

Testing the low latitude/high defense hypothesis for broad-leaved tree species

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Abstract We tested the hypothesis that leaves of broad-leaved tree species are more highly defended at low latitudes than at high latitudes. We used canonical discriminant analysis to compare tree species from Panama (9°N, 39 species), Missouri, USA (38°N, 37 species), and southern Ontario, Canada (44°N, 34 species) with respect to two structural and five nutritional traits, taking into account each species' tolerance to shade. Trees from the three locations differed significantly, with Panamanian species the most distinct. Defenses of shade-tolerant species were significantly greater than those of shade-intolerant species, but only for the Panamanian sample, which is consistent with the low latitude/high defense hypothesis. Because we sampled many of the same tree species from Missouri and southern Ontario, and many tree species in the same taxonomic families in Missouri and Panama, we were able to control for the potential confounding effects of phylogeny. Overall defense levels, calculated by summing the *z*-scores for individual traits in each location, were significantly higher for Panama compared to Missouri, and marginally so for Missouri compared to southern Ontario, again consistent with the low latitude/high

defense hypothesis. Traits contributing to these differences were mostly structural factors (e.g., fiber) and to a lesser degree nutritional traits, while secondary compounds made no independent contribution to differences in overall defense levels (four traits compared between Panama and Missouri). Contrary to our expectation, the number and types of secondary compounds per species reported in the literature for our species did not differ between temperate and tropical locations, while the diversity of these compounds was greater for the temperate species. Overall, our results provide some support for the hypothesis that leaf defenses against herbivory are better developed in tropical than in temperate trees, but the differences were due to structural and nutritional factors rather than secondary compounds.

Keywords Broad-leaved trees · Herbivore pressure · Latitudinal gradients · Plant defense evolution · Leaf traits

Introduction

Decreasing species richness at higher latitudes has been documented repeatedly for many taxa in both terrestrial and aquatic habitats (e.g., MacArthur 1969; Rosenzweig 1995; Gaston 1996; Roy et al. 1998; Hillbrand 2004). The types of interactions that structure communities may also differ between tropical and temperate latitudes. Dobzhansky (1950) proposed that biotic interactions dominated in tropical climates, while abiotic factors become increasingly important with distance from the equator. This view influenced much of the early thinking (e.g., Pianka 1966) regarding latitudinal gradients in species richness. In particular, biotic interactions are thought to increase the complexity of tropical environments and stimulate diversification (Schemske 2002; Schemske et al. 2009). Yet few comparative

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studies have documented differences in the incidence and strength of biotic interactions between tropical and temperate regions, or how these differences might be related to species richness and trait evolution.

Data on consumption of the leaves of forest trees by insects show that annual leaf area loss in tropical latitudes exceeds that in temperate latitudes (Coley and Aide 1991; Coley and Barone 1996). A possible mechanism for such differences is that insect herbivore populations suffer relatively less impact from abiotic factors in tropical evergreen forests. Consequently, they consume more biomass in tropical compared to temperate forests, where harsh winter conditions reduce insect populations.

Provided that herbivore pressure (and/or impacts from other consumers, fungi and bacteria: Packer and Clay 2000; Mangan et al. 2010) is elevated in tropical regions, tropical plants should evolve stronger herbivore defenses than temperate plants (Gillett 1962; Hay and Fennical 1988; Coley and Aide 1991): the “low latitude/high defense” hypothesis. In support of this hypothesis, global surveys of alkaloids (Levin and York 1976), and of tannins and terpenoids (Hay and Fennical 1988; Coley and Aide 1991; Bolser and Hay 1996), have demonstrated higher concentrations in tropical than in temperate plants. In addition, palatability trials show that marine (Bolser and Hay 1996) and salt-marsh (Pennings et al. 2001, 2009) herbivores prefer plants from higher latitudes. If herbivore defenses are effective, leaf damage would be even greater in tropical plants than in temperate plants if it were not for their better developed physical and chemical protection. Not all data are supportive: only 9 of 56 studies exhibited higher defense levels at low latitude in a recent meta-analysis (Moles et al. 2011a), only 2 of 14 traits surveyed across 75 sites were significantly higher at lower latitudes (Moles et al. 2011b), and leaf mechanical properties were not related to latitude (Onoda et al. 2011).

An additional complication arises from the manner in which defenses have been compared between plants of tropical and temperate forests. Differences could appear for two reasons: (1) different environmental factors, i.e., differences in herbivore and disease pressure, and tritrophic interactions, could select for differences in defense, or (2) differences could arise because unrelated plant species found in two regions have different levels of defense for reasons other than their interactions with herbivores (Siska et al. 2002). Because no systematic effort has been made to sample tropical and extratropical forest plants while controlling for phylogeny (relatedness), we cannot yet distinguish these alternative causes for the observed patterns in terrestrial ecosystems [but see Bolser and Hay (1996) and Pennings et al. (2001, 2009) for studies controlling for phylogeny in marine and salt-marsh systems, respectively]. The importance of phylogeny and geography is emphasized by Seigler et al. (1989) in their summary of the distribution

of cyanogenesis in the Fabaceae: most cyanogenic species belong to phylogenetically derived tribes, and most of these occur in temperate regions.

The goal of this study was to quantify potential differences in leaf quality along a latitudinal gradient while controlling for relatedness among species. Specifically, we expected that leaves from tropical plants would be more heavily defended than those from temperate plants. In addition, we suspected that more closely related plant species would have more similar leaf traits (e.g., Ehrlich and Raven 1964; Ricklefs and Matthew 1982; Ricklefs 2008), but were uncertain whether this would confound any difference observed between tropical and temperate localities. For this reason, we included tropical and temperate species belonging to the same families to distinguish between the effects of relationship and environment on plant traits. In addition, we predicted that tropical and temperate samples would differ more than temperate samples of the same species from different latitudes because of the dominating effect of winter cold temperatures in temperate regions. An additional complicating factor is the influence of resource availability—both light (Coley et al. 1985) and soil nutrients (Fine et al. 2006)—on the evolution of defense. Adaptation to low light and low nutrients are predicted to lead to higher defense allocation because of slower growth and greater leaf longevity. We classified species sampled as either shade tolerant or intolerant, and used this factor in our analyses. We predicted that differences in defense allocation between shade-tolerant and shade-intolerant species would be greater for species of low latitude sites, again because of expected higher herbivore pressure in the tropics.

Our sampling included two temperate locations (one in Missouri and one in southern Canada), as well as one tropical location in Panama. In addition, to supplement data on secondary compounds, we used independent data from a published database (NAPRALERTsm) to compare the number of discovered compounds and compound types per plant species, and overall compound richness for the tropical and temperate tree species of our study. Presuming the occurrence of higher herbivore pressure and greater numbers of species attacking individual plant species in the tropics (Dyer et al. 2007), we would expect greater diversity of compounds in tropical compared to temperate species due to repeated rounds of herbivore-driven diversification (Ehrlich and Raven 1964).

Methods

Latitude leaf data sets

We assembled four data sets of leaf traits for forest trees (see Table 1 and the Electronic supplementary material,

Table 1 Site, years sampled, location, and sample size for the four latitudinal data sets used in these analyses

Data set	Site	Years sampled	Elevation (m a.s.l.)	Latitude/longitude	Number of species sampled
C	Lake Opinicon, Ontario, Canada	1977	137–198	44°34'N, 76°19'W	34
M	St. Louis, Missouri, USA	2003–2004	150–195	38°–39°10'N 90° 19' W	37
P1	Barro Colorado Island, Panama	1982	61	9°09'N 79°51'W	21
P2	Barro Colorado Island, Panama	2003–2004	61	9°09'N 79°51'W	19

C Canada, M Missouri, P1 Panama data set 1, P2 Panama data set 2, *m a. s. l.* meters above sea level

ESM). The first two, one from Lake Opinicon, Ontario, Canada (34 species) and one from Barro Colorado Island (BCI), Panama (Panama set 1: 21 species), were collected from various tree species without regard to phylogenetic relationship. The third data set, also from BCI (Panama set 2: 19 species) and the fourth, from the St. Louis, Missouri, USA region (37 species), were collected specifically with the goal of testing latitudinal differences while controlling for phylogeny. Thus, the second Panamanian sample consisted of species chosen from plant families that occur in Missouri, while the Missouri sample included species also sampled in Canada. The Canadian samples (Ricklefs and Matthew 1982) were collected in 1977 from within a 24 km radius of the Queen's University Biological Station on Lake Opinicon, near Chaffey's Locks, Ontario (44°34'N). Samples were collected at three times during the growing season (late May to early June, early July, and early August). Mean values for leaf traits from these three collections were used for comparison with the Panama and Missouri data. The first set of Panamanian samples was collected from Barro Colorado Island (BCI, 9°09'N) in 1982 by G. Stevens, with leaf quality traits measured as in Ricklefs and Matthew (1982). The second set of Panamanian samples was collected from BCI, in June 2003 and May 2004, from trees on the 50 ha forest dynamics plot (<http://ctfs.si.edu/datasets/bci/>). Samples were obtained from 2–3 trees per species when possible. Finally, Missouri leaves (generally shade grown, reached from the ground, sampling 2–3 trees per species, 38°–39°10'N), were collected in July and August of 2003, with the exception of three species (*Cornus florida*, *Sideroxylon lanuginosum*, and *Ailanthus altissima*) collected in August 2004. All leaves were fully mature at the time of collection. Most samples were collected in Cuivre River State Park (<http://www.mostateparks.com/cuivre.htm>), a secondary forest near Troy, MO, USA. Additional samples came from three other locations (all secondary forest): Forest Park, St. Louis, MO; Rockwood Reservation, Eureka, MO; and the Potosi Ranger District of the Mark Twain National Forest, Missouri.

With the exceptions listed below, the methods for analyzing Canadian leaves and the first Panamanian samples (Ricklefs and Matthew 1982) were followed for those from

the second Panamanian and Missouri samples. Missouri and Panama leaves were collected on ice, and processed for disk-making to estimate specific leaf area, water content (10 disks 1.9 cm in diameter weighed fresh and then weighed again after drying), and toughness [g/mm^2 based on three punches with a penetrometer (point 1.5 mm radius) for each of 3–4 leaves]. Toughness estimates for Canadian leaves were recalculated to g/mm^2 from the values reported in Ricklefs and Matthew (1982) using the equation $Y = 0.632X - 8.182$, calibrated with the instruments used in the two studies. Panamanian species were stored initially at -20°C , until they were transported to the University of Missouri—St. Louis, where they were stored at -80°C . The Missouri collections were stored throughout at -80°C . Leaves were lyophilized and then ground to a fine powder. Macro- and micronutrients, acid detergent fiber (ADF), and neutral detergent fiber (NDF) were measured at the Soil, Water and Plant Testing Laboratory of Colorado State University (<http://www.soiltestinglab.colostate.edu/>). Lignin and ash weight were estimated as in Allen et al. (1974) for all four data sets, and ADF as in Allen et al. (1974) and NDF as (holocellulose + lignin) (Allen et al. 1974) for the Canadian species and the first Panamanian sample.

We measured condensed tannins using a microscale modification of the acid–butanol assay (Rossiter et al. 1988), and gallotannins or hydrolyzable tannins using a microscale modification of the potassium iodate technique (Schultz and Baldwin 1982), with purified sorghum tannin and tannic acid, respectively, serving as standards. Tannin measurements for the Missouri and second Panamanian samples could not be compared directly to those from the Canadian and first Panamanian samples because substantially different methods were used for the preparation and storage of leaf material. We estimated alkaloid concentration by filtering and weighing the dried precipitate resulting from mixing Dragendorff's reagent with 0.5 g dried leaf material after initial extraction in sulfuric acid (Marquis and Batzli 1989). Saponins were measured as in Dearing and Schall (1992), using *Trifolium repens* as a standard. Cyanide was measured in 0.5 g of dried leaf material using Feigl–Anger paper and results were compared to the known cyanogenic species *Prunus serotina* (Brinker and Seigler 1989). Most

species showed no trace of cyanogenesis, and those that did (*P. serotina*, *P. virginiana*, *Aralia spinosa*, *Dendropanax arboreum*, and *Tabebuia rosea*) were equal in color development to that of *P. serotina*. Species means for traits measured are given in the ESM.

Statistical analysis

All ANOVA analyses were conducted in SAS (SAS 1989). Accepted *P* values for all ANOVAs were based on type III sums of squares. Missing values were assigned the mean value for all plants in an analysis, regardless of site or shade tolerance. Species were classified as either shade tolerant or shade intolerant based on Fowells (1965) and web updates (http://www.na.fs.fed.us/spfo/pubs/silvics_manual/table_of_contents.htm) and the experience of the authors (Missouri and Canada), and the consensus of advice from P.D. Coley, S.J. Wright, and Comita et al. (2010) for Panamanian species (ESM). The contribution of phylogeny to the variation in trait values was estimated using the Panama (second set) and Missouri samples with nested ANOVA, testing the order effect over the family-within-order effect. Only orders that had multiple families were included, and only families within those orders that had multiple species. Canonical discriminant analysis based on the subset of eight traits (log-transformed: nitrogen, water, magnesium, phosphorus, lignin, potassium, ADF, and NDF) measured in all three locations, including both Panama data sets, was used to test for differences among sites. In further analyses, traits were first standardized to a mean of zero and a standard deviation of 1.0 to produce *z*-scores. These *z*-scores were then summed across all traits (Fine et al. 2006). Those traits considered to contribute to defense were given a positive sign (secondary compounds and structural traits), while nutritional traits were given negative signs. ANOVA, with family and site (Panama second set vs. Missouri) or family, species, and site (Missouri vs. Canada) as the main effects, were conducted on the summed *z*-scores for each species. ANOVAs also were conducted for each trait separately using family and site (Panama second set vs. Missouri) or species and site (Missouri vs. Canada) as the main effects.

NAPRALERTsm database

We searched the Natural Products Alert database (<http://www.napralert.org/>) for studies that have identified secondary compounds from plant species included in this study. The database covers studies published between 1975 and 2003, and a subsample of those published subsequently. We found records for leaf compounds identified from 18 of the tropical species and 28 of the temperate species in our samples. We used analysis of covariance with latitude

(temperate vs. tropical), order, and family nested within order as the main effects, and number of studies reported per plant species as the covariate, to predict the total number of compounds and number of unique classes of compounds. Because neither family, order, latitude, nor the interaction of latitude with the number of studies was significant, we report the simple regressions of the number of compounds and the number of unique classes of compounds on the number of studies per tree species for temperate and tropical species combined. Finally, we asked whether the rarified “species richness” (Coleman curves) of compounds differed at 16 species (the smaller of the two samples) between the temperate and tropical samples, as calculated by Estimates 8.0 (Colwell 2006).

Results

Latitude data

Nested analysis of variance applied to the Missouri and Panama samples supported an effect of phylogeny on the traits measured (Table 2). Of the 23 traits measured, four showed a significant effect ($P \leq 0.10$) of family, and three showed a significant effect of order ($P \leq 0.10$). Including the species of the Fabales, Lamiales, Laurales, Malpighiales, and Rubiales (all of which had a single family but multiple species per family, results not shown) led to significant ($P \leq 0.10$) order effects for percent nitrogen ($P = 0.029$), magnesium ($P = 0.033$), zinc ($P = 0.066$), and boron content ($P = 0.022$), and a significant family effect for percent ash content ($P = 0.092$).

Canonical discriminant analysis of plant species based on traits measured at all three sites (nitrogen, water, magnesium, phosphorus, lignin, potassium, ADF, and NDF) showed significant overall separation of the six combinations of site and shade tolerance (Wilk’s lambda $F_{40,404} = 5.62$, $P < 0.0001$, Fig. 1). The contribution of site to the overall separation was greater than that of shade tolerance. Specifically, all between-site comparisons were significant ($P \leq 0.0008$), except for the comparison between shade-intolerant species from Canada versus Missouri ($P = 0.066$) (Table 3). In contrast, trees differed significantly with respect to shade tolerance only for the Panama samples ($P = 0.0051$), a result consistent with the prediction that differences would be greater at lower latitudes (Table 3). The three sites separated along canonical variable 1, which accounted for 79.4% of the variance (Fig. 1). Temperate species were distributed towards the negative end of this axis. Water, lignin, magnesium, ADF, and NDF contributed positively, and phosphorus contributed negatively to scores on axis 1. Tree species of the two shade-tolerance levels separated along axis 2 (11.4% of the total variance

Table 2 Effect of order, and family nested within order, on measured plant traits

Trait	Family	<i>N</i>	Order	<i>N</i>
Nutritional traits				
N ^a	0.6730	12	0.1713	5
Mn ^b	0.1769	12	0.5574	5
Ca ^a	0.0565	12	0.7417	5
P ^a	0.5492	12	0.2135	5
K ^a	0.3093	12	0.7278	5
Na ^a	0.2259	12	0.6105	5
Fe ^b	0.5209	9	0.3967	4
Mg ^a	0.9621	12	0.0246	5
Zn ^b	0.8582	9	0.0717	4
Cu ^b	0.0513	9	0.8259	4
B ^b	0.0948	9	0.1776	4
Mo ^b	0.7309	12	0.2692	5
Ash ^a	0.0999	12	0.1372	5
H ₂ O ^a	0.3071	12	0.0454	5
Structural traits				
Specific leaf area ^c	0.4264	12	0.1552	5
Lignin ^a	0.6437	12	0.2698	5
Non-detergent fiber (NDF) ^a	0.3154	12	0.1730	5
Acid-detergent fiber (ADF) ^a	0.5471	12	0.4234	5
Toughness ^d	0.6942	12	0.4804	5
Secondary compounds				
Alkaloids ^e	0.1723	9	0.9105	4
Saponins ^f	0.1966	9	0.5246	4
Condensed tannins ^g	0.1990	10	0.2128	4
Hydrolyzable tannins ^h	0.3449	10	0.1734	4

All based on type III sums of squares. Order was tested over the family effect. *N* = number of families or orders included in the analysis

- ^a Percent dry weight leaf mass
- ^b Parts per million
- ^c mg/2.0 cm²
- ^d g/7.065 mm²
- ^e Parts per thousand
- ^f cm of foam
- ^g Sorghum tannin equivalents per mg dry weight leaf mass
- ^h Tannic acid equivalents per mg dry weight leaf mass

explained), with positive loadings of nitrogen, water, and phosphorus associated with shade-intolerant species [Fig. 1; trait loadings for canonical variables 1 and 2, respectively, were nitrogen (0.22, 0.54), water (0.76, 0.63), magnesium (0.96, -0.08), phosphorous (-0.84, 0.50), potassium (0.60, -0.12), neutral detergent fiber (0.94, -0.31), acid detergent fiber (0.89, 0.30), and lignin (0.90, 0.27); species scores are given in the ESM]. All seven variables differed significantly among site/shade-tolerance combinations (univariate ANOVAS, *P* ≤ 0.043, *df* = 5, 99).

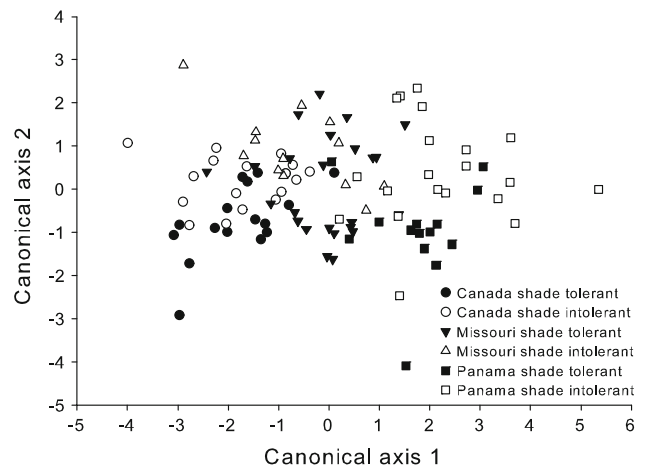


Fig. 1 Canonical discriminant analysis of tree species based on their location and shade tolerance

Patterns of overall defense using *z*-scores showed support for the low latitude/high defense hypothesis, especially for the Missouri versus Panama comparison (*F*_{1,20} = 6.78, *P* = 0.017), while the Canada versus Missouri comparison (*F*_{1,13} = 4.10, *P* = 0.0638) was marginally significant after controlling for either family or species and family, respectively. In both cases, the mean *z*-score for the higher latitude site was lower than that of the lower latitude site, with the stronger effect occurring between Missouri and Panama, supporting our prediction of greater effects between Missouri and Panama (Fig. 2). The species effect (*P* = 0.012) was significant for *z*-scores for the Canada versus Missouri comparison, while the family effect was not significant for either the Missouri versus Panama comparison (*P* = 0.69) or the Canada versus Missouri comparison (*P* = 0.31). *z*-score values were not significantly affected by shade tolerance for either comparison (Missouri vs. Panama: *P* = 0.70, Canada vs. Missouri: *P* = 0.58), although the values were in the direction predicted by defense theory (high *z*-score values for shade-tolerant species; see the ESM). Using residuals from the shade-tolerance ANOVA to test for effects for site and phylogeny did not change the results for either comparison.

The low latitude/high defense hypothesis predicts that nutritional values of leaves for herbivores would be lower in tropical latitudes, while secondary compounds and structural traits, including leaf toughness, thickness, and fiber, would be higher. Four of the five structural traits differed significantly between Panama and Missouri in the predicted direction (Table 4). Likewise, three of the five structural traits differed significantly between Missouri and Canada in the predicted direction, but leaf toughness contradicted the prediction of the low latitude/high defense hypothesis (Table 5). Overall, the fiber contents of leaves increased consistently towards lower latitudes, with toughness also

Table 3 Matrix of pairwise squared Mahalanobis distances between site/shade-tolerance combinations (upper right) and *P* values (numerator degrees of freedom = 8, denominator degrees of freedom = 95) for the respective comparisons (lower left)

	Canada shade tolerant	Canada shade intolerant	Missouri shade tolerant	Missouri shade intolerant	Panama shade tolerant	Panama shade intolerant
Canada shade tolerant	–	1.1915	4.5589	4.3885	13.3589	16.9757
Canada shade intolerant	0.3218	–	3.7795	2.2452	14.0814	16.1344
Missouri shade tolerant	<0.0001	0.0002	–	1.4183	4.8551	6.6344
Missouri shade intolerant	0.0008	0.0660	0.2116	–	9.5417	9.4151
Panama shade tolerant	<0.0001	<0.0001	<0.0001	<0.0001	–	3.1175
Panama shade intolerant	<0.0001	<0.0001	<0.0001	<0.0001	0.0051	–

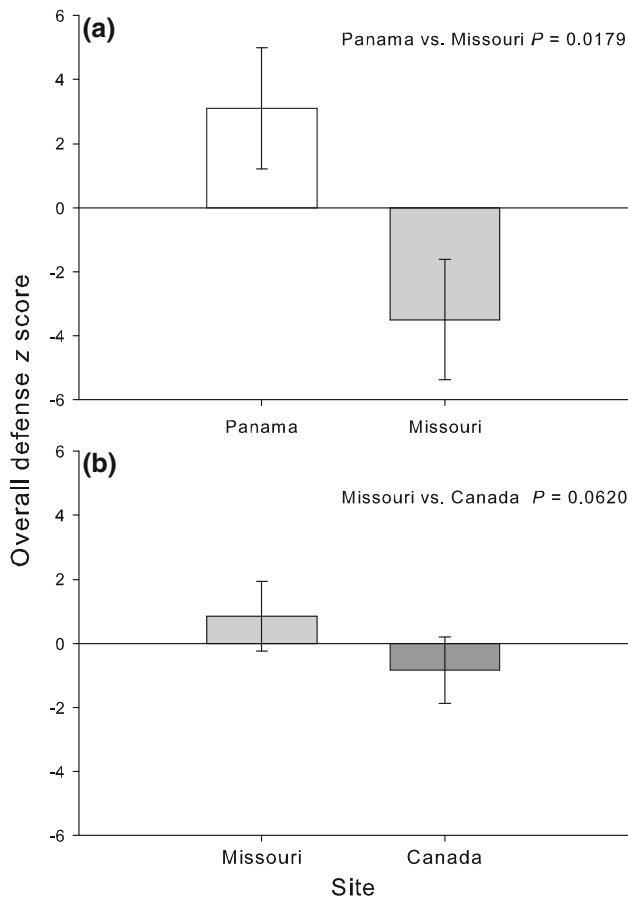


Fig. 2 Comparison of mean (\pm SE) *z*-scores for overall defense for the high versus low latitude sites for **a** Panama versus Missouri ($P = 0.0179$) and **b** Missouri versus Canada ($P = 0.0620$), after first controlling for the effect of family and species, respectively

contributing to differences in leaf traits between the temperate and tropical samples.

Except for sodium, 4 out of 14 other nutritional components of leaves had significantly higher percent dry mass values in Missouri compared to Panama in the direction predicted by the hypothesis (Table 4). In contrast, differences in nutritional components between Missouri and Canada were smaller and mixed with respect to the predictions of the low latitude/high defense hypothesis (Table 5).

None of the secondary-compound traits varied significantly between Panama and Missouri, after controlling for a family effect (Table 4).

In comparisons between Missouri and Panama, the taxon (family) effect was relatively weak, with $P < 0.01$ only for the concentration of magnesium, NDF, and ADF (Table 4). In comparisons between Missouri and southern Ontario, taxon (species) exhibited significant effects ($P < 0.01$) for 8 of the 11 traits measured (Table 5). Overall, taxon effects tended to be weaker than site effects in both comparisons, but species was more frequently significant ($P < 0.05$) in the Missouri versus Canada comparisons than family was for the Panama versus Missouri comparisons ($\chi^2 = 11.52$, $P = 0.0007$).

NAPRALERTsm database

Neither family, order ($P = 0.058$), latitude, or the interaction of latitude with the covariate (number of studies) was a significant predictor of the number of compounds found in the NAPRALERTsm database (latitude, $P = 0.78$; interaction, $P = 0.74$) or the number of unique classes of compounds reported in that database (latitude, $P = 0.94$; interaction, $P = 0.79$). Number of studies (X) was a significant predictor ($P < 0.0001$) of the number of compounds ($Y = 1.28 + 2.68X$) (Fig. 3a) and the number of unique classes of compounds ($Y = 1.46 + 0.334X$) (Fig. 3b). Finally, the rarefied richness of secondary compounds ($\pm 95\%$ confidence intervals) at 16 species was significantly higher for the sampled temperate species (238.8 ± 3.5) than for the tropical species (168.5 ± 11.0) (Fig. 4).

Discussion

Overall, our analysis provides support for the low latitude/high defense hypothesis, especially for the importance of structural components of defense. Differences in overall defense, as estimated by *z*-scores, were higher at low latitude than higher latitude for both comparisons. In addition, differences were stronger between Panama and Missouri

Table 4 Trait means (\pm SE) and *P* values for ANOVA for the effects of site and family on leaf traits of Panama versus Missouri sampled species

Trait	Panama	<i>N</i>	Missouri	<i>N</i>	Family	Site	Hyp
Nutritional traits							
N	2.6 \pm 0.1	20	3.0 \pm 0.2	13	0.3499	0.1398	No
Mn	109.6 \pm 24.8	20	150.2 \pm 59.8	13	0.0905	0.4769	No
Ca	1.1 \pm 1.0	20	1.7 \pm 0.1	13	0.4788	0.0005	Yes
P	0.16 \pm 0.01	20	0.25 \pm 0.02	13	0.6486	0.0055	Yes
K	0.72 \pm 0.06	20	0.59 \pm 0.02	13	0.2073	0.0781	No
Na	0.08 \pm 0.02	20	0.0025 \pm 0.0004	13	0.2228	0.0027	No
Fe	75.7 \pm 5.1	20	97.8 \pm 8.1	13	0.6663	0.0206	Yes
Mg	0.44 \pm 0.03	20	0.34 \pm 0.03	13	0.0042	0.0282	No
Zn	13.9 \pm 3.6	20	15.1 \pm 5.8	13	0.3879	0.5212	No
Cu	11.6 \pm 0.4	20	10.5 \pm 0.6	13	0.2388	0.1586	No
B	28.7 \pm 3.1	20	66.5 \pm 15.7	13	0.4513	0.0037	Yes
Mo	0.19 \pm 0.08	20	0.14 \pm .05	13	0.7841	0.7795	No
H ₂ O	70.2 \pm 1.8	20	66.9 \pm 1.2	14	0.6173	0.1773	No
Structural traits							
Specific leaf area	3.8 \pm 0.3	20	3.9 \pm 0.4	14	0.4341	0.7869	No
Lignin	18.2 \pm 1.5	20	13.6 \pm 1.4	14	0.2646	0.0459	Yes
NDF	42.4 \pm 2.7	20	26.0 \pm 1.8	14	0.0026	<0.0001	Yes
ADF	29.9 \pm 2.3	20	20.3 \pm 1.3	13	0.0195	0.0004	Yes
Toughness	277.1 \pm 26.7	20	134.6 \pm 17.9	13	0.7338	0.0011	Yes
Secondary compounds							
Alkaloids	0.0011 \pm 0.0003	20	0.0011 \pm 0.0001	14	0.5869	0.7171	No
Saponins	0.68 \pm 0.19	20	0.68 \pm 0.31	14	0.0824	0.8354	No
Condensed tannins	0.029 \pm 0.005	20	0.032 \pm 0.005	14	0.1404	0.8735	No
Hydrolysable tannins	0.36 \pm 0.08	20	0.54 \pm 0.19	14	0.7124	0.3997	No

Hyp = support for the low latitude/high defense hypothesis (see text). Units are as in Table 1
N number of species sampled per site

Table 5 Trait means (\pm SE) and *P* values for analysis of variance, based on type III sums of squares, for the effects of site and species on various leaf traits of Missouri versus Canada sampled populations

Trait	Missouri	<i>N</i>	Canada	<i>N</i>	Species	Site	Hyp
Nutritional traits							
N	2.26 \pm 0.12	14	2.01 \pm 0.08	14	0.0261	0.0242	No
Ca	1.49 \pm 0.11	14	0.67 \pm 0.08	14	0.0144	<0.0001	No
P	0.15 \pm 0.01	14	0.20 \pm 0.02	14	0.0007	0.0002	Yes
K	0.53 \pm 0.03	14	0.73 \pm 0.06	14	0.0911	0.0035	Yes
Mg	0.32 \pm 0.02	14	0.24 \pm 0.02	14	0.0429	0.0021	No
H ₂ O	58.8 \pm 1.5	14	57.1 \pm 1.6	14	0.0004	0.1225	No
Structural traits							
Specific leaf area	4.7 \pm 0.4	14	6.6 \pm 0.4	14	0.2555	0.6645	No
Lignin	15.0 \pm 0.9	14	9.7 \pm 0.8	14	0.0310	<0.0001	Yes
NDF	36.1 \pm 1.9	14	26.8 \pm 1.3	14	0.0390	<0.0001	Yes
ADF	25.0 \pm 1.0	14	15.0 \pm 2.0	15	0.0816	<0.0001	Yes
Toughness	163.2 \pm 18.2	15	204.1 \pm 9.4	15	0.0142	0.0098	No

Hyp = support for the low latitude/high defense hypothesis (see text). Units are as in Table 1
N number of species compared per site

than between Missouri and Canada, consistent with an effect of the temperate winter on the evolution of defense. Finally, in further support of the hypothesis, differences between shade-tolerant and shade-intolerant species were significant only at the Panama site. However, in contradiction to the low latitude/high defense hypothesis, concentrations,

number, and diversity of secondary compounds did not differ significantly between the tropical and temperate sites. Analysis of the NAPRALERT database, which includes only secondary compounds, showed no support for the low latitude/high defense hypothesis. We expected an effect of latitude on the compound types we assessed because they

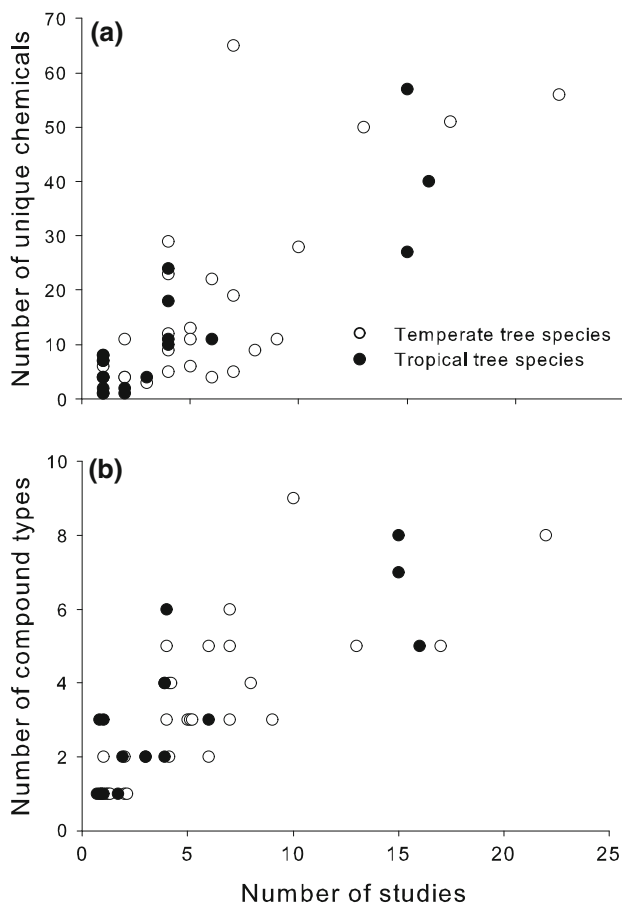


Fig. 3 Effects of species location (tropical vs. temperate) and sample size on the number of **a** unique secondary compounds and **b** classes of secondary compounds as reported in the NAPRALERT database (<http://www.napralert.org/>) for the species in this study. Overlapping values in (b) are jittered by 0.1 on the x-axis to reveal their presence

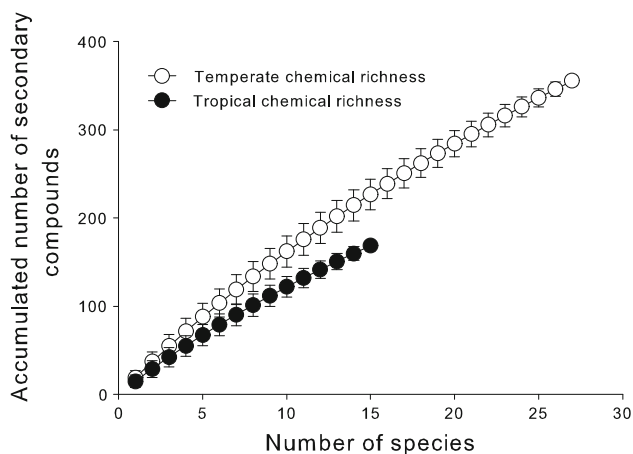


Fig. 4 Effect of location (tropical vs. temperate) on the rarified chemical richness ($\pm 95\%$ confidence intervals) for secondary compounds reported in the NAPRALERT database (<http://www.napralert.org/>) for the species in this study

have been shown previously to correlate with interspecific differences in herbivore damage: tannins (Coley 1983; Cooke et al. 1984), alkaloids (Dolinger et al. 1973), and saponins (Agrell et al. 2006).

Some leaf traits varied significantly among taxa, particularly among orders and particularly for nutrients (Table 2). In addition, the species effect explained a significant amount of variation in z -scores for the Missouri–Canada comparison. Both of these findings demonstrate the need to take into account phylogeny when testing the low latitude/high defense hypothesis. The strong taxon effects on variations in water and magnesium might reflect phylogenetically conserved leaf traits that are to some extent independent of defenses against herbivores.

After controlling for taxonomic relationship, several traits exhibited significant differences between southern Ontario and Missouri (Table 5), and especially between Missouri and Panama (Table 4). The most significant differences were in the direction predicted by the low latitude/high defense hypothesis, namely higher concentrations of structural elements (particularly fiber, lignin, and leaf toughness) and lower concentrations of nutritional elements and compounds (particularly calcium, phosphorus, iron, and boron) in tropical compared to temperate species. Contrary to our expectations, for the Panama/Missouri data set, none of the classes of secondary compounds differed significantly in concentration between the two locations after controlling for family level taxon (Table 4). Our analysis of data gathered from the NAPRALERTSM database also failed to support the prediction of a greater variety of secondary compounds in tropical compared to temperate regions: the number of reported compounds or compound classes per species was related only to the number of studies and did not differ between tropical and temperate tree species (Fig. 3). Further, when corrected for sample size, the number of compounds identified for tropical species was less than that for temperate species (Fig. 4).

A canonical discriminant analysis of the three sample areas based on five nutritional and three structural traits measured at each site, and ignoring taxonomic relationship, revealed significant differences between the sites, with negligible overlap between the temperate and tropical sites (Fig. 1). In this analysis, the tropical species were distinguished uniquely from temperate species primarily by having greater structural components and higher concentrations of potassium and lower concentrations of phosphorus (see also Table 4). The differences in fiber and lignin are consistent with greater structural toughness, potentially as a deterrent against herbivory, but the higher concentration of potassium in tropical leaves is in contrast to most of the nutritional variables, which have lower concentrations in tropical species (Table 4).

Species also separated in the discriminant analysis based on shade tolerance, but to a lesser extent than by latitude

(Fig. 2). Defense theory predicts that species adapted to low light should invest in greater defense because of the greater cost of leaf tissue for shade-adapted plants on the one hand, and the lower tolerance to loss of leaf tissue in those same species on the other (Coley et al. 1985). We found that shade-tolerant species differed significantly from shade-intolerant species only in Panama. This result is consistent with the low latitude/high defense hypothesis, i.e., selection by herbivores across the light gradient is greatest closer to the equator. Future studies should take into account leaf lifespan and resource availability (as influenced by light and soil nutrients). Plants growing essentially next to each other but on different soil types can have very different defense investment profiles (Fine et al. 2006).

At the Panama site, herbivory is inversely related to the concentrations of structural compounds in leaves, and positively related to measures of nutritive value (e.g., Coley and Aide 1991; Coley and Barone 1996). Similar relationships have not been demonstrated among species of temperate forest trees (e.g., Karban and Ricklefs 1984), and differences between tropical and temperate species have not been studied in this regard. One confounding factor, among others (Pennings et al. 2009), is a difference in leaf life span of plants sampled in Panama versus those from Missouri and Canada. Coley and Aide (1991) found that leaf life span was most strongly associated with leaf quality differences between temperate forest leaves, tropical dry forest leaves, and leaves of gap and shade-adapted species of tropical wet deciduous forest. Leaves of species of the last category live, on average, 3–5 times as long as those of plants in the other categories. Leaf life span cannot be the entire story, however. For example, leaves of Brazilian cerrado plants are significantly tougher, have higher tannin concentrations, and have lower water and nitrogen levels than leaves of Barro Colorado Island trees (Marquis et al. 2002). However, leaves of most studied species from the Brazilian site live for approximately one year (Marquis et al. 2001), while those of shade-adapted species on BCI live on average for 32 months (Coley and Aide 1991). Traits of woody plant species possibly differ as much among different tropical habitat types as they do between temperate and tropical locations. Furthermore, parasitism and predation might ameliorate the influence of herbivore pressure, and should be studied. Ant predation on herbivores might be greater in tropical than temperate forests (Novotny et al. 2006), but parasitism of Lepidoptera larvae varies in relation to variability in local climate and not with latitude (Stireman et al. 2005).

One limitation of this study is that only three locations were sampled, and the Missouri and southern Ontario sites are temperate and harbor many of the same species. Thus, our principal latitudinal comparison is between the temperate Missouri location and the tropical Panama location. To

our knowledge, however, this is the first study to compare leaf traits of broad-leaved trees that are likely to influence herbivore attack along a latitudinal gradient, while controlling for the possible confounding effects of phylogeny, which we show are clearly important. Future investigations of the low latitude/high defense hypothesis should involve more sites along the latitudinal gradient and also record the rate of leaf area removal to document herbivore pressure. There is a clear need for more detailed studies of genera of plants that span wide latitudinal ranges and the development of methods that allow comparisons of secondary compounds across unrelated species. Experimental studies of leaf palatability to standardized herbivores would also be informative.

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