

Jonathan M. Adams · Brian Rehill · Yangjian Zhang
Jonathan Gower

A test of the latitudinal defense hypothesis: herbivory, tannins and total phenolics in four North American tree species

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Abstract It is widely believed that insect herbivory is less intense at higher latitudes, due to winter mortality which would tend to keep insect herbivores from reaching density-limitation of their populations. One prediction of this theory is that plants should tend to be better defended at lower latitudes. Here we investigated latitudinal trends in herbivory and tannins, in four species of common North American trees. Our comparisons spanned 15° of latitude in *Acer rubrum*, *Fagus grandifolia*, and *Quercus alba*, and 10° latitude in *Liquidambar styraciflua*. Sun leaves on forest edges were sampled, at phenologically equivalent times of year. Analysis revealed significant differences between populations, including those at similar latitudes, but no significant latitudinal trend in herbivory, condensed and hydrolyzable tannins, or total phenolics measured as Folin–Denis reactives in any of the four species. Our findings contradict the theory that low latitude plants are better defended, in that lower latitude populations of the four tree species showed no greater amounts of phenolics. The possible implications for community ecology are discussed.

Keywords Tannins · Phenolics · Herbivory · Trees · Latitude · North America

Introduction

Biogeographical theory predicts increased herbivory (i.e., greater loss of leaf area to herbivores) in the tropics versus the temperate zone, and in warmer versus colder parts of the temperate zone (MacArthur 1972; Vermeij 1978; Jablonski 1993). It is suggested that greater population densities of herbivorous insects and, therefore, more herbivory occur because in tropical climates dormant season death (i.e., winter dieback) of herbivores is absent or greatly reduced and/or plant productivity is generally greater than at higher latitudes (Dobzhansky 1950; Coley and Aide 1991; Coley and Barone 1996). With frequent declines in populations, the links in the web of interactions between species are said to be weaker at higher latitudes (Dobzhansky 1950; MacArthur 1969). Due to greater herbivory, plants are thought to be more highly defended or otherwise less palatable at low latitudes as a result of natural selection (e.g., MacArthur 1972; Hay and Fenical 1988; Coley and Aide 1991; Bolser and Hay 1996; Coley and Barone 1996). Also, plant anti-herbivore defenses at low latitudes could be better developed because plants there have had more generations to evolve such defenses, compared to plants at high latitudes where glaciations frequently greatly reduced species diversity (Fischer 1960). This view now seems so widely held that it is taken as self evident rather than frequently referred to, although it has appeared at the core of work on latitudinal herbivory gradients (Lowman 1984; Coley and Barone 1996; Siska et al. 2002; Andrew and Hughes 2005).

Another prediction of the theory—which we investigate here—is that plants should be better defended in climates that do not have as strong a winter cold season or a dry season. This prediction is difficult to test on an interspecific level because plant defenses differ so widely between species. Coley and Barone (1996) have suggested that tannin concentrations are greater in tropical tree leaves than in temperate tree leaves, presumably making tropical leaves better defended against herbivores.

Jonathan M. Adams, Brian Rehill, and Yangjian Zhang are equal joint lead authorship on this paper.

J. M. Adams (✉) · Y. Zhang
Department of Biological Sciences,
Rutgers University, Newark, NJ 07102, USA
E-mail: jonadams@andromeda.rutgers.edu

B. Rehill · J. Gower
Department of Chemistry,
United States Naval Academy,
Annapolis, MD 21402, USA
E-mail: rehill@usna.edu

Because of the huge interspecific variability in the quantity and efficacy of plant defenses, possibly a more promising approach is to compare populations of the *same species* from different latitudes, since natural selection should lead to greater investment in defenses in environments where herbivory is more severe.

This latter prediction has already been tested in a small number of studies. Work on three species of saltmarsh plants, comparing populations in the south-eastern and north-eastern USA across about 10° of latitude, found that leaves collected from the wild in the south-eastern USA were tougher and lower in nitrogen, and generally less palatable to generalist caterpillars (Siska et al. 2002). A common garden study confirmed that these differences had a genetic basis (Salgado and Pennings 2005). However, a study of an Australian shrub across 15° of latitude found no evidence of any trend in either herbivory or leaf toughness (Andrew and Hughes 2005). Furthermore, in some species of birch (*Betula*) and aspen (*Populus*), twigs from more northern latitudes are less palatable to their major browser, snowshoe hares, than those from southern latitudes (Swihart et al. 1994). This is the inverse of what would be expected from theory. Thus intraspecific tests of the latitudinal defense hypothesis show no consistent trend.

We chose to test the predictions of the latitudinal herbivory theory in several species of North American trees, sampled at several points along the latitudinal temperature gradient in the eastern United States (Fig. 1). In eastern North America, forest is the dominant ecosystem, and most plant biomass is in the form of trees. Although plant defenses vary widely, most tree foliage contains varying amounts of tannins and other phenolics. These compounds have well estab-

lished effects on herbivores and associations with herbivory and herbivore communities (e.g., Feeny 1970; Schultz and Baldwin 1982; Rossiter et al. 1988; Shure and Wilson 1993; Dudt and Shure 1994; Lill and Marquis 2001; Abrahamson et al. 2003; Forkner et al. 2004) although other functions have also been proposed (Zucker 1982; Ayres et al. 1997; Close and McArthur 2002). While phenolic defenses, especially hydrolyzable and condensed tannins, are not the only chemical defenses of the tree species used in this study, they are well studied, and present in great quantities, often >10% of leaf dry weight. Tannins likely reduce herbivory and herbivore fitness by a variety of mechanisms (Zucker 1982; Deveau and Schultz 1992; Barbehenn et al. 2003), although tannin interactions with pathogens and insecticides can increase herbivore survival (Hunter and Schultz 1993; Appel and Schultz 1994). Because trees are long lived, they would seem more likely to adapt to long-term average levels of insect attack spanning centuries, rather than herbaceous plants which may respond more to local and short-term variations in herbivory. Thus trees seem more likely to show adaptation to truly broad scale patterns in community selective forces. In this study, we assessed herbivory and measured foliar tannin concentrations of four North American trees along a latitudinal transect.

Methods

Sampling times

Timing of sampling during the growing season

Sampling at about the same time in all localities (north and south) would not only be logistically difficult but would also not take into account the major differences in phenology from north to south. Such phenological differences could account for any differences in phenolic content, because foliar phenolic concentrations often change as leaves age (Hunter and Lechowicz 1992; Rehill et al. 2006). The growing season in Florida begins months earlier than in northern New England, and any tree (e.g., *Fagus grandifolia*, *Acer rubrum*, *Quercus alba*) harvested in northern New England will have had less time for the leaves to mature, and have been exposed to several weeks less potential herbivory than one in Florida. Thus sampling took place in early summer (about 12 weeks after the mean bud break time as averaged across a range of deciduous species; Borchert et al. 2005) at phenologically analogous stages at different latitudes. Equivalent sampling times were estimated using two methods:

Method 1 Growing degree days (GDD) are calculated using the formula: $\{(T_{\max} + T_{\min})/2\} - B = \text{Growing Degree Days (GDD)}$ where **B** represents a base tem-

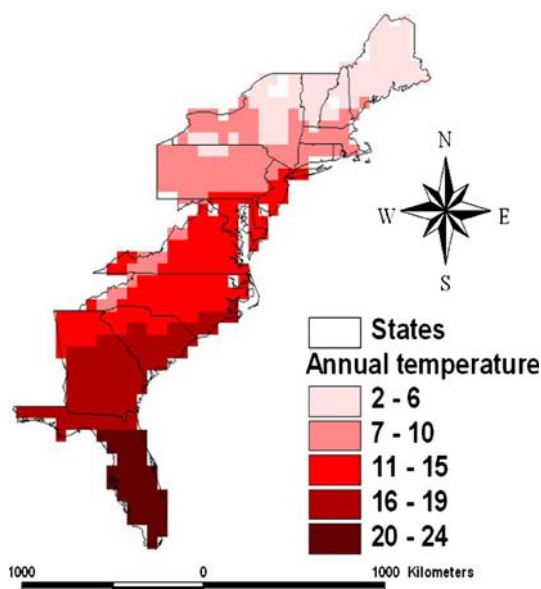


Fig. 1 Mean annual temperature through the eastern USA

Table 1 Starting from a “standard” date of 10th July in New Jersey at 41°N, the following sampling times are estimated to be equivalent in terms of biological activity and leaf development, using two different methods

Latitudinal band	Latitude	Date from growing degree days (GDD)	Date from Borchert et al. (2005) formula
Northern Florida, Southern Georgia	30–32°N	25th May	28th May
Northern Georgia, South Carolina	33–35°N	10th June	15th June
New Jersey–Southern New York State–Southern New England	40–42°N	10th July	10th July
Northern Vermont, Northern NY	43–45°N	28th July	25th July

perature value of 10°C. For the base 10/30 method, the following adjustments are made:

- 1 temperatures below 10°C are set at 10°C; and
- 2 temperatures above 30°C are set at 30°C.

The reasons for these “cut-offs” in GDD calculations are that very little growth and biological activity take place below 10°C, whereas above 30°C there is no evident benefit to plant growth and other biological activity. However, these cutoff limits do vary among GDD indices. Climate station data were obtained from: <http://www.engr.udayton.edu/weather/>

Method 2 Phenological delay is calculated using the predictive formula of Borchert et al. (2005) which has a predictive R^2 value of 0.97 for bud break time through North America and montane Mexico (Week of bud break = $10.3 \times (0.77 \times \text{mean temperature of coldest month})$). This empirical formula was based on observations all the way from montane eastern Mexico to Canada. While the formula characterizes bud break, we use the relationship between seasonal delay and temperature (using climate data from <http://www.engr.udayton.edu/weather/>) to determine equivalent delay in early summer sampling times 12 weeks after bud break, working northward from Florida (Table 1).

Averaging the two sources of phenological estimation the dates for collecting were:

1. Northern Florida, southernmost Georgia. 24th–26th May.
2. Northern Georgia–South Carolina. 10th–12th June.
3. New Jersey–southern New York State. 9th–13th July.
4. Northern Vermont and New York State. 20th July–25th July.

Phenolic analyses

The four species studied here were sampled from within four latitudinal bands in the eastern USA—northern Florida and southern Georgia (30–31°N), northern Georgia and South Carolina (33–34°N), New Jersey and

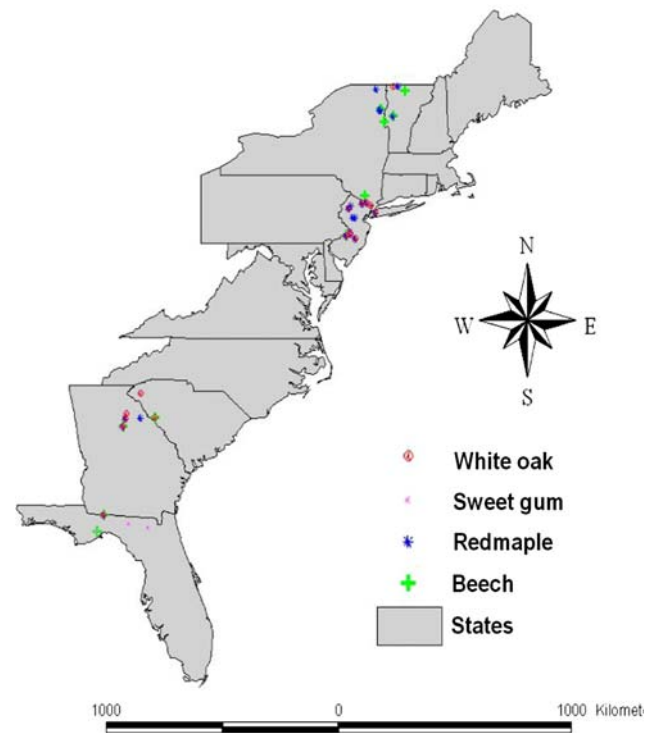


Fig. 2 Sampling sites of red maple (*Acer rubrum*), beech (*Fagus grandifolia*), sweetgum (*Liquidambar styraciflua*), and white oak (*Quercus alba*) in the eastern USA

southern New York (40–42°N), and northern New York and Vermont (43–45°N) (Fig. 2). At least two populations of each species were sampled within each band.

Within each latitudinal band, state parks and forest areas known to contain populations of one or more of the species were selected on a semi random basis across a wide spatial scatter, based on their proximity within 30 km of main interstate routes allowing ease of access. Within each park, a random quadrat was selected for sampling. Populations of any or all of the four selected species found within this quadrat were sampled. If a particular species could not be sampled we moved to other quadrats until all of the park had been covered.

In sampling along latitudinal gradients, more important than the detailed position of the samples within the tree is that the sampling is carried out consistently. If all other factors apart from latitude are held constant, it is reasonable to suppose that any latitudinal

trend will show itself. In this study we chose to sample only sun leaves overhanging forest edges, because these are easily accessible and the sampling environment is also more readily standardized.

Sampling was carried out only along the edges of small roads or tracks 5–15 m in width, forested along both sides of the road as part of a contiguous area of at least 10 ha of forest on either side of the road. Along this forest edge we sampled up to ten small branches of about 30 leaves consisting only of “sun leaves”, observed to be in direct sunlight on the forest edge during the middle part of the day (10 a.m.–4.30 p.m.). We randomly selected one such branch per tree at around 4–5 m height. At this height, damage level cannot easily be seen from the ground, ensuring that branches were not chosen by the amount of damage. The branch was removed using a pole pruner, and placed in a plastic bag. Within a quadrat we sampled the first individual encountered if in an accessible location for sampling. The next tree of the same species at least 10 m away was then sampled, and so-on along the forest edge at least every 10 m, until up to ten trees in all had been sampled. All samples (one branch per tree) were placed together in a large plastic bag, labeled by site, and taken for separate sampling by branch for leaf disks and phenolics.

Six leaves per branch, chosen arbitrarily within the branch, were sampled for phenolics within 24 h of being harvested. Two small disks of leaf material, on either side of the midrib, were taken using an office hole puncher. One disk of each pair was placed into a 2.0 mL microcentrifuge tube containing 70% acetone with 1 mM ascorbate, for subsequent phenolic analysis, while the other was placed dry in a 2.0-mL microcentrifuge tube. This procedure was repeated with all six selected leaves so that eventually six punched disks were present in the acetone solution, and six in the dry tube. The samples were kept under dry ice until they could be stored at -80°C , up to the time of analysis. To estimate the dry weight of the disks used for phenolic analysis, the disks in the dry tube were heated in an oven at 70°C until constant mass. This permitted estimation of phenolic concentrations per dry weight. Since leaf disks were of the same area, leaf disk mass was also used as a surrogate for leaf specific mass (mass per unit area), as a potential surrogate for leaf toughness.

Thirty remaining leaves from each species at each site were placed in a labeled paper bag, dipped in liquid N for flash freezing, and transferred immediately to a -80°C freezer. These leaves would later be used to prepare the standards for tannin analyses.

The sampling and extraction procedures were based on those discussed in Abrahamson et al. 2003.

Before extraction, leaf disks were cut with dissecting scissors then homogenized for 30 s using a Tissue Master 125 homogenizer (Omni International, Marietta, Georgia, USA). Samples were extracted exhaustively by sonication for 30 min, centrifugation at 3,600g RCF and 4°C for 10 min, decanting the supernatant to a separate microcentrifuge tube, and addition of 0.5 mL of 70%

acetone with 1 mM ascorbate for a total of four cycles. After extraction, the acetone was removed by rotary evaporation and samples were made up to a constant volume of 0.5 mL.

Samples were analyzed for condensed tannins with the acid-butanol technique (Rossiter et al. 1988; Rehill et al. 2006), for hydrolyzable tannins using the potassium iodate technique (Bate-Smith 1977; Schultz and Baldwin 1982) and for total phenolics with the Folin–Denis technique (Folin and Denis 1915; Waterman and Mole 1994); all modified for leaf discs as discussed in (Abrahamson et al. 2003). The Folin–Denis assay measures the total concentration of phenolic hydroxyl groups, and, therefore, includes both condensed and hydrolyzable tannins and smaller non-tannin phenolics. For each tree species, a standard was prepared using equal amounts of lyophilized, ground leaf powder from all collection sites, except those located in Florida, by a modification of the method of Hagerman and Butler 1980 as discussed in (Rehill et al. 2006). Since all four tree species contain both hydrolyzable and condensed tannins, and this purification method does not separate the two classes of compound, the results of assays are quantified as percentage tannin equivalents, indicating reactivity relative to the purified standard.

Herbivory assessment

Two hypotheses that are relevant to latitudinal variation in tannin levels were tested by assessing average herbivory at each site:

- 1 herbivory should be greater at lower latitude sites; and
- 2 variation in tannin levels might be related to (controlled by or responsible for) variation in herbivory.

We assumed that only insects, not mammals, were the cause of the observed leaf area loss for two major reasons. First, the only arboreal folivorous mammal in eastern North America, the porcupine (*Erethizon dorsatum*), typically only climbs and consumes trees away from the forest edge, where we sampled, and occurs only in ca. 1/3 of the sites in our study. Second and most importantly, the leaf samples were taken 4–5 m from the ground, far above the height that deer or other ground-dwelling herbivores in eastern North America can reach.

Seventy-five leaves from amongst the branches gathered at each site were picked randomly, and scanned on a flatbed scanner. Percentage area loss in each of four herbivory damage categories was assessed using Sigma-scan.

The categories were as follows:

1. Chewing. Areas missing from the leaf whether at the edge or as a hole in the interior of the leaf.
2. Skeletonizing. Network of veins remaining with the intervening tissue eaten out.

3. Leaf mining. Papery area, often as a curving line, where the leaf tissue has been eaten out leaving the epidermes intact.
4. Galls. Lumps of many different forms, resulting from insects or mites laying eggs which resulted in the leaf tissue growing to envelop the herbivore.

These categories were checked against the illustrations given in Cranshaw (2004), an authoritative source on insect damage types.

It is possible that some leaves were completely eaten by insect herbivores while still on the trees, and thus may have gone unnoticed in the sampling here. Visual inspection of harvested branches indicated that no leaves were completely eaten down to the petiole, suggesting that complete consumption is relatively uncommon. The pattern of leaf damage in samples taken in a related study on freshly fallen autumn leaves suggests that there is a lognormal distribution of damage—with high percentage area loss being rare (Fig. 3; Zhang and Adams, unpublished data). It is reasonable to expect that given that most leaves are only lightly damaged, an increase in overall damage levels would result in an increase in more heavily damaged leaves and a shift in the lognormal distribution, and thus a higher average herbivory, even if completely eaten leaves go uncounted. Thus we should expect that “unseen” complete consumption of leaves can be detected by proxy, though an overall increase in average herbivory on incompletely consumed leaves.

Statistical analyses

All statistical analyses were performed with SPSS 12.0 for Windows (SPSS, Chicago, IL, USA). Sample sites were considered the experimental unit, thus for comparisons of chemistry measures versus latitude, the mean for each site is used. On all graphs of chemistry mea-

ures, the mean \pm one standard error of the mean is given for each site.

Results

Phenolics

Levels of hydrolysable tannins, Folin–Denis reactives, and condensed tannins varied widely (see Figs. 4–6) and differed significantly among sites for all four tree species except for Folin–Denis reactives for beech, which showed only a marginally significant difference (Table 2). Despite the clear differences in phenolics among sites, no strong latitudinal trends emerged for condensed tannins, hydrolyzable tannins, or total phenolics.

Herbivory

No significant latitudinal trend was observed in leaf area loss due to herbivory versus latitude (Fig. 7). Phenolic

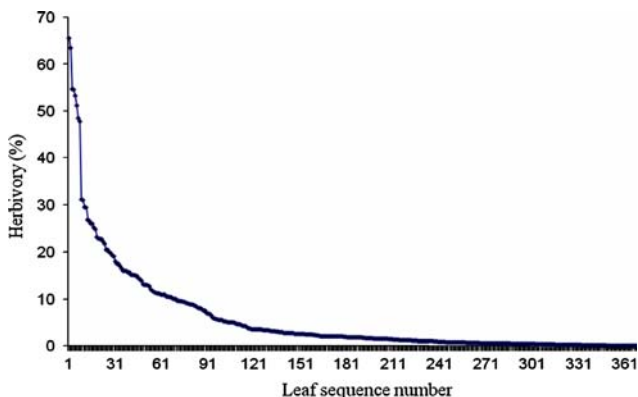


Fig. 3 Rank order of leaf damage per leaf (% area lost, with most heavily damaged leaves placed first) in leaves in temperate sample of leaf litter. This curve represents the combined samples of 75 leaves from freshly fallen leaf litter from five randomly selected temperate North American sites. (Adams and Zhang, unpublished data)

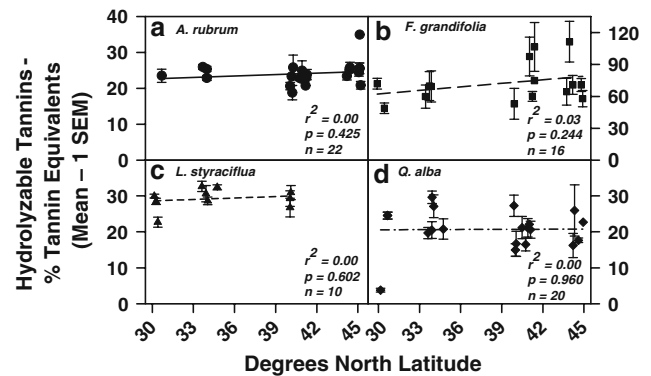


Fig. 4 Hydrolysable tannin concentrations versus site latitude for: **a** *Acer rubrum*, **b** *Fagus grandifolia*, **c** *Liquidambar styraciflua*, and **d** *Quercus alba*. Note that scales differ among species

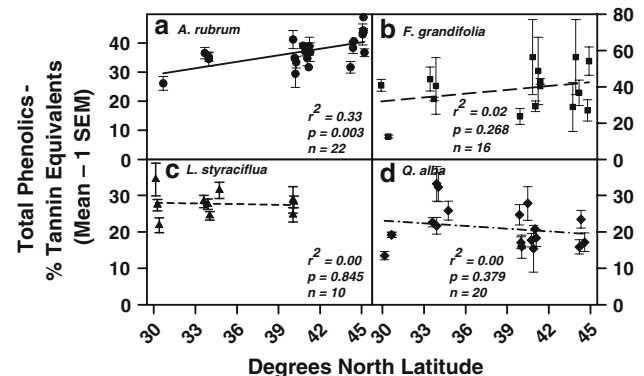


Fig. 5 Total phenolics versus latitude for: **a** *Acer rubrum*, **b** *Fagus grandifolia*, **c** *Liquidambar styraciflua*, and **d** *Quercus alba*. Note that scales differ among species

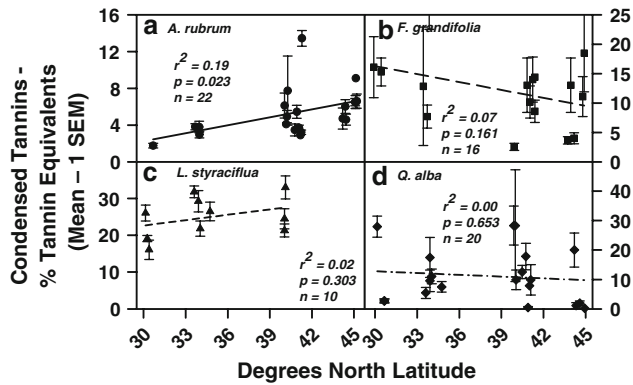


Fig. 6 Condensed tannins versus latitude for: **a** *Acer rubrum*, **b** *Fagus grandifolia*, **c** *Liquidambar styraciflua*, and **d** *Quercus alba*. Note that scales differ among species

Table 2 Summary of general linear model analysis of hydrolyzable tannin, total phenolics, and condensed tannin concentrations among sites

Phenolic metabolite	Tree species			
	Red maple	Sweetgum	White oak	Beech
Hydrolyzable tannins				
<i>df</i>	28, 79	11, 44	19, 60	16, 60
<i>F</i>	1.99	4.89	4.41	11.3
<i>P</i>	0.009	<0.001	<0.001	<0.001
Total phenolics				
<i>df</i>	28, 79	11, 44	18, 61	16, 61
<i>F</i>	2.68	2.21	2.93	1.72
<i>P</i>	<0.001	0.031	<0.001	0.068
Condensed tannins				
<i>df</i>	28, 79	11, 44	19, 60	16, 61
<i>F</i>	3.38	5.24	3.41	2.63
<i>P</i>	<0.001	<0.001	<0.001	0.003

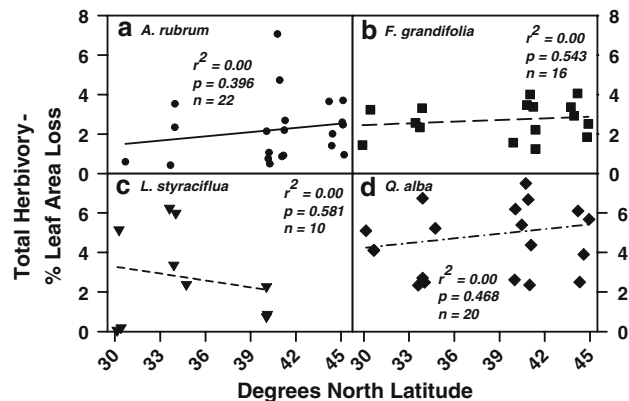


Fig. 7 Total herbivory (measured as percentage of leaf area lost to herbivory) versus latitude for: **a** *Acer rubrum*, **b** *Fagus grandifolia*, **c** *Liquidambar styraciflua*, and **d** *Quercus alba*

concentrations in the leaves were not associated with total herbivory for all four tree species (data not shown). However, several minor trends appeared for several types of herbivory. For red maple, leaf skeletonizing was

negatively associated with total phenolics ($r = -0.58$, $P = 0.010$) and galling was positively related to condensed tannin concentration ($r = 0.71$, $P = 0.001$). Also, galling was negatively associated with latitude in beech ($r = -0.62$, $P = 0.054$).

Discussion and conclusions

Phenolics

The results for all four tree species showed only a few latitudinal trends in hydrolyzable tannins, condensed tannins, or total phenolics. For red maple, both total phenolics and condensed tannins increased with latitude; for all other species and measures, no trends emerged. We hasten to add that few latitudinal trends emerged despite the significant differences in mean phenolic concentrations among sites detected in this study (Table 2), indicating that our methods were sensitive enough to detect differences among sites, and therefore also to detect any trends. These observations are at odds with the predictions of the hypothesis (so widely held, perhaps, as to be termed a “theory”) that insect attack on plants is heavier towards the lower latitudes, requiring more investment in defenses.

While these phenolic compounds may instead serve as a sunscreen against ultraviolet light (Close and McArthur 2002; Sullivan 2005), this theory would also predict an increase in mean concentrations in sun leaves towards lower latitudes. UV light flux increases by approximately 1–2% per 1° latitude towards the Equator (Close and McArthur 2002), so there should be an 15–30% difference in UV flux between 30 and 45°N, over which three of the species were sampled, and 10–20% increase over 10° in the case of sweetgum. However, none of the compounds differ over this latitudinal range in the way predicted by the UV protectant hypothesis.

Herbivory

This study also found no indications of any widespread latitudinal trends in herbivory—leaf chewing, skeletonizing, mining, and galling showed only a few minor trends, which is also at odds with the predictions of the theory that biotic control is greater at low latitudes. Phenolics and total herbivory levels were also not significantly associated, suggesting that phenolic concentrations at the sites do not greatly affect herbivory, nor does herbivory detectably affect phenolic concentrations.

Foliar phenolics may affect insects by a number of mechanisms including oxidative damage (Johnson 2005; Barbehenn et al. 2006); alteration of immunocompetence (Haviola et al. 2007), and recruitment of natural enemies (Muller et al. 2006). Although some recent studies have demonstrated significant relationships between foliar phenolics and various herbivore communi-

ties (e.g., leaf chewers in *Quercus alba* and *Quercus velutina*, Forkner et al. 2004, leaf miners in hybrid oaks, Yarnes et al. 2008), we detected only a few, minor associations between phenolics and feeding guilds, such as the positive correlation between galling and condensed tannins in red maple, similar to trends seen in red oaks by Abrahamson et al. (2003). The lack of a relationship between phenolics and herbivory is consistent with other studies (e.g., Ayres et al. 1997) which have also demonstrated no broad spectrum anti-herbivore effects of foliar phenolics such as condensed tannins.

How do these results fit within the context of other work? Latitudinal trends in plant defenses have been found in several species of saltmarsh plants in eastern North America, sampled over around 10° latitude (Siska et al. 2002; Salgado and Pennings 2005). Leaves of wide-ranging species from “southerly” localities in coastal Georgia were compared with leaves on the coasts of New England. The southerly leaves were tougher, lower in N and less palatable to caterpillars than the northerly ones. These differences carried over into common garden experiments where the different populations were grown side by side. The results of this study, on forest trees in the same geographical region, suggest that such trends in plant defenses may not be typical of other ecosystem types. Furthermore, among geographical regions and ecosystem types, latitudinal trends in herbivory and plant defense do not always emerge from either field or common garden studies (see “Introduction”). This calls into question just how widespread these latitudinal trends really are—despite the pervasive perception that they are ubiquitous.

Further studies are necessary to determine whether the lack of a trend found in these four tree species is generally true of forest trees. It would be interesting to compare temperate and tropical trees for tannin content across taxonomic boundaries, but the difficulties of standardization between species are formidable, in terms of both quantification and biological effect. As an extension of this study it would be instructive to measure tannin concentrations in more southerly populations of the same species. *Liquidambar* and *Fagus* have populations in the cloud forests of south-eastern Mexico, essentially an environment without frost. *Acer rubrum* also extends to southern Florida, a subtropical climate. Comparing populations of these three species over a greater latitudinal range would provide a stronger test of the theory of latitudinal differences in plant defense.

We also only sampled at one time during the early part of the growing season at each locality. Although it is generally agreed that herbivory is concentrated into the earliest weeks of a leaf’s lifetime (Coley and Barone 1996), it is possible that differences in herbivory or plant defenses show up later in the season. Forests and parts of forest canopies could potentially be sampled in a myriad of ways: our method was chosen since it permitted sampling in a standardized way in a multitude of locations. Significantly, the present work represents one

example of closely standardized, consistent study. Ideally, follow up studies to this work should focus on measuring tannins and herbivory at other stages in the season and positions within the forest canopy. Other work could broaden the focus to study secondary compounds besides tannins (e.g., terpenoids) which are thought to play a role in the defenses of these and other tree species.

Despite the uncertainties, if any latitudinal trends in total herbivory and phenolics exist in these tree species, and we did not detect them due to sample sizes or details of sampling strategy, these latitudinal trends contribute small amounts of the variation in leaf area loss and defenses among sites relative to other sources. This is instructive since it demonstrates that any selective forces for greater plant defenses at lower latitudes are fairly weak. The other sources of variation in herbivory and plant defenses likely include local cycles in the populations of multiple herbivore species, based on biotic and abiotic factors such as natural enemy population cycles and the weather (respectively), and differences in the mean level of plant defenses among sites, both within and among regions. The interaction of these two major sources of variation over evolutionary time may have produced idiosyncratic, species-specific patterns of plant defense and total herbivory. Also, variation in factors based on local environment (e.g., soils, weather, and population cycles of herbivores) may make field studies of latitudinal trends relatively insensitive, requiring prohibitively large sample sizes to detect any trends.

Plant defenses are typically under strong genetic control, including chemical defenses (see Hamilton et al. 2001 and references therein). If consistently greater herbivory over evolutionary time has selected for greater plant defenses for populations at lower latitudes, we would expect to detect differences among populations found along a broad latitudinal transect if environmentally based variation were minimized. Therefore, a common garden approach may be required to detect any genetically based variation in plant defenses due to latitudinal trends.

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