



Leaf defense syndromes in tropical ferns

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Abstract Vascular plants exhibit defense syndromes, a variety of interdependent defensive traits against herbivores, which may considerably differ between plant groups. Although ferns are an abundant component of tropical forest understories, studies of fern–herbivore interactions are scarce, and none has focused on the underlying defense syndromes. To examine the potential defense syndromes of 34 species of tropical ferns of Brazilian forests, we measured ten leaf traits and examined their correlation with parallelly assessed leaf damages. The first three components of categorical PCA were related (1) with SLA, water content, nitrogen, and phosphorus (33.2% of variance); (2) with tannins and saponins, but negatively with trichome density (22.5%); and (3) with

phenol concentrations (16.1%). We identified three groups of fern species with similar leaf damages but different defense syndromes: (I) 14 species were of high nutritional quality (= high SLA, N and water content), but a variable trichome density; (II) 4 species were of low nutritional quality, but had high phenol concentrations, and often a high trichome density; and (III) 16 species were of intermediate nutritional quality and had a low trichome density or were glabrous. Most species (groups I and III) including tree ferns used chemical defenses to protect their highly valuable, nutritious leaves. Group II, exemplified by bracken fern, combined however a low nutritional quality with a powerful chemical defense, including high phenol concentrations, and many trichomes. Because leaf damages did not differ significantly among groups, we conclude that each defense syndrome provides species with a similar resistance against their herbivores.

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Introduction

Herbivores represent one of the main forces that exerts selective pressure on plants (Stamp 2003). Nevertheless, herbivores have to face complex plant defense syndromes consisting of a variety of combinations of

defensive features (Futuyma and Agrawal 2009; Rasmann and Agrawal 2009), which may comprise nutritional quality, regrowth capacity (i.e., tolerance), physical, chemical, and phenological traits (Coley and Barone 1996; Ruiz et al. 2008; Lamarre et al. 2014). Traits may serve two or more functions and are considered as defense mechanisms even if this is not their primary purpose (Strauss and Agrawal 1999). The diversity and combination of distinct defense traits confers plants a considerable herbivore resistance that is reflected in an average annual leaf consumption of 5.3% of the produced leaf tissues across all major lineages of vascular plants (Turcotte et al. 2014). Several authors who have investigated the expression of defense traits and their variation among plant lineages (e.g., Coley et al. 1985; Carmona et al. 2011) have developed evolutionary hypotheses of plant defenses.

The plant defense syndrome hypothesis postulates that plants evolved multiple convergent traits simultaneously (i.e., set of co-varying traits) rather than individual traits (Agrawal and Fishbein 2006), and consequently rejects functional redundancy among traits, although other studies supported possible trade-offs among traits (e.g., Steward and Keeler 1988). The former hypothesis grouped traits based on palatability and defense status of the plant into three general herbivore syndromes: ‘tolerance or escape’, ‘nutritional and defense’, and ‘low nutritional quality’ (Agrawal and Fishbein 2006). The tolerance or escape syndrome typically occurs in resource-rich environments, where highly palatable plants invest little in chemical defenses, but respond to herbivore damage with vigorous growth. In contrast, plants with a nutrition and defense syndrome balance high palatability with efficient leaf defenses, whereas plants of resource-poor environments frequently express the syndrome of low nutritional quality by providing little reward for herbivores (Agrawal and Fishbein 2006). The defense syndrome hypothesis has been corroborated for different clades of angiosperms (see Agrawal and Fishbein 2006; Travers-Martin and Müller 2008; Johnson et al. 2014) and gymnosperms (Moreira et al. 2016), as well as within communities from woody species of the Brazilian Cerrado (Silva and Batalha 2011). Nevertheless, the generality and variability of the described defense syndromes has not been tested for ferns.

The lineage of ferns (Polypodiopsida) is supposedly equipped with an array of efficient biochemical defenses, which has been considered the main reason for their high resistance against herbivores (Soeder 1985; Page 2002). There is little information about the role of physical defense traits in ferns. As the second-most diverse lineage of vascular plants, with about 10,578 species worldwide (PPG I 2016), ferns are especially abundant in tropical forests (Sharpe et al. 2010), where they can comprise up to 15% of the understory cover (Harms et al. 2004). Although ferns and seed plants are sister groups of the same evolutionary age, most modern ferns (e.g., Polypodiales) evolved after the angiosperms (Schneider et al. 2004) and have been suggested to serve only a small number of herbivorous insects as food source (Balick et al. 1978; Mehltreter 2010). However, ferns and angiosperms experience comparable rates of herbivory (Turcotte et al. 2014).

Ferns have been mainly ignored during the development of theories of plant–herbivore interactions. Studies of fern–herbivore interactions have primarily reported species interactions (e.g., Mehltreter et al. 2003; Farias et al. 2018a; Santos et al. 2019), measured levels of herbivore damage (Mehltreter et al. 2006; Farias et al. 2018b) and their effects on plants (e.g., Mesipuu et al. 2009), and evaluated the role of nectaries to provide leaf protection by ants (e.g., Koptur et al. 2013). Here, we investigate the defense syndromes of ferns for a better understanding of the combination of their structural, physical, and chemical defenses. The main goal of this study was to examine the presence, composition, and variety of potential defense syndromes in tropical ferns and their effect and correlation with observed leaf damage.

Methods

Study sites and fieldwork

This study was performed on 34 species of ferns at three tropical forests located in the State of Pernambuco, Northeastern Brazil. Study sites were classified as Lowland Forest (I—8° 34' S; 35° 7' W, below 100 m a.s.l.) and Submontane Forest (II—8° 29' S; 35° 41' W, III—7° 35' S; 35° 29' S, between 450–550 m a.s.l.). The climate was hot and humid with a mean annual temperature of 22–24 °C and an annual rainfall

of 1200–1800 mm. The forests had a canopy height of 15–25 m and displayed a significant floristic richness, because inventories of sites II and III listed 400 and 220 angiosperms, and 90 and 93 ferns, respectively.

We established a total of 22 rectangular plots of 10×20 m (200 m^2) with a minimum distance of 100 m between them. In each plot, we collected 10–15 fully expanded, undamaged leaves from ten mature (= spore-producing) individuals of each fern species (Cornelissen et al. 2003). Because in the tropics, ferns have on average longer leaf life spans (19.2 months) than seed plants (10.0 months; Wright et al. 2004; Mehltreter and Sharpe 2013; Farias et al. 2015), we aimed to study defensive leaf traits that act during most of the life time of a leaf and, consequently, sampled fully expanded rather than young developing leaves.

Leaf damages

We estimated the levels of herbivory by classifying each mature leaf of each plant/plot into six damage categories depending on the relative percentage of removed leaf area (0–1%, > 1–5%, > 5–15%, > 15–25%, > 25–50%, > 50–75%, and > 75% (Mehltreter et al. 2006). The herbivore damage for each plant was obtained as the average of the median values of damage classes of all leaves on a plant. The herbivore damage per species was calculated as the average of all plant individuals of that species.

Measuring leaf traits

Leaf area was measured by scanning entire fresh leaves. Then, leaves were dried at 50 °C for 72 h, ground, and kept in dry conditions until further chemical analyses. We measured ten leaf traits: SLA (specific leaf area), water content, trichome density, nitrogen, phosphorus, alkaloids, phenols, saponins, tannins, and triterpenoids. These traits have been widely recognized to play a role in plant defense (Hanley et al. 2007).

SLA was obtained by dividing the one-sided area of a fresh leaf (cm^2) by dry weight (mg^{-1}). The water content (%) was calculated as the difference of fresh weight and dry weight (mg^{-1}), divided by fresh weight, and multiplied by 100 (Pérez-Harguindeguy et al. 2013). The trichome density was estimated under the stereomicroscope as the average trichome cover of

three squares of 4×6 mm on each side of the leaf, halfway between leaf margin and midvein. For each species the average of all six squares was used to classify trichome density in four categories: glabrescent, slightly hairy (1–25% of grids with trichomes), moderately hairy (26–50%), and strongly hairy (> 50%).

Leaf nitrogen and phosphorus concentrations were obtained by analyzing samples following the adapted protocols of Kjeldahl (Bremner and Mulvaney 1982) and Bezerra-Neto and Barreto (2011), respectively.

Extracts of three solvents (hexane, ethyl acetate and methanol) were obtained from 500 mg of dry leaf material. Then, we performed chemical qualitative tests for alkaloids (Dragendorff's reactions), saponins (boiling distilled water, shaken vigorously and some drops of HCL), and tannins (ferric reaction), as described by Matos (1997) from all three types of extracts. Results were presented as presence/absence data because test results of species did not differ among extracts of different solvents. Using methanolic extracts, triterpenoids were measured by thin layer chromatography (TLC) with toluene–chloroform–ethanol (40:40:10) as mobile phase, lupeol as a standard substance, and anisaldehyde-sulfuric acid for triterpenoid detection (Wagner and Bladt 1996). Phenol concentrations were obtained after mid-boiling 100 mg of dry leaves in 80% methanol, using a 10% Folin–Ciocalteu reagent according to Amorim et al. (2008), and interpolation of the samples' absorbance against a calibration curve of tannic acid (0.1 mg/ml^{-1}) in a spectrophotometer (Genesys 10S UVVIS, Thermo Scientific, Waltham, USA) calibrated at a wavelength of 760 nm.

Data analyses

We examined the relationships among all defense traits using a Categorical Principal Components Analysis (CATPCA). This analysis is particularly useful for data sets with a combination of nominal, ordinal, and numeric variables that might be non-linearly correlated to each other (Jolliffe 2002). We applied the principal normalization to the dataset as it optimizes the association between variables. The component loadings (i.e., correlations between the quantified variables and the principal components) were used as a descriptor for traits that contributed most to the variation of each axis, reporting

values > 0.5 . We reported the first three principal components because these axes were associated with eigenvalues > 1 , according to Kaiser's criterion (Legendre and Legendre 2012). The analyses were performed using SPSS software version 22.0 (SPSS Inc. Chicago, USA).

We examined the similarity of traits among species by performing a hierarchical cluster analysis, using Euclidian distances and Ward's method for linkage. Differences of traits between the formed clusters were evaluated by means of a t-test. We compared the rates of herbivory between the species of each cluster generated from previous analyses by use of a generalized linear mixed model (GLMM). In GLMM, herbivore damage was the response variable, species number of the cluster was the random effect and the generated clusters were fixed effects. We used a significance level of $p \leq 0.05$. Data were analyzed using R v.3.3.3 (R Development Core Team 2009).

Results

Relationship of defensive leaf traits

Eight of the ten leaf traits of ferns were grouped into three principal components, which explained 71.7% of the total variation (CATPCA, Table 1, Fig. 1). The first component had positive loadings for SLA, water content, nitrogen, and phosphorus; the second component showed negative loadings for trichome density and positive loadings for the presence of tannins and saponins (Table 2, Fig. 1); and the third component had positive loadings for phenol concentrations (Table 2). Two of the ten measured defense traits (Table 1) did not present any variance, because alkaloids were absent, and triterpenoids present in all 34 studied species.

Fern defense syndromes

We identified three species groups with different defense syndromes (hierarchical cluster analysis of defense traits, Fig. 2). The first group included 14 species with higher SLA, water content, and nitrogen, and variable trichome density (Table 3, Fig. 2). The second group contained four species with lower SLA, water content and nitrogen, high phenol concentrations, and often high trichome density (Table 3,

Fig. 2). The third group consisted of 16 glabrous or slightly hairy species with intermediate values of nutritional quality (Table 3, Fig. 2). Triterpenoids, saponins, and tannins did not differ significantly among the three groups (Table 3).

At the family level, five of the nine families ($n = 15$) with more than one studied species, presented a homogeneous defense syndrome: Tectariaceae and Thelypteridaceae belonged exclusively to group I, and Blechnaceae, Lindsaeaceae, Marattiaceae to Group III (Table 3). Dryopteridaceae was the only family that included species having all three kinds of defense syndromes (Table 3).

Leaf damages

Ferns presented leaf damages from 0 to 32.3% (Table 1), with an average of 2.0%. Approximately 90% of fern species experienced less than 5% leaf damage. Only *Tectaria incisa* (10%) and *Meniscium serratum* (32.3%) had significantly higher leaf damages than the other species ($F = 10.06$; $p < 0.001$). Leaf damages did not differ between species groups of the three defense syndromes ($F = 2.07$; $p = 0.142$).

Discussion

In the studied tropical fern species, all leaves were defended by a variable number of chemical traits (i.e., triterpenoids, tannins, saponins, phenols content). In addition, some species simultaneously expressed an abundance of trichomes, potentially combined with traits of low nutritional quality such as lower SLA (i.e., high tissue density, see Kitajima and Porter 2010), water, and nitrogen content. Our results corroborate that ferns present similar leaf defense syndromes than seed plants (see Agrawal and Fishbein 2006; Travers-Martin and Müller 2008; Johnson et al. 2014; Moreira et al. 2016), and that in both plant groups physical and chemical defenses act together (Hanley et al. 2007; Carmona et al. 2011). Therefore, the defense strategy of ferns is not built exclusively on a single defensive type such as chemical traits, as it has been previously considered (see Balick et al. 1978; Cooper-Driver 1985). This result was expected, because plants require comprehensive defense strategies that act simultaneously against vertebrates and invertebrates, as well as against generalist and

Table 1 Leaf traits of 34 tropical fern species with distinct defense syndromes: (I) high, (II) low, and (III) intermediate nutritional quality

Family/species	Leaf damage (%)	Defense syndrome	SLA (cm ² /g)	Water (%)	N (% DW)	P (g/kg DW)	Trichomes	Phenols (g/kg DW)	Tannins	Saponins
Anemiaceae										
<i>Anemia hirta</i> (L.) Sw.	0.40	I	303.7 ± 23.7	52.5 ± 10.1	1.98 ± 0.12	0.84 ± 0.04	2	33.6 ± 3.3	+	–
<i>Anemia villosa</i> Humb. & Bonpl. ex Willd.	0.00	II	68.8 ± 18.5	47.4 ± 5.0	2.03 ± 0.17	0.83 ± 0.03	3	39.8 ± 4.4	+	+
Blechnaceae										
<i>Blechnum occidentale</i> L.	0.53	III	223.0 ± 32.3	72.6 ± 1.5	2.20 ± 0.25	0.83 ± 0.05	1	30.4 ± 1.4	+	+
<i>Salpichlaena volubilis</i> (Kaulf.) J. Sm.	1.70	III	169.2 ± 15.6	73.6 ± 2.0	2.02 ± 0.08	0.76 ± 0.00	0	26.1 ± 2.2	+	+
<i>Telmatoblechnum serrulatum</i> (Rich.) Perrie et al.	4.60	III	220.7 ± 43.9	68.9 ± 1.7	1.90 ± 0.24	0.83 ± 0.01	0	32.1 ± 2.8	+	+
<i>Neoblechnum brasiliense</i> (Desv.) Gasper & V.A.O. Dittrich	1.73	III	229.3 ± 11.9	76.4 ± 2.2	2.11 ± 0.14	0.84 ± 0.02	0	29.8 ± 2.7	+	+
Cyatheaaceae										
<i>Cyathea microdonta</i> (Desv.) Domin	7.85	I	395.1 ± 38.3	76.6 ± 5.5	2.88 ± 0.07	0.93 ± 0.02	1	31.1 ± 0.4	+	+
<i>Cyathea phalerata</i> Mart.	3.10	I	282.2 ± 45.5	78.1 ± 2.2	2.77 ± 0.06	0.83 ± 0.00	0	30.6 ± 3.3	+	+
<i>Cyathea praecincta</i> (Kunze) Domin	1.20	III	242.0 ± 39.9	76.1 ± 1.9	2.13 ± 0.05	0.84 ± 0.01	0	28.6 ± 1.9	+	+
Dennstaedtiaceae										
<i>Peridium arachnoideum</i> (Kaulf.) Maxon	1.10	II	102.8 ± 13.6	66.7 ± 4.8	1.67 ± 0.10	0.85 ± 0.00	3	30.0 ± 2.8	+	+
Didymochlaenaceae										
<i>Didymochlaena truncatula</i> (Sw.) J. Sm.	0.00	I	262.3 ± 23.2	74.4 ± 1.5	3.21 ± 0.20	0.89 ± 0.06	1	28.3 ± 5.0	–	+
Dryopteridaceae										
<i>Ctenitis distans</i> (Brack.) Ching	6.00	I	270.3 ± 32.7	66.2 ± 1.0	2.57 ± 0.24	0.77 ± 0.01	1	28.8 ± 2.0	+	+
<i>Cyclodium meniscioides</i> (Willd.) C. Presl	2.80	III	195.2 ± 32.5	80.4 ± 1.8	2.01 ± 0.06	0.85 ± 0.03	1	28.8 ± 1.8	+	–
<i>Diplazium cristatum</i> (Desr.) Alston	0.40	I	306.1 ± 55.6	79.0 ± 3.9	3.50 ± 0.16	0.99 ± 0.05	0	26.9 ± 1.0	–	–
<i>Elaphoglossum iguapense</i> Brade	1.30	II	107.1 ± 16.1	71.4 ± 1.5	1.62 ± 0.18	0.80 ± 0.00	0	28.5 ± 1.9	+	+
<i>Polybotrya osmundaceae</i> Willd.	0.50	III	237.6 ± 11.1	71.5 ± 6.2	2.32 ± 0.10	0.76 ± 0.01	0	27.7 ± 2.9	+	+
<i>Olfersia cervina</i> (L.) Kunze	0.60	III	224.1 ± 27.5	78.8 ± 0.3	1.98 ± 0.09	0.83 ± 0.01	0	36.7 ± 2.1	+	+
Gleicheniaceae										
<i>Gleichenella pectinata</i> (Willd.) Ching	3.85	II	85.2 ± 14.9	56.4 ± 6.9	1.49 ± 0.09	0.74 ± 0.00	3	32.3 ± 2.2	+	+
Lindsaeaceae										
<i>Lindsaea lancea</i> (L.) Bedd.	3.50	III	199.9 ± 26.3	63.1 ± 2.3	2.44 ± 0.13	0.75 ± 0.00	0	26.6 ± 1.0	–	+

Table 1 continued

Family/species	Leaf damage (%)	Defense syndrome	SLA (cm ² /g)	Water (%)	N (% DW)	P (g/kg DW)	Trichomes (% DW)	Phenols (g/kg DW)	Tannins	Saponins
<i>Lindsaea quadrangularis</i> Raddi	0.40	III	193.4 ± 34.4	63.5 ± 5.7	2.43 ± 0.10	0.77 ± 0.01	0	28.2 ± 0.3	+	+
Marattiaceae										
<i>Danaea geniculata</i> Raddi	2.35	III	216.9 ± 15.3	78.4 ± 2.0	2.63 ± 0.53	0.82 ± 0.02	0	33.3 ± 0.4	+	+
<i>Danaea nodosa</i> (L.) Sm.	0.40	III	231.7 ± 1.9	76.0 ± 3.1	1.58 ± 0.18	0.82 ± 0.00	0	29.7 ± 2.5	—	+
Metaxyaceae										
<i>Metaxya parkeri</i> (Hook. & Grev.) ex J. Sm.	0.30	III	192.6 ± 7.5	69.9 ± 0.5	1.88 ± 0.04	0.74 ± 0.00	1	31.2 ± 1.6	+	+
Polypodiaceae										
<i>Pechluma robusta</i> (Fée) M. Kessler & A. R. Sm.	1.40	III	145.1 ± 23.8	60.4 ± 6.2	2.89 ± 0.07	0.89 ± 0.08	0	50.4 ± 3.3	+	+
<i>Serpocaulon</i> sp.	2.75	I	283.2 ± 97.9	75.6 ± 5.8	2.49 ± 0.23	0.76 ± 0.00	3	27.6 ± 0.7	—	—
Pteridaceae										
<i>Adiantopsis radiata</i> (L.) Fée	0.15	III	199.3 ± 9.6	60.3 ± 1.4	3.12 ± 0.19	0.79 ± 0.01	1	41.7 ± 3.8	+	+
Saccolomataceae										
<i>Saccoloma elegans</i> Kaulf.	1.20	III	210.9 ± 50.2	73.5 ± 5.5	3.18 ± 0.21	0.83 ± 0.00	0	28.0 ± 0.6	+	+
Tectariaceae										
<i>Tectaria incisa</i> Cav.	11.7	I	520.5 ± 81.3	83.3 ± 2.3	3.22 ± 0.17	0.99 ± 0.02	1	30.8 ± 1.7	+	+
<i>Triplophyllum dicksonioides</i> (Fée) Holttum	1.10	I	267.1 ± 10.8	70.4 ± 1.3	3.08 ± 0.09	0.78 ± 0.00	1	26.3 ± 0.4	+	+
Thelypteridaceae										
<i>Christella hispidula</i> (Deene.) Holttum	2.90	I	364.4 ± 15.0	74.6 ± 5.7	2.40 ± 0.09	0.79 ± 0.01	3	26.3 ± 1.0	—	—
<i>Goniopteris biolleyi</i> (Christ) Pic.Serm.	0.90	I	368.3 ± 40.6	72.7 ± 0.9	4.09 ± 0.66	0.89 ± 0.04	2	28.2 ± 1.9	+	+
<i>Meniscium macrophyllum</i> Kunze	5.10	I	298.7 ± 99.0	81.9 ± 3.8	2.02 ± 0.20	0.78 ± 0.01	0	28.5 ± 1.8	+	+
<i>Meniscium serratum</i> Cav.	32.3	I	252.5 ± 53.5	74.0 ± 4.8	2.47 ± 0.41	0.93 ± 0.03	2	31.2 ± 1.8	+	+
<i>Stenopteris polypodioides</i> (Raddi) Salino & T.E. Almeida	1.40	I	251.8 ± 21.2	68.7 ± 2.5	2.25 ± 0.12	0.83 ± 0.03	2	27.8 ± 0.8	+	+

Numeric values (mean ± SD)

Trichomes: 0—glabrescent, 1—slightly hairy, 2—moderately hairy and 3—strongly hairy. Presence (+) or absence (—) of tannins and saponins

*In all species triterpenoids were present and alkaloids absent

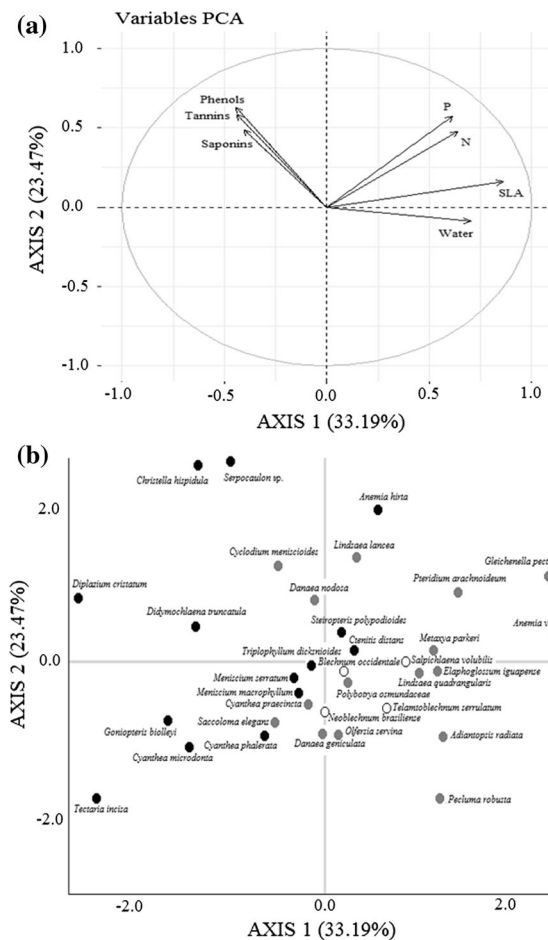


Fig. 1 Ordination of (a) defensive leaf traits and (b) 34 fern species and on the first two axes of a Categorical Principal Components Analysis (CATPCA). Significant loadings: axis 1—SLA, water content, nitrogen, and phosphorus (+), axis 2—trichomes (–), tannins and saponins (+), axis 3—phenol content (–). Fern group I (black circles, II (white circles), III (gray circles)

specialist insect herbivores. However, individual defense traits aimed against vertebrates (e.g., alkaloids) differ from defense traits against invertebrates (Tanentzap et al. 2011), and those designed to repel generalist insects are distinct from those to fight off specialist insects (Jared and Agrawal 2012). Ferns are mainly attacked by insects (Mehltreter 2010), and consequently defense syndromes of ferns have to combine several traits against different kinds of insects to provide a broader herbivore protection.

The low nutritional quality exhibited in few of the studied ferns (group II, $n = 4$) has been reported for slow-growing gymnosperms (Moreira et al. 2016), woody species in areas with poor soils (Silva and Batalha 2011) and evergreen trees of dry tropical forests (Pringle et al. 2011). Plants characterized by low nutritional quality often combine low SLA and low water content with low nutrient concentrations (e.g., nitrogen and/or phosphorus). Together with the set of chemical traits, they represent a defense syndrome that affects host-plant choice (acceptability) and host-plant suitability for herbivores. In general, insects select their food plants depending on their own developmental performance, which usually is correlated with physical and chemical traits related to plant quality (Scriber 2009). Low concentrations of nitrogen and phosphorus, for example, affect negatively the performance of insects (see Mattson 1980; Stamp and Casey 1993; Sankaran and McNaughton 2005; Penning and Simpson 2008) due to the high nutrient requirements of their body tissues for maintenance, growth, mobility, and reproduction (Perkins et al. 2004; Huberty and Deno 2006; Lemoine et al. 2014).

The low nutritional quality as a potential defense against herbivorous insects is especially effective

Table 2 Relationships among defense traits of 34 tropical fern species (Categorical Principal Components Analysis = CATPCA)

	Component 1	Component 2	Component 3
Variance accounted	33.19%	22.47%	16.06%
Eigenvalues	2.65	1.79	1.28
SLA	0.86*	0.01	0.05
Water content	0.72*	− 0.12	− 0.46
N	0.68*	0.24	0.37
P	0.64*	0.29	0.46
Trichomes	− 0.40	− 0.62*	0.35
Phenols	− 0.38	0.47	0.67*
Tannins	− 0.31	0.72*	− 0.12
Saponins	− 0.27	0.71*	− 0.33

The first three components had eigenvalues above 1

* = significant loadings

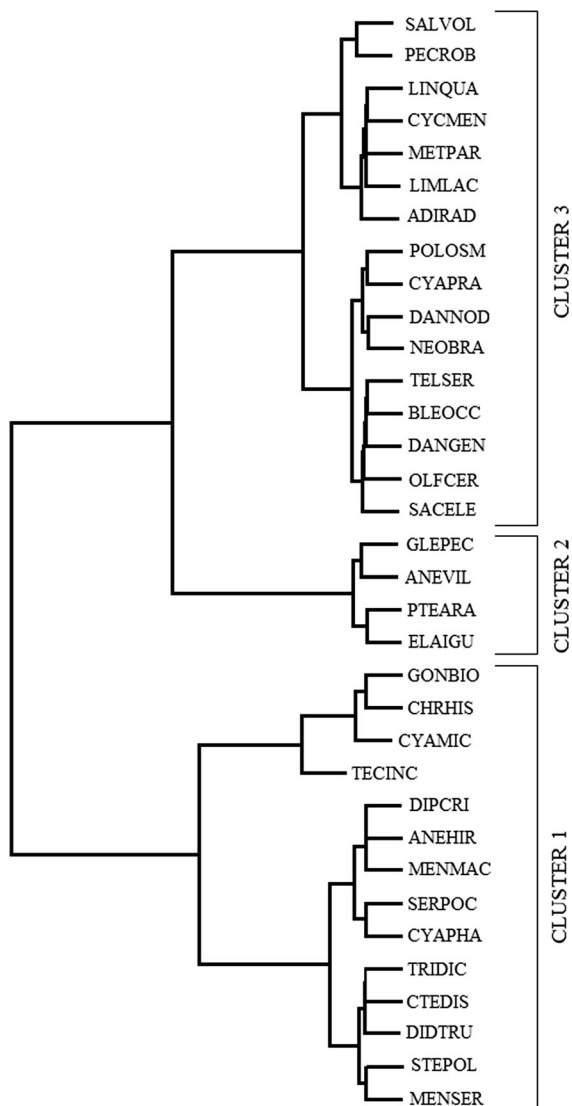


Fig. 2 Species clustering based on defensive leaf traits of 34 fern species from tropical Brazilian forests (Euclidean distances, Ward's grouping linkage method). Species names are abbreviated with each of the first three letters of the genus and the epithet

when combined with chemical defenses, because herbivores can compensate for nutrient deficiency by a longer foraging time and higher consumption rates, but this compensation mechanism of insects does not work any longer if plants possess a defense syndrome that includes chemical defenses (Haukioja et al. 1991; Augner 1995). One example of such a combination of defense mechanisms is the common tropical bracken fern, *Pteridium arachnoideum* that exhibits a powerful

array of several chemical defense traits, combined with a low nutrient content and trichomes on the lower leaf surface (Robinson et al. 2010). On the other hand, the most studied fern species expressed a defense syndrome comprising high nutritional quality (group I, $n = 14$ and group III, $n = 16$), as is the case of the tree ferns (Cyatheaceae) and species of Thelypteridaceae, which seem to depend on the efficiency of chemical defenses, and sometimes even physical traits (e.g., trichomes) to prevent the damage and loss of highly nutritious leaf tissues.

The mean herbivore damage of studied ferns (2.0%) was lower than the average of 5.8% reported in a meta-analysis performed by Turcotte et al. (2014). However, we observed a considerably larger variation between species (0–32.3%), compared to Turcotte et al. (2014) with values between 4.0 and 8.3%. Our results reinforce conclusions of former investigations that ferns present similar mean leaf damages than angiosperms, and that high leaf damages are often concentrated on a few plants or species (Hendrix and Marquis 1983; Mehltreter and Tolome 2003; Winkler et al. 2005; Turcotte et al. 2014). The few species with high leaf damages did neither rely on a particular defense syndrome nor did species with distinct defense syndromes have different leaf herbivory, which indicates that all three syndromes apparently confer their fern species a similarly effective resistance against herbivores. However, Johnson et al. (2014) considered that plant traits must respond and adapt to many other selective pressures, including environmental stress. Consequently, more successful defense syndromes might be otherwise disadvantageous or too expensive to evolve further. As a result, differences in the efficiency of defense syndromes are hard to elucidate, and researchers may report seemingly contradictory findings (see Agrawal and Fishbein 2006; Travers-Martin and Muller 2008; Pearse 2011). Finally, the similar efficiency of different defense syndromes might be also due to a predominance of generalist herbivores in the tropics (Novotny et al. 2002) that cannot be kept at bay with a single defense trait.

Based on the 'defense syndrome triangle' of Agrawal and Fishbein (2006), studied fern species were classified into two syndromes: 'low nutritional quality' (group II) and 'nutrition and defense' (group I and III). The resistance by constitutive traits seems to be the main defense strategy of tropical ferns, which are mostly evergreen. Most deciduous fern species

Table 3 Trait values of groups of ferns with different defense syndromes (mean \pm SD) of (I) high, (II) low, and (III) intermediate nutritional quality

Defensive traits	Group I	Group II	Group III
SLA	316.15 \pm 74.12 ^a	90.94 \pm 17.55 ^c	208.17 \pm 25.82 ^b
Water content	73.43 \pm 7.65 ^a	60.48 \pm 10.76 ^b	71.45 \pm 6.56 ^a
N	2.78 \pm 0.59 ^a	1.70 \pm 0.23 ^c	2.30 \pm 0.45 ^b
P	0.85 \pm 0.08 ^a	0.80 \pm 0.04 ^a	0.81 \pm 0.04 ^a
Trichomes	Absent to frequent	Often frequent	Absent or scarce
Phenols	29.01 \pm 2.16 ^a	32.62 \pm 5.02 ^a	31.80 \pm 6.34 ^a

Group I ($n = 14$), II ($n = 4$) and III ($n = 16$). Tannins and saponins did not differ among groups. Letters indicate significant differences between groups ($p < 0.05$)

shed their leaves to overcome the dry season; however, some ferns, such as *Alsophila firma* (Baker) D. S Conant possess unusual escape traits as a defense strategy, because this tree fern species drops its leaves during the rainy season to avoid the presumably higher herbivore pressure of unidentified insect species during that time of the year (Mehlreter and García-Franco 2008). The escape strategy is commonly reported for angiosperms, especially those of seasonal forests. For instance, *Hybanthus prunifolius* (Humb. & Bonpl. ex Schult.) Schulze-Menz (Violaceae) anticipates the peak period of herbivore abundance at the beginning of the wet season by flushing its leaves still during the climatically harsh, dry season (Aide 1992). This strategy might have been an adaptation to a strong herbivore pressure of some specialized insects, because it represents a high energetic cost to replace each year simultaneously the plant's entire set of leaves.

Both strategies, resistance and escape are costly defensive strategies, the former by investing into production of chemical defenses, and the latter by allocating resources into production of new leaves to compensate for lost tissue (Herms and Mattson 1992; Sagers and Coley 1995). However, the allocation of plant defenses evolves to maximize the benefit–cost ratio associated with each defense syndrome (Simms and Rausher 1987). Slow-growing understory ferns might have a low capacity to compensate for herbivore-damaged leaf tissues, because of the low efficiency of the photosynthesis in the dark shade (Page 2002) and should be expected to avoid herbivory by investing in defenses.

Future studies should investigate if herbivore damage and leaf traits differ among fern species with distinct defense syndromes, and under which abiotic conditions (e.g., drought, high irradiance) ferns follow an escape strategy. Many tropical ferns (and angiosperms; see Kursar and Coley 2003) expand their leaves rapidly (i.e., proxy to growth) and simultaneously within a month to oversaturate herbivore populations (Sharpe 1997; Farias et al. 2015). In those cases, we should gain a better understanding of the escape strategy by studying the biology and life cycles of their dominant herbivorous insect species.

In some fern species, trichomes added to the effect of other plant defenses, although they were less important than other leaf traits, and subdivided ferns into further categories of defense syndromes. Trichomes provided a powerful addition to the leaf defense of some ferns in group II, that even with low nutritional quality and defensive chemical traits, still invested in high amounts of trichomes. This combination of defensive leaf traits including trichomes might be, however, related to the habitat conditions of three of the fern species in group II (*Anemia villosa*, *Gleichenella pectinata* and *Pteridium arachnoideum*) that occur typically in sunny, dry habitats, such as clearings and forest edges (Mickel and Smith 2004). These habitats favor carbon-based, physical, and chemical defensive traits, as reported in seed plants (Tuomi et al. 1987) and under those circumstances, trichomes may primarily serve as a protection against photoinhibition and desiccation, as has been shown for laminar scales (Watkins et al. 2006). In contrast, only two species of group I with high nutritional quality, and without tannins and saponins, were also strongly

covered by trichomes. Therefore, the presence of trichomes in ferns occurs independently of other chemical and physical traits of resistance, as it was suggested by Moreira and Pearse (2017) for oak species (genus *Quercus*). The occurrence of trichomes with any combination of defensive traits in ferns may be supported by their multifunctional role (see Dalin et al. 2008). For instance, trichomes on the upper leaf surface can reduce the colonization of foliicolous lichens (Lücking 1998), increase fungal infections (Calo et al. 2006), and lower the efficacy of predators (Schoonhoven et al. 2005). Consequently, we conclude that the abundance of trichomes on plants is influenced by diverse biotic variables, such as herbivore pressure (Agrawal 2000), or by abiotic variables, such as light availability (Molina-Montenegro et al. 2006).

The elevated concentration of phenols in ferns of group II is surprising, because many plant species with low nutritional quality supposedly reduce chemical defenses when they present strong physical defenses, such as leaf toughness (low SLA) and trichomes (Hanley et al. 2007). However, in other studies, plants followed an opposite pattern, presenting a positive correlation between leaf toughness and phenol concentrations (Read et al. 2009), like the ferns of group II. Because phenol production increases at higher light levels (Mole et al. 1988) and these fern species develop better in sunnier conditions (Mickel and Smith 2004), higher phenol production in those ferns may not be necessarily linked with herbivore pressure but light conditions. Finally, alkaloid tests were negative for all fern species of our study but were positive for some ferns in other phytochemical investigations (e.g., Feio et al. 2013). Few authors, however, have reported the presence and structural composition of alkaloids in ferns (e.g., Zhou et al. 1998), and future studies will have to discover how widespread alkaloids are in ferns, and if they play a similar role in herbivore defense as they do in angiosperms (see Hartmann and Ober 2008).

Conclusion

Our data corroborate that tropical ferns exhibit at least two of the defense syndromes proposed by Agrawal and Fishbein (2006): ‘nutritional and defense’ (most of the species) and ‘low nutritional quality’. Most fern

species invest heavily in a combination of chemical traits to protect their nutritious, long-lived leaves. Trichomes, however, constitute a more flexible defense trait that may or may not co-occur with other types of defenses. Different defense strategies of ferns were similarly effective, because plants of each defense syndrome suffered similar levels of leaf damage.

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