshi, & Singh 2011; Eamus 1999; Mooney & Gulmon 1982). In general, these studies addressed the trade-off in resource allocation between photosynthesis and growth and the investment in defense against herbivores and pathogens (Mooney & Gulmon 1982; Pringle et al. 2011). Several leaf traits can be used to infer adaptation in a selective context, and thus, plant defenses can be approached as multiple attributes (e.g., defense syndromes) that interact synergistically to maximize plant fitness (see Agrawal & Fishbein 2006).

In tropical seasonal terrestrial ecosystems, water stress is a fundamental problem and different adaptive strategies enable both deciduous and evergreen species to colonize such habitats (Eamus & Prior 2001; Franco et al. 2005). Evergreen (drought-tolerant) plants retain their leaves for longer periods and need to be adapted to water conservation during the severe dry season. On the other hand, deciduous (drought-avoiding) plants drop their leaves during the dry season to reduce water loss, and optimize their growth and photosynthesis rates during the rainy season (Eamus & Prior 2001; Tomlinson et al. 2013). The morpho-physiological traits related to such contrasting phenological strategies usually also lead plant species in each group to differ significantly in their resource-use efficiency (e.g., CO₂ and nitrogen) (Eamus & Prior 2001; Sobrado 1991). Deciduous species invest less in the production of non-photosynthetic leaf tissues and maintain higher water use efficiency to sustain higher photosynthetic rates per unit of water loss in relation to evergreen species (Eamus 1999; Franco et al. 2005; Sobrado 1991). Thus, it is likely that deciduous and evergreen species in seasonal environments will possess distinct leaf traits related to water use and conservation (Franco et al. 2005; Pringle et al. 2011; Tomlinson et al. 2013).

In terrestrial environments, herbivory is predominantly controlled by bottom-up forces (Coley & Barone 1996;

Stiling & Moon 2005), mainly through leaf traits that may converge according to habitat conditions (Powers & Tiffin 2010) and plant functional group (Dirzo & Boege 2008; Pringle et al. 2011). Thus, evergreen and deciduous plants may exhibit different sets of leaf traits primarily related to water stress that also influence herbivory attack. Usually, drought tolerance by evergreen species involves producing leaves with high levels of structural carbon-based compounds (Eamus 1999; Mooney & Gulmon 1982; but see Pringle et al. 2011). On the other hand, drought-avoiding by deciduous species involves producing leaves with lower C/N ratio (Franco et al. 2005; Sobrado 1991). Also, the constant availability of leaves on evergreen plants increases their apparency and risk of being encountered by herbivores. There is evidence that this increased herbivory pressure favored higher investment in structural defenses (e.g., fibers, lignin and high carbon:nitrogen ratio) on evergreen plants in seasonal forests (Dirzo & Boege 2008; Janzen & Waterman 1984), which would be less edible for herbivores compared to deciduous plants (Dirzo & Boege 2008; Pringle et al. 2011).

In tropical dry forests (TDFs), the consumption of plant biomass is performed primarily by insects, which synchronize their life cycles with the production of new leaf tissues by both evergreen and deciduous plants (Pezzini et al. 2014; Villalobos, González-Carcacía, Rodríguez, & Nassar 2013) at the beginning of the rainy season (Dirzo & Domínguez 1995). Regardless of the phenological strategy, foliage nutritional quality usually changes during leaf maturation and senescence, with increasing levels of fiber, phenolics and tannins (Boege 2005; Janzen & Waterman 1984; Silva & Neves 2014) and reduced nitrogen content (Silva, Espírito-Santo, & Melo 2012), resulting in lower consumption of mature leaves. Such variations should be more drastic on long-lived leaves of evergreen species, which persist from the rainy to the dry season and experience the severe and extended water deficits during the dry season and may be forced to even more conservative water use (see Eamus & Prior 2001; Tomlinson et al. 2013). The short-lived leaves of deciduous species only occur during the rainy season and undergo less pronounced seasonal changes along this 4–6 months period.

In general, only a small fraction of the TDFs flora is evergreen, ranging between 1.1 and 9.7% (Villalobos et al. 2013). Thus, most studies comparing leaf traits and herbivory between phenological groups in TDFs sampled plants occurring in evergreen and deciduous habitats (Dirzo & Boege 2008; Dirzo & Domínguez 1995; Pringle et al. 2011). However, the present study evaluates deciduous and evergreen plants within the same habitat, where deciduous trees predominate (Madeira et al. 2009) and drop approximately 90–100% of their leaves during the dry season (Pezzini et al. 2014). In this scenario, we tested in the field the relevance of two standing hypotheses: leaf syndromes and herbivory levels differ (i) between phenological groups (Eamus 1999; Dirzo & Boege 2008; Pringle et al. 2011) and (ii) during the leaf ontogeny (Coley & Barone 1996; Marquis, Diniz, & Morais 2001). We predicted that (1) evergreen species possess a set of leaf traits related to water conservation and defense, showing low levels of herbivory compared to deciduous trees, whose leaves are selected for traits involved in water and light use efficiency; (2) mature leaves of species from both phenological groups have higher levels of defenses and lower levels of nitrogen and herbivory when compared to young leaves; and (3) leaf damage is positively affected by leaf nutritional quality, and negatively affected by leaf physical and chemical defenses in both phenological groups.

Materials and methods

Study area

This study was conducted at the Mata Seca State Park (MSSP), created in 2000, and managed by the Instituto Estadual de Florestas (IEF – Forestry State Institute). The MSSP has an area of 15,466.44 ha and is located in the valley of the São Francisco River, Minas Gerais state, Brazil, between 14°48'36" and 14°56'59" S and 43°55'12"–44°04'12" W, at an altitude between 400 and 500 m above sea level. The climate of the region is considered as tropical semi-arid (Köppen's classification), with an average temperature of 24 °C, and average annual precipitation of 871 mm (Antunes 1994). The dry season extends from May to October, and rains are unevenly distributed along the rainy season from November to April (Pezzini et al. 2014).

Approximately 1,525 ha of the MSSP are covered with abandoned pastures in early stages of regeneration, whereas the remaining area supports old-growth secondary and primary dry forest (Madeira et al. 2009). This study was conducted in a secondary TDF area, which was abandoned about 60–65 years ago, after being cleared for cattle raising. In this TDF there are two vertical strata: the first is composed of deciduous trees of rapid growth that reach a maximum height of 10–12 m and form a closed canopy. The second stratum is composed of a dense understory with lianas, adult trees of shade-tolerant species and juvenile trees.

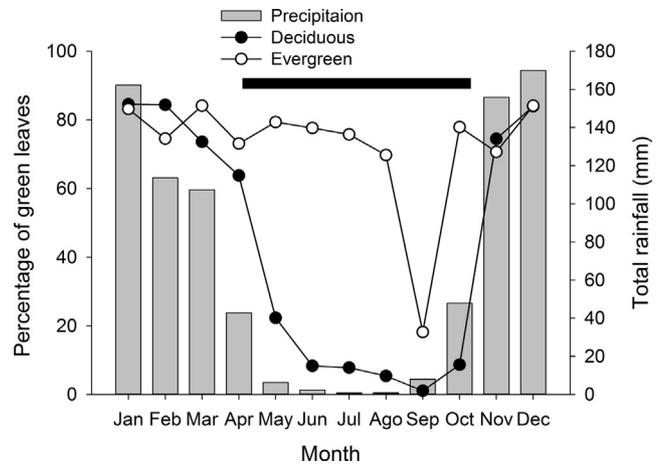


Fig. 1. Mean percentage of green leaves in the crown of deciduous and leaf-exchanger evergreen species (2007–2012) and average monthly rainfall (2007–2012) at the Mata Seca State Park (MSSP). Deciduous species: *S. glandulosum*, *H. reticulatus* and *C. duar-teanum*; evergreen species: *A. polyneuron*, *G. marginata* and *Z. joazeiro*. The horizontal black bar corresponds to the dry season.

Sampling design

We selected the three most abundant deciduous species and the three evergreen species occurring in the study area (Madeira et al. 2009; Pezzini et al. 2014) for comparisons. The evergreen species were *Aspidosperma polyneuron* Müll. Arg. (Apocynaceae), *Goniorrhachis marginata* Taub. (Fabaceae-Cesalpinoideae) and *Ziziphus joazeiro* Mart. (Rhamnaceae). These plants are in fact “leaf-exchanger” evergreen species (*sensu* Villalobos et al. 2013): they drop all their leaves at the end of the dry season, replacing them within a few days (Pezzini et al. 2014). The level and duration of deciduousness vary among plant species, but leaf-exchanger evergreen species can hold their leaves during the dry season (Fig. 1). The three deciduous species were *Sapium glandulosum* L. Morong (Euphorbiaceae), *Handroanthus reticulatus* Mattos (Bignoniaceae) and *Combretum duar-teanum* Cambess. (Combretaceae). These deciduous species had a high importance value (IV – an index that combines density, basal area and frequency) in the study site (see Madeira et al. 2009).

Individuals of the selected leaf-exchanger evergreen species retained their leaves from October to September (11.75 months) and can be found along a successional gradient from early forests (ca. 11 years of regeneration) to old-growth forests (>60 years of regeneration) (see Madeira et al. 2009; Pezzini et al. 2014). The deciduous species sampled here had leaves from November to April (6 months) and occur in intermediate and old-growth forests (Fig. 1). All sampled species are hosts for a variety of herbivores, including adults and immature Coleoptera, Hemiptera, Orthoptera and larvae of Lepidoptera (J. O. Silva, unpubl. data).

We sampled 10 adult individuals (heights between 5 and 8 m) of each species between September 2011 and

August 2012, totaling 60 individuals. The individuals had a minimum distance of 100 m from the forest edge and of 10 m between each other. We accessed tree crowns with the aid of a ladder and arbitrarily marked a cohort of 40 undamaged leaf buds for each individual during the period of leaf flushing of each group: September for evergreen and November for deciduous trees. Thirty days after marking, 10 young and fully-expanded leaves from this cohort were collected on each individual. Another 10 mature leaves from the same cohort were collected from each individual right before senescence: in April and August for deciduous and evergreen plants, respectively.

Quantification of herbivory and leaf traits

All the collected leaves were individually stored in a cooler with ice and transported to the laboratory. Among the 10 leaves collected per individual in each sampling period, five leaves were used for herbivory quantification (percentage of leaf area removed). Each leaf was photographed against a white board with centimeter marks, and had its total and damaged leaf areas calculated with the software ImageJ (Rasband 2006). Percent leaf damage was calculated as $[(\text{removed area}/\text{total area}) \times 100]$ and then averaged per plant for each leaf age class (young and mature).

The same leaves used to determine percent leaf damage were used to measure leaf thickness (mm), water content (%) and specific leaf area (SLA; leaf area per unit of leaf mass). Leaf thickness was measured with a digital micrometer (Mitutoyo Co., Kanagawa, Japan), calibrated to 0.001 mm (see Telhado, Esteves, Cornelissen, Fernandes, & Carneiro 2010), at three positions on the leaf blade, avoiding the primary and secondary veins. For the determination of water content, three leaf discs were fresh-weighed, dried at 50 °C for 72 h (Cornelissen et al. 2003) and re-weighed. Then, we calculated water content per leaf as following: $[(\text{fresh weight} - \text{dry weight})/\text{fresh weight}] \times 100$. Finally, the SLA was obtained by the ratio between the leaf area (cm²) and the leaf dry weight (g). All data were averaged per individual.

The other five leaves collected per plant in each sampling period were also dried at 50 °C for 72 h, and ground into a fine powder to quantify two types of secondary metabolites: concentration of total phenolic compounds (mg/g) and tannins with protein precipitation capacity (PPC; mg/g). These compounds were chosen due to their deterrent effects on herbivores in TDFs (Boege 2004, 2005; Dirzo & Boege 2008). The same leaves were used to assess nitrogen content (%), which is considered one of the most limiting resources for herbivores (Mattson 1980). The concentration of total phenolic compounds was quantified following the Folin–Dennis method (Swain & Hillis 1959), using gallic acid as standard. Samples were extracted with 50% aqueous methanol, by using a solvent-to-tissue ratio of 1-ml solvent per 50 mg of plant dry weight. Total phenolic concentration was averaged per plant and expressed as milligrams of phenolic compounds

per grams of plant dry mass. The same extracts were used for PPC quantification through the radial diffusion method (Hagerman 1987) using tannic acid as standard. The nitrogen content was determined through the micro-Kjeldahl method (Allen, Grimshaw, Parkinson, & Quarmby 1974). Three replicates were performed per plant for each chemical analysis (phenolics, PPC and nitrogen), and subsequently, the data were averaged for each individual. These methods were chosen due to their easily accessible results, relatively low cost and wide use in ecological studies (Oliveira, Espírito-Santo, Silva, & Melo 2012).

Statistical analyses

We used mixed-effect models (LME) to verify the effects of phenological group (evergreen versus deciduous) and leaf age (young versus mature) on leaf thickness, SLA, water and nitrogen contents, levels of phenolics, PPC and leaf damage (Crawley 2007). The data were collected repeatedly on the same tree and leaf cohort in subsequent dates, which violates the assumption of independence of the samples. To overcome this problem, the data were grouped by plant and the error variances were calculated for each different group (Crawley 2007). The phenological group and leaf age were used as explanatory variables (fixed effect), whereas the resulting groups per plant were treated as random effects (date/identity of the individual). A model was constructed for each response variable (leaf traits and herbivory). Leaf traits and herbivory were also compared between leaf age categories within each phenological group and species.

To verify the effects of the leaf traits on percent leaf area removed, we constructed generalized linear models (GLM). This procedure was performed only with data obtained from young leaves, for two reasons: (1) the highest percentage of leaf consumption by herbivores occurs during the expansion of young leaves (Coley & Barone 1996; Kursar & Coley 2003), which coincides with the beginning of the rainy season for TDFs (Boege 2005; Filip, Dirzo, Maass, & Sarukhán 1995) and (2) as leaf damage accumulates with time, most of the damage recorded on mature leaves had been caused when leaves were young and had different trait values. Therefore, the relationship between leaf cumulative herbivory and late leaf traits could be spurious for mature leaves. A complete GLM model with quasibinomial error distribution was built, followed by stepwise comparisons (backward selection), with the removal of non-significant variables. We used quasibinomial distribution instead of ordinary binomial because data were overdispersed. In the former, the *F* test is recommended (see McCullagh & Nelder 1989). The model was adjusted for orthogonal partitioning of variance (type III sum of squares), to verify the proportion of variance exclusively explained by each variable regardless of sequential order of the explanatory variables in the model. The percentage of leaf area removed was used as the response variable, whereas phenological group, leaf thickness, SLA, water and nitrogen content and

concentration of phenolics and PPC were used as explanatory variables. The models were submitted to residual analysis to verify the adequacy of the error distribution. All analyzes were performed using the software R 2.15 (R Development Core Team 2013).

Results

All leaf traits differed between phenological groups. Deciduous plants exhibited higher SLA and water and nitrogen contents, whereas leaf-exchanger evergreen plants had greater leaf thickness and concentration of phenolic compounds and PPC (Table 1 and Fig. 2). In addition, a higher accumulated percentage of leaf damage was recorded on deciduous (6.48%) compared to evergreen (3.20%) trees (Table 1 and Fig. 3).

Leaf traits also differed between young and mature leaves regardless of the phenological group, except for leaf thickness (Table 1). Within phenological groups, young leaves of both deciduous and evergreen trees had higher water and nitrogen contents, and lower concentration of phenolic compounds than mature leaves (Fig. 2). Moreover, young leaves of evergreen plants had higher SLA and lower PPC concentration than mature leaves (Fig. 2). For both phenological groups, cumulative damage increased along leaf aging, but most of the damage occurred when leaves were young: 5.9% and 2.18% of leaf area were removed in this stage for deciduous and evergreen plants, respectively (Fig. 3). A much lower leaf damage increment (given by the average difference in damage between two consecutive periods) was recorded during the mature stage: 0.58% and 1.02% for deciduous and evergreen plants, respectively (Fig. 3). The same patterns of leaf traits and herbivory were detected when each species was analyzed individually (Appendix A: Table 1).

In general, the percentage of leaf area removed on each plant was positively related to the concentration of phenolic compounds and nitrogen content for both phenological groups (Table 2, Fig. 4, Appendix A: Table 2). When plant species were analyzed separately, few leaf traits affected the percentage of leaf area removed among deciduous species and no effects were detected for evergreen species (Appendix A: Table 3).

Discussion

In the studied TDF, deciduous plants had higher SLA, water and nitrogen contents, whereas leaf-exchanger evergreen plants had higher leaf thickness and phenolic and PPC concentrations. Also, herbivory levels were higher in deciduous compared to evergreen species. This is in accordance with the prediction of the first hypothesis tested here: plants from different phenological groups possess distinct leaf syndromes, which reflect physiological trade-offs and/or evolutionary adaptations (Eamus 1999; Pringle et al. 2011;

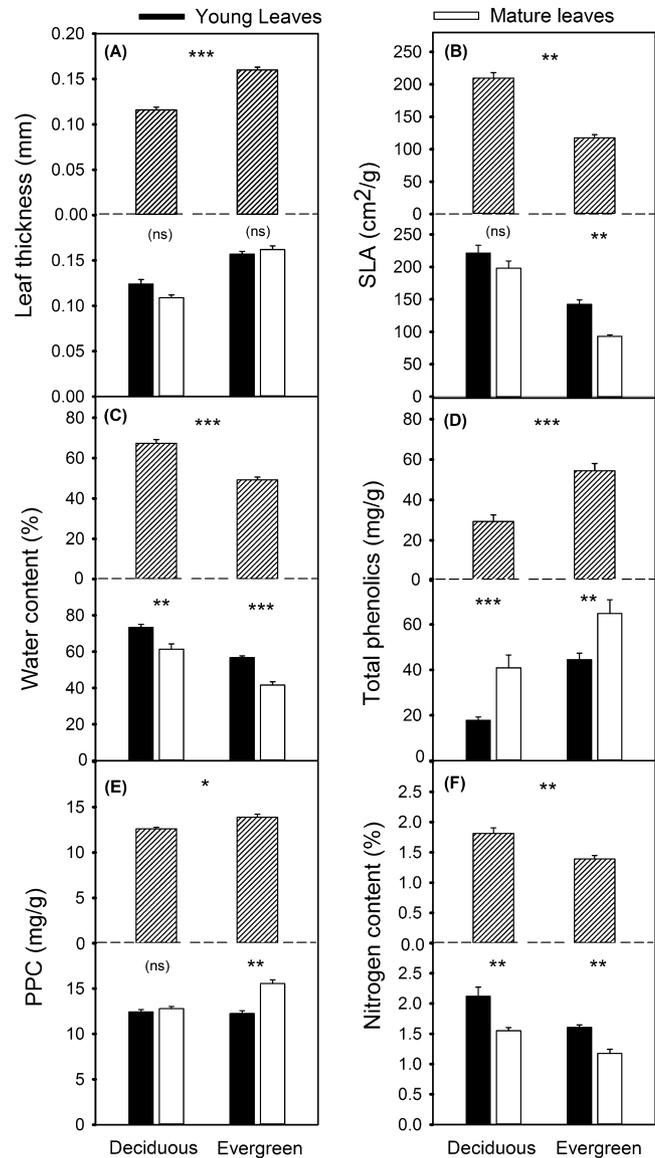


Fig. 2. Leaf traits of deciduous and leaf-exchanger evergreen trees regardless of leaf age (hatched columns; upper panel), and separated according to leaf age (black and white columns; lower panel). Error bars indicate one standard error ($n=60$). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and (ns) non-significant.

Tomlinson et al. 2013) and affect herbivory intensity. The set of leaf traits exhibited by evergreen species are typical of drought-tolerant species, whereas deciduous plants possessed traits related to drought avoidance (Eamus 1999; Franco et al. 2005). Leaves of evergreen plants usually have longer lifespan and higher costs of construction and maintenance than leaves of deciduous species (Chaturvedi et al. 2011; Eamus 1999; Sobrado 1991). Construction costs are the sum of all the carbon and energy utilized in producing a net gain in dry weight (Eamus 1999; Eamus & Prior 2001; Sobrado 1991). Longer-lived leaves are more exposed to herbivore attack than short-lived leaves, thus an investment in secondary compounds for defense may be higher in leaves of

Table 1. Analyses of variance of the linear mixed-effect models (LME) constructed to test the effects of phenological group (deciduous versus evergreen), leaf age (young versus mature) and the interaction between phenological group and leaf age on leaf thickness, specific leaf area (SLA), water content, phenolic compounds, protein precipitation capacity (PPC), nitrogen, and accumulated herbivory ($n = 60$ for each model).

| Response variable | Explanatory variable | numDF | denDF | <i>F</i> | <i>P</i> |
|--------------------------|--------------------------|-------|-------|----------|----------|
| Thickness (mm) | Phenological group | 1 | 58 | 118.38 | <0.001* |
| | Leaf age | 1 | 57 | 1.68 | 0.199 |
| | Phenological group × age | 1 | 56 | 7.4 | 0.08 |
| SLA (cm ² /g) | Phenological group | 1 | 58 | 87.15 | <0.001* |
| | Leaf age | 1 | 57 | 28.83 | <0.001* |
| | Phenological group × age | 1 | 56 | 3.66 | 0.075 |
| Water (%) | Phenological group | 1 | 58 | 75.05 | <0.001* |
| | Leaf age | 1 | 57 | 102.66 | <0.001* |
| | Phenological group × age | 1 | 56 | 1.312 | 0.256 |
| Phenolic (mg/g) | Phenological group | 1 | 58 | 32.72 | <0.001* |
| | Leaf age | 1 | 57 | 24.59 | <0.001* |
| | Phenological group × age | 1 | 56 | 0.105 | 0.745 |
| PPC (mg/g) | Phenological group | 1 | 58 | 26.87 | <0.001* |
| | Leaf age | 1 | 57 | 41.22 | <0.001* |
| | Phenological group × age | 1 | 56 | 7.41 | 0.09 |
| Nitrogen (%) | Phenological group | 1 | 58 | 25.05 | <0.001* |
| | Leaf age | 1 | 57 | 34.76 | <0.001* |
| | Phenological group × age | 1 | 56 | 0.64 | 0.425 |
| Herbivory (%) | Phenological group | 1 | 58 | 40.06 | <0.001* |
| | Leaf age | 1 | 57 | 7.46 | <0.04* |
| | Phenological group × age | 1 | 56 | 3.64 | 0.083 |

* Explanatory variable retained in the minimum adequate model ($P < 0.05$).

evergreen than deciduous trees (Dirzo & Boege 2008; Pringle et al. 2011).

As a whole, the leaf syndromes presented by evergreen species seem to confer adaptation to a conservative water use and also to avoid the loss of nutrients via herbivory in costly leaves (Dirzo & Boege 2008; Pringle et al. 2011). Indeed, mature leaves of evergreen species lost half the cumulative area removed from deciduous species (3.20% versus 6.48%). However, these herbivory levels are quite low for tropical ecosystems and may not be relevant to plant fitness.

In TDFs, leaf damage ranges from 3% to 73%, with a mean of 17% (Filip et al. 1995). Moreover, studies involving herbivory impact on plant fitness in TDFs recorded that only defoliation above 25% was sufficient to reduce plant growth and seed production (Dirzo & Domínguez 1995). Hence, herbivory probably has a low impact on fitness of the evaluated species and may not be strong enough to affect plant trait adaptive selection.

Although our results detected striking differences between phenological groups, several studies emphasized the impor-

Table 2. Analyses of deviance of the generalized linear model (GLM, type III sum of squares) showing the effects of the phenological group, leaf thickness, specific leaf area (SLA), water content, phenolic compounds, protein precipitation capacity (PPC) and nitrogen content on leaf area damaged (%). Only data from young leaves were used in this analysis ($n = 60$).

| Response variable | Explanatory variables | DF | Deviance | AIC | <i>F</i> | <i>P</i> |
|-----------------------|--------------------------|----|----------|--------|----------|----------|
| Leaf area removed (%) | Null model | | 84.33 | 204.31 | | |
| | Phenological group | 1 | 138.91 | 211.55 | 16.64 | 0.001* |
| | Thickness (mm) | 1 | 85.54 | 203.13 | 0.72 | 0.405 |
| | SLA (cm ² /g) | 1 | 85.17 | 203.56 | 1.09 | 0.301 |
| | Water (%) | 1 | 84.78 | 202.55 | 0.21 | 0.650 |
| | Phenolics (mg/g) | 1 | 103.16 | 213.99 | 11.17 | 0.011* |
| | PPC (mg/g) | 1 | 84.42 | 202.37 | 0.06 | 0.817 |
| | Nitrogen (%) | 1 | 95.26 | 209.37 | 6.48 | 0.014* |

* Explanatory variable retained in the minimum adequate model ($P < 0.05$).

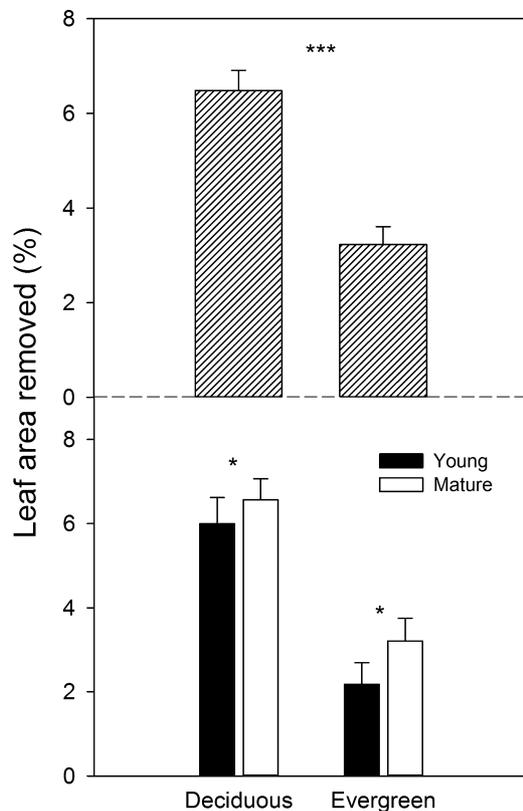


Fig. 3. Mean percentage of leaf area removed for deciduous and leaf-exchanger evergreen trees regardless of leaf age (hatched columns; upper panel), and separated according to leaf age (black and white columns; lower panel). Error bars indicate one standard error ($n = 60$). * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

tance of conducting phylogenetically controlled comparisons (see Dirzo & Boege 2008; Powers & Tiffin 2010; Pringle et al. 2011). Testing defensive leaf traits and herbivory between phenological groups can be a difficult task, due to interspecific variation among attributes evaluated for species within each phenological group. Unfortunately, our data are limited and do not allow an assessment of phylogenetic effects, since only three evergreen species were found in the study site (see Madeira et al. 2009; Pezzini et al. 2014). Nevertheless, variations in leaf traits between species of different phenological groups were quite contrasting (see Fig. 2, Appendix A: Table 1). Likely, there was an adaptive convergence in leaf traits in plant species from the same phenological group, shaped by seasonal variation in abiotic conditions (e.g., water availability) and the risk of attack by herbivores, regardless of the species phylogeny (see Pringle et al. 2011).

Overall, young leaves had higher water content, nitrogen and leaf damage increment, and lower concentration of phenolic compounds when compared to mature leaves. This pattern was found within phenological groups, and reinforced at the species level (see Appendix A: Table 1). Young leaves have higher nitrogen content because of their greater protein levels and presence of expanding cells without secondary walls (Kursar & Coley 2003; Mattson 1980; Silva

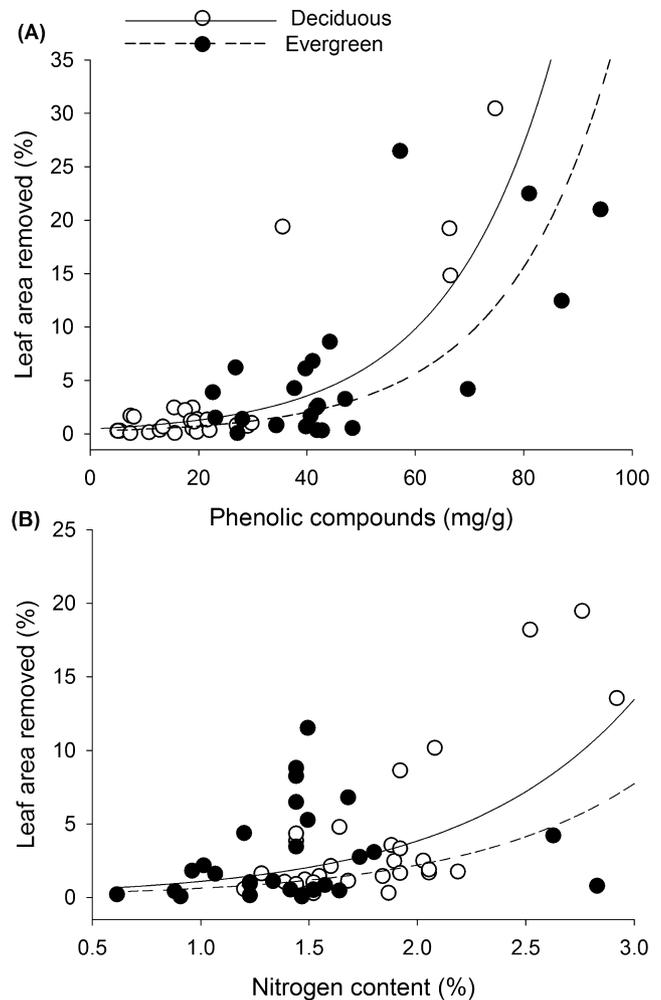


Fig. 4. Relationship between leaf damage and (A) concentration of phenolic compounds and (B) nitrogen content on young leaves of deciduous and leaf-exchanger evergreen trees ($n = 60$). The curves were adjusted based on parameters estimated from the analysis of minimal adequate GLM model (see Appendix A: Table 2). Open (deciduous) and solid (evergreen) circles represent values obtained after summation of raw residuals with the expected values for each variable, assuming average value for other covariate.

et al. 2012). After full expansion, there is a reduction in the amount of nitrogen imported into the leaf, and an accumulation of carbon-based compounds (e.g., phenolics and PPC) (Boege 2005; Kursar & Coley 2003), resulting in lower leaf damage increment (Silva et al. 2012). Between complete maturation of leaves and senescence, leaf nitrogen content is reduced through translocation to other plant organs (Gotsch, Powers, & Lerdau 2010; Mattson 1980). Such physiological processes make mature leaves progressively less edible for herbivores.

Our results corroborate some studies performed in TDFs (mainly on deciduous plants), indicating a consistent temporal pattern of variation in leaf traits and herbivory along leaf aging (Boege 2005; Silva et al. 2012). Most leaf damage occurs at the beginning of the rainy season when the leaves

were young, highly nutritious and less defended. At the end of the rainy season, almost all leaves of deciduous plants were mature, with lower water and nitrogen contents, and higher phenolic concentration. For evergreen plants in TDFs, higher levels of carbon-based defenses (e.g., leaf thickness, phenolic and PPC concentrations) were reported in mature leaves during the dry season, while leaf damage increment was lower during this period (Janzen & Waterman 1984; Gotsch et al. 2010; Silva & Neves 2014). Evergreen plants also produce higher amounts of fibers and PPC during the dry season (Janzen & Waterman 1984), increasing the C/N ratio and their sclerophylly-related traits. Such temporal patterns are probably a phenotypic response to increase photoprotection during the dry season (Janzen & Waterman 1984; Silva & Neves 2014; Tomlinson et al. 2013), since the activity of leaf-chewing insects is low during this period even on evergreen species (Silva & Neves 2014). Moreover, our study supported that long-lived leaves of evergreen species, which persisted from the rainy to the dry season, showed more variation in leaf traits.

Although bottom-up forces (i.e., foliage quality) are considered the main drivers of herbivory intensity (Coley & Barone 1996; Stiling & Moon 2005), it is important to take into account the temporal variation in diversity of herbivores in seasonal environments. Thus, the higher leaf damage on young leaves can also be related to the herbivore community composition and abundance. Previous studies in TDFs reported a synchronization of the life cycle of herbivorous insects with the leaf production at the beginning of the rainy season, a strategy that is also related to predator avoidance (Janzen 1981; Neves, Silva, Espírito-Santo, & Fernandes 2014). Although herbivore diversity was not quantified in the present study, it is possible that the higher leaf damage inflicted on young leaves is related to selective pressures influencing insect's life cycle in the highly seasonal TDFs.

Among the leaf traits evaluated in this study, only phenolic compounds and nitrogen content significantly affected the leaf area removed when all species were considered together. We found a positive relationship between the concentration of phenolic compounds and leaf area removed. Although phenolic compounds are known for their toxic and/or repellent action against herbivores (Salminen & Karonen 2011), they perform several functions such as protection against excessive solar radiation and pathogen attack (Close & McArthur 2002; Marquis et al. 2001; Salminen & Karonen 2011). Our results can be explained by three non-exclusive hypotheses: (1) the production of phenolic compounds is related to the conservation of leaf water and nutrients; (2) the effects of phenolics may be masked by higher nutritional value (e.g., leaf nitrogen) of young leaves; and (3) phenolics may be produced as induced defense after leaf damage. However, long-term effects of the phenolic compounds on insect performance are necessary to clarify their role on herbivores in TDFs. Although widely used in ecological studies, the method used here to quantify phenolics has some limitations

(Appel, Govenor, D'ascenzo, Siska, & Schultz 2001). This procedure prevents extrapolation to all phenolic compounds (Salminen & Karonen 2011) by selecting only for those that have oxidative potential. For this reason, the detectable antiherbivory effects of phenolic compounds should be interpreted with caution.

From the traits analyzed here, leaf nitrogen content was the only one that affected percentage of leaf area removed for both phenological groups in the manner predicted by our hypotheses. Since leaf nitrogen is a limiting resource for folivores, some specialist insect herbivores could have evolved abilities to find and utilize the most nitrogen-rich leaves (Mattson 1980). Despite the relevance of low foliage nutritional quality in affecting herbivory intensity, the large deviance verified for phenological groups (see Table 2) suggests that other leaf attributes (e.g., alkaloids and cyanogenic glycosides) are also important in determining herbivory levels. Hence, the investigation of a wider chemical array is necessary to understand the role of bottom-up forces in controlling leaf damage patterns in this ecosystem.

Leaf-exchanger evergreen and deciduous trees coexist in the studied TDF, but species from the same phenological group seem to have experienced an evolutionary convergence in leaf syndromes. Although the traits related to each syndrome are primarily related to drought adaptation (i.e., water conservation for evergreen species and water use efficiency for deciduous species), some of them also play a role in controlling insect herbivory. The mechanisms driving insect herbivory intensity in TDFs need further investigation, and multiple comparisons including other phenological groups and traits more directly related to plant defense would help understanding the evolution of leaf syndromes in seasonal ecosystems.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.baae.2015.02.005>.

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