WHY DO TOOTHED LEAVES CORRELATE WITH COLD CLIMATES? GAS EXCHANGE AT LEAF MARGINS PROVIDES NEW INSIGHTS INTO A CLASSIC PALEOTEMPERATURE PROXY

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Leaf teeth are conspicuous and often diagnostic features of many plant species. In mesic environments with sufficient nutrient resources, the percentage of toothed species in extant floras generally correlates negatively with temperature; consequently, fossil leaf teeth are widely used to estimate continental paleotemperatures. However, the function of leaf teeth with respect to climate is poorly understood. Here, we test the hypothesis that teeth enhance rates of carbon uptake at the beginning of the growing season when temperatures are limiting. We measure the seasonal patterns of leaf-margin photosynthesis and transpiration for 60 woody species from two temperate regions with differing climates (Pennsylvania and North Carolina). Three significant results are, first, physiological activity at leaf margins is greatest early in the growing season (first 30 d); second, toothed margins are more active with respect to photosynthesis and transpiration than untoothed margins; finally, leaf margins are more active in species native to colder Pennsylvania. The toothed species increase transpiration and photosynthate production early in the growing season relative to untoothed species and do so more in the Pennsylvania sample, maximizing carbon gain when temperature is limiting but moisture and nutrient availability are not. This mechanism may provide a proportionally increasing selective advantage to toothed species with decreasing temperature that is reflected in empirical correlations used for paleotemperature estimation.

Keywords: leaf teeth, photosynthesis, transpiration, functional ecology, paleoclimate, leaf-margin analysis.

Introduction

Many size and shape characteristics of leaves correlate strongly with temperature and moisture variables (Webb 1968; Lewis 1972; Parkhurst and Loucks 1972; Givnish 1979). In particular, under mesic conditions with sufficient nutrient resources, the percentage in a flora of woody angiosperm species (exclusive of monocots) that have toothed leaf margins (leaf-margin percentage) generally has a significant negative correlation with mean annual temperature (MAT; Bailey and Sinnott 1915, 1916; Wolfe 1979, 1993; Wilf 1997; Gregory-Wodzicki 2000; Greenwood et al. 2004). Paleobotanists have long applied correlations between leaf-margin percentage and MAT, derived from extant floras, to reconstruct paleotemperatures from fossil floras (Bailey and Sinnott 1915; Spicer and Parrish 1986; Parrish and Spicer 1988; Greenwood and Wing 1995; Utescher et al. 2000; Wing et al. 2000; Jacobs 2002; Liang et al. 2003; Uhl et al. 2003; Wilf et al. 2003; Fricke and Wing 2004; Roth-Nebelsick et al. 2004). Given the wide application of this proxy, called leaf-margin analysis, it is surprising that the selective mechanisms driving the response of leaf margins to temperature are only poorly known (Wolfe 1993; Roth et al. 1995; Baker-Brosh and Peet 1997; Wilf 1997; Wing et al. 2000; Feild

et al. 2005) nearly a century after the correlation was first noted (Bailey and Sinnott 1915). Why are leaves more likely to have teeth in colder climates? More experimentally tested explanations are needed to increase confidence in temperature reconstructions based on fossil leaves and to possibly facilitate the development of new paleoclimate proxies.

Previous Work on the Function of Leaf Teeth

Most explanations for the function of leaf teeth have not focused directly on the correlation with MAT. Givnish (1978, 1979) proposed that leaf thickness controls the presence versus absence of teeth because photosynthetic tissue in thin leaves is more dependent on major veins for transporting nutrients; a geometric consequence of this constraint is less leaf area between major veins near the margins, as seen in the incisions of teeth and lobes. Teeth may also be related to herbivory; theoretical considerations suggest that teeth and lobes may deter leaf feeding (Brown and Lawton 1991), but in practice this has not yet been demonstrated (Rivero-Lynch et al. 1996). Leaf teeth have also been related to the deciduous habit. Baker-Brosh and Peet (1997) dosed young leaves from a temperate deciduous forest with atmospheric $^{14}CO_2$ and, using autoradiographic imaging, reported enhanced photosynthesis in the teeth or lobes of eight of 14 species. These authors also observed that teeth were disproportionately large in young leaves (see also Feild et al. 2005), and they suggested that enhanced leaf-margin gas exchange early in the growing season is an adaptation for exporting photosynthate

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during leaf expansion, when abundant light in the canopy is available.

Finally, water availability is thought to influence the development of teeth; it has long been noted that physiologically dry (including severely cold) habitats support proportionally fewer toothed species than comparable wet habitats (Bailey and Sinnott 1916; Wolfe 1993), and teeth appear to be sites of increased transpiration (Canny 1990; Wolfe 1993). Recent experiments with the perennial herb *Chloranthus japonicus* Siebold suggest that teeth, via guttation, may help prevent flooding by positive root pressure of intercellular airspaces and consequent reduction of photosynthesis (Feild et al. 2005). This could be adaptive not only in wet environments, where positive root pressure is common, but also in cold climates because hydathodes help maintain optimal leaf turgor, which maximizes leaf expansion rates and could help refill freeze-thaw embolisms (Feild et al. 2005).

Gas-Exchange Hypothesis

This study builds on the carbon acquisition model of Baker-Brosh and Peet (1997) by focusing on the gas exchange (photosynthesis and transpiration) of leaf teeth and how this may be related to the well-established correlation between proportional diversity of toothed species and MAT. Surface edges are associated with strong gradients, particularly where the surface area/volume ratio is highest, such as corners of mineral crystals, lightning rods, or, of interest here, leaf teeth. Assuming an even distribution of stomata across a leaf (but see below), evaporation rates should be highest in leaf teeth (Canny 1990). Wind currents should enhance this effect because teeth generate turbulence over the leaf surface, thinning the boundary layer (Schuepp 1993). Most toothed species also have major veins extending to their tooth apices (Hickey and Wolfe 1975; Roth et al. 1995), and the diameters of tracheary elements near these apices are not significantly smaller than those of more proximal elements (Canny 1990). Moreover, teeth generally contain disproportionately large numbers of stomata and hydathodes (fixed pores), which presumably enhance rates of sap flow and water loss (Canny 1990; Wolfe 1993; Roth et al. 1995; Feild et al. 2005).

Leaf teeth may also support elevated rates of photosynthesis early in the growing season (Baker-Brosh and Peet 1997). Because rates of photosynthesis and transpiration are coupled via stomatal conductance (Wong et al. 1979), high sap flow rates should be associated with vigorous photosynthesis and rapid growth, particularly within teeth early in the growing season (Mauseth 1988; Wilson et al. 1991), but few data exist that bear on these issues.

Here, we quantitatively test the hypothesis that leaf teeth are more common in cold climates because they are sites of enhanced gas exchange early in the growing season, boosting photosynthesis and maximizing growth potential when temperature is limiting but moisture and nutrient resources are not (herein referred to as the gas-exchange hypothesis). We measure leaf-margin photosynthesis and transpiration through a growing season for two groups of native woody species from Pennsylvania and North Carolina. First, we compare the seasonal responses of toothed and untoothed species. Second, we compare cohorts of leaves that form at different times during the growing season; if a function of leaf teeth is to boost carbon production early in the growing season, leaves that flush after this critical interval may not have physiologically active margins, even when they are young. Finally, we compare the responses of the two climatically distinct plant groups; the gas-exchange hypothesis predicts that leaves from colder climates have the most active margins.

Material and Methods

Plant Selection and Growth

Two regions were targeted: cold-temperate York County in south central Pennsylvania (PA; MAT = 11.8°C, mean annual precipitation = 104 cm; climate data from York SSW3 Pumping Station; species toothed in regional flora = 72%; climate and plant data from Wilf 1997) and warm-temperate Chatham County in central North Carolina (NC; MAT = 15.2°C, mean annual precipitation = 111 cm; climate data from Durham Station as described in Royer et al. 2005; species toothed in regional flora = 55% as described in Dolph

Table 1

Species List for Pennsylvania Sample (York County)

Plant name[a]	Margin type[b]	Leaf habit[c]	Growth habit[d]
Acer negundo L.	0	D	T
Acer rubrum L.	0	D	T
Acer saccharinum L.	0	D	T
Acer saccharum Marsh.	0	D	T
Alnus serrulata (Ait.) Wild.	0	D	S, T
Betula lenta L.	0	D	T
Betula nigra L.	0	D	T
Celtis occidentalis L.	0	D	S, T
Cornus amomum P. Mill.	1	D	S
Fraxinus americana L.	0	D	T
Hamamelis virginiana L.	0	D	S, T
Ilex verticillata (L.) Gray	0	D	S, T
Lindera benzoin (L.) Blume	1	D	S, T
Liriodendron tulipifera L.	1	D	T
Nyssa sylvatica Marsh.	1[e]	D	T
Photinia pyrifolia (Lam.) Robertson & Phipps	0	D	S
Platanus occidentalis L.	0	D	T
Quercus coccinea Muenchh.	0	D	T
Quercus prinus L.	0	D	T
Quercus velutina Lam.	0	D	T
Rhododendron maximum L.	1	E	S, T
Rhus typhina (L.) Sudworth	0	D	S, T
Salix nigra Marsh.	0	D	T
Sambucus nigra L. ssp. *canadensis* (L.) R. Bolli	0	D	S, T

[a] Binomial nomenclature and authorities are after USDA (2004).

[b] 0 = toothed; 1 = untoothed. For facultatively toothed species, classification above is based on individuals used in study only.

[c] D = deciduous; E = evergreen. For facultatively evergreen species, classification above is based on individuals used in study only.

[d] T = tree (single-stemmed plants with a maximum height >5 m); S = shrub (multistemmed plants with a maximum height <5 m).

[e] Facultatively toothed or evergreen species (classification in table is based on individuals used in study only).

and Dilcher 1979). For both regions, native woody nonmonocot angiosperm species were selected to reflect the overall proportions of toothed species in their respective floras, where teeth are defined as vascularized extensions of the leaf margin incised less than one-quarter of the distance to the midvein, thus excluding lobes but not teeth on lobes (Ash et al. 1999; Royer et al. 2005; PA: $n = 24$ species, 79% toothed; NC: $n = 42$ species, 60% toothed; see tables 1, 2 for species lists). This approach facilitates the testing of individual species as well as groups of species; the latter is important because most leaf-climate methods are based on all appropriate species in a flora (Wolfe 1979, 1993; Wilf 1997).

The two plant groups were analyzed in different years (PA: 2003; NC: 2004). Three individually potted plants of each species were purchased from nurseries located within or near their native ranges (Octoraro Native Plant Nursery, Kirkwood, PA; Cure Nursery, Pittsboro, NC; Elk Mountain Nursery, Asheville, NC) and placed in the Penn State Forest Resources Laboratory Greenhouse, which has conditioned, charcoal-filtered air. Daily mean temperatures matched outside conditions within 2°C, and daily mean relative humidity was $84\% \pm 6\%$ (1 SD). An important exception was that greenhouse temperatures were maintained above 10°C, neutralizing any major differences in ambient temperature at time of measurement during the two spring flushes (<1°C average difference for the first 2 wk), which began on March 19 (NC) and April 16 (PA). Plants were watered to saturation three times weekly and fertilized monthly with slow-release pellets or sticks (N/P/K = 18/9/18). All plants were between 2 and 5 yr old when measured. Removing environmental and ontogenetic conditions as sources of variation is the most direct means by which to compare physiological responses of plants adapted to different natural climates.

Gas-Exchange Measurements

A nondestructive method was developed to quantify leaf-margin photosynthesis and transpiration using a portable open gas-exchange system (LI-6400; LI-COR, Lincoln, NE). First, a leaf or portion of leaf with its margin exposed inside the leaf chamber (herein referred to as "leaf") was measured in a standardized environment: 22°C air temperature, 1000 μmol m^{-2} s^{-1} PAR, 1.2 ± 0.4 kPa (1 SD) leaf–air vapor pressure deficit, 420 ± 38 μmol mol^{-1} (1 SD) CO_2 concentration. Next, a 1–2-mm band around the margin (herein referred to as "margin"), which included all teeth if present, was sealed with grease (Crisco; Smucker, Orrville, OH). Finally, the leaf was reinserted into the leaf chamber in the same position and remeasured. The difference between the two quantities represents leaf-margin gas exchange. Grease was removed after the measurements with pure glycerin soap.

The method's reliability was assessed with several tests. First, grease alone, in quantities exceeding that applied to leaves, did not emit CO_2 or H_2O above the detection limits of the gas-exchange system. Leaves completely sealed in grease also did not emit CO_2 or H_2O. Second, for two species (*Cornus amomum* and *Acer saccharum*), a region of leaf comparable in size to the leaf chamber (6 cm^2) was sealed with grease while the gas exchange of an unaltered region on the same leaf was monitored continuously for 5 min;

Table 2

Species List for North Carolina Sample (Chatham County)

Plant name[a]	Margin type[b]	Leaf habit[c]	Growth habit[d]
Aesculus sylvatica Bartr.	0	D	S, T
Amelanchier canadensis (L.) Medik.	0	D	S, T
Asimina triloba (L.) Dunal	1	D	S, T
Callicarpa americana L.	0	D	S
Calycanthus floridus L.[e]	1	D	S
Carpinus caroliniana Walt.	0	D	S, T
Catalpa bignonioides Walt.[e]	1	D	T
Celtis laevigata Willd.	0[f]	D	S, T
Cephalanthus occidentalis L.[e]	1	D	S, T
Chionanthus virginicus L.	1	D	S, T
Clematis virginiana L.[e]	0	D	V
Clethra alnifolia L.[e]	0	D	S
Cornus alternifolia L. f.[e]	1	D	S, T
Cornus amomum L. Mill.	1	D	S
Cornus florida L.	1	D	S, T
Crataegus marshallii Egglest.	0	D	S, T
Cyrilla racemiflora L.	1	E[f]	S, T
Diospyros virginiana L.	1	D	T
Euonymus americana L.	0	D	S
Fothergilla gardenii L.[e]	0[f]	D	S
Fraxinus pennsylvanica Marsh.	0[f]	D	T
Hamamelis virginiana L.	0	D	S, T
Ilex decidua Walt.	0	D	S, T
Ilex glabra (L.) Gray	0	E	S
Itea virginica L.	0	D	S
Lindera benzoin (L.) Blume	1	D	S, T
Magnolia acuminata (L.) L.	1	D	T
Magnolia virginiana L.[e]	1	E[f]	S
Morella cerifera (L.) Small[e]	0[f]	E	S
Oxydendrum arboreum (L.) DC	0[f]	D	T
Photinia pyrifolia (Lam.) Robertson & Phipps	0	D	S
Platanus occidentalis L.	0	D	T
Quercus michauxii Nutt.	0	D	T
Quercus nigra L.	1	E[f]	T
Quercus phellos L.	1	D	T
Rhododendron atlanticum (Ashe) Rehd.[e]	1	D	S
Rhododendron viscosum (L.) Torr.[e]	1	D	S
Salix sericea Marsh.	0	D	S, T
Sambucus nigra L. ssp. *canadensis* (L.) R. Bolli	0	D	S, T
Vaccinium sp.[g]	0	E	S
Viburnum dentatum L.[e]	0	D	S, T
Xanthorhiza simplicissima Marsh.[e]	0	D	S

[a] Binomial nomenclature and authorities are after USDA (2004).

[b] 0 = toothed; 1 = untoothed. For facultatively toothed species, classification above is based on individuals used in study only.

[c] D = deciduous; E = evergreen. For facultatively evergreen species, classification above is based on individuals used in study only.

[d] T = tree (single-stemmed plants with a maximum height >5 m); S = shrub (multistemmed plants with a maximum height <5 m); V = woody vine (USDA 2004).

[e] Species is native to counties contiguous with Chatham County but not to Chatham County (Radford et al. 1968).

[f] Facultatively toothed or evergreen species (classification in table is based on individuals used in study only).

[g] Plant identified by nursery as *Vaccinium corymbosum* L.

gas-exchange rates did not differ from pretreatment rates. Third, gases were monitored in two *Cornus florida* leaves for 3 d after a grease treatment. For both leaves, photosynthesis and transpiration returned to pretreatment levels within 1 d. Finally, leaves from eight species that were never sealed in grease were periodically measured for photosynthesis and transpiration during the 2003 growing season; these patterns were similar to those of leaves that were sealed in grease.

Measurements for each species began when the first leaves were 5 d old (cohort 1, where a cohort is a set of leaves of similar age) and were repeated every 10 to 40 d on the same leaves until August or early September. Leaves that flushed during the middle of the growing season were also monitored (cohorts 2 and 3). If a leaf was damaged or lost, a neighboring leaf of similar age was substituted.

Leaf sizes and shapes were quantified using a digital camera (Nikon 995; Nikon, Melville, NY; 2048×1536 pixels) and imaging software (SigmaScan Pro 5; SPSS Science, Chicago). For all leaves, the areas of the "leaf" and "margin" were determined (fig. 1). Accounting for leaf area in this manner removes any potential biases stemming from variations among leaves in their margin and leaf areas. To quantify the relative importance of leaf-margin gas exchange within individual leaves, all area-based gas-exchange data are expressed in terms of the margin/leaf ratio (e.g., fig. 2). For the first two rounds of measurements in the North Carolina sample and the final round in both samples, additional size and shape variables related to leaf teeth were measured in complete leaves, such as number of teeth, number of teeth/perimeter, perimeter/leaf area, tooth area, average tooth area, tooth area/leaf area, and tooth area/leaf perimeter, as defined by Huff et al. (2003) and Royer et al. (2005).
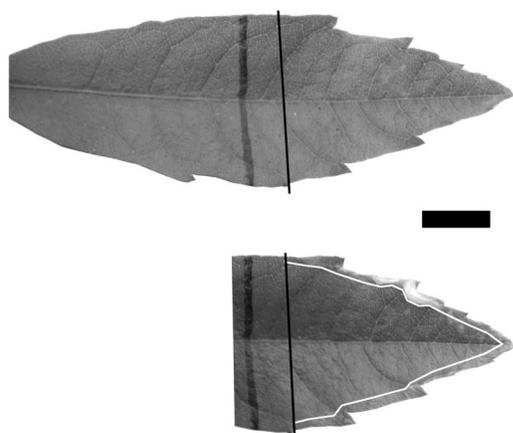
Fig. 1 Example of leaf-area selection for gas-exchange measurements. The top image is a portion of a *Morella cerifera* leaf. The vertical black line drawn directly on the leaf marks the outer edge of the leaf chamber (see "Material and Methods" for more details); the vertical black line superimposed on the leaf represents the inner edge (the seal of the leaf chamber is 6 mm wide). All leaf area from this second black line to the leaf tip was measured for gas-exchange properties. The lower image is the same leaf after the margins were sealed with grease. The white line superimposed on the leaf marks the inner edge of the band of grease; all leaf area inside of this line was measured for gas-exchange properties during occlusion of the margin. Scale bar = 1 cm.
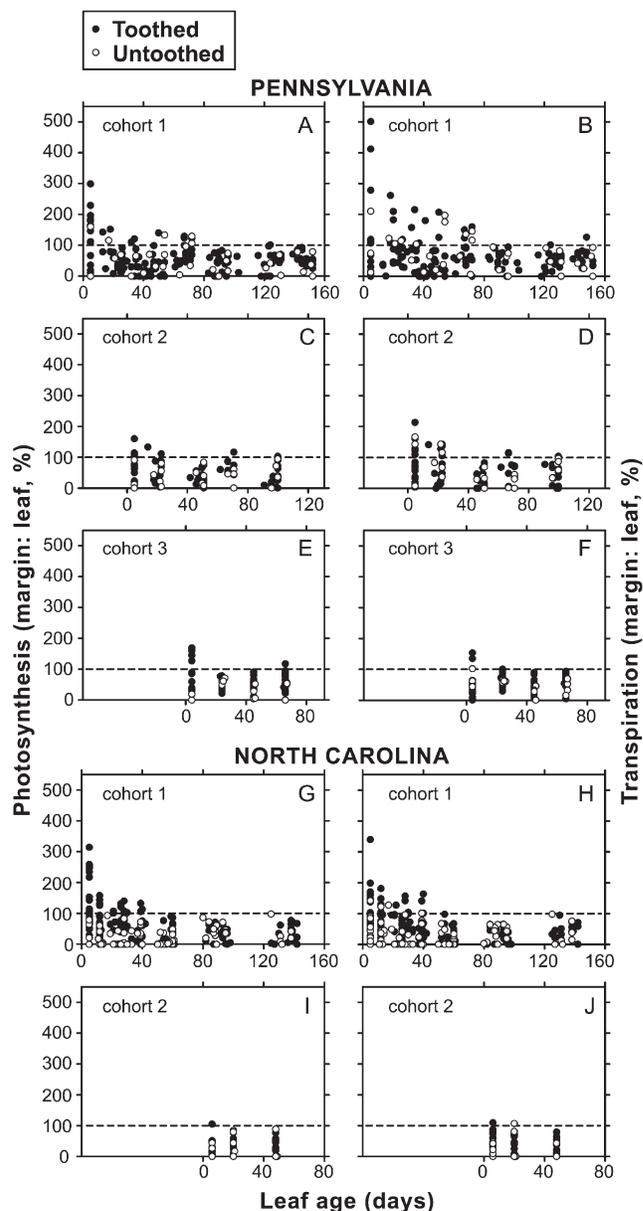
Fig. 2 Rates of leaf-margin gas exchange (panels on left side: photosynthesis; panels on right side: transpiration) through a growing season for woody plant species from two climatically distinct regions (*A–F*, Pennsylvania; *G–J*, North Carolina). All data are normalized to their respective leaf rates (see "Material and Methods") and expressed as percentages. Horizontal dashed lines at 100% represent parity, above which rates of leaf-margin gas exchange are higher per unit area than their associated whole-leaf rates. Each cohort is a set of leaves of similar age; the horizontal axes for cohorts 2 and 3 have been shifted by the average number of days after which the leaves flushed relative to cohort 1.

Statistical Analyses

All species data reported here represent the means of two leaves, where each leaf is from a different plant. Statistical tests are based on species means of the first cohort of leaves using a one- or two-way ANOVA, unless noted otherwise.

For all tests, photosynthesis and transpiration data were pooled because they did not differ significantly in either of the sites (PA, NC: $P > 0.30$ for all tests discussed in text) and did not introduce significant interaction terms. Margin types (toothed, untoothed) and/or sites were pooled in some tests when they did not introduce significant interaction terms.

Results

Seasonal Patterns

Strong seasonality in leaf-margin gas exchange is apparent in the first cohort for both the Pennsylvania and North Carolina samples (figs. 2, 3). Most notably, in the first round of measurements, when the first leaves to flush are 5 d old (leftmost column of data points in fig. 2A, 2B, 2G, 2H), leaf margins in a large percentage of species exhibit higher area-normalized gas-exchange rates than their respective whole leaves (45% and 32% for Pennsylvania and North Carolina, respectively; data plotting above 100% in fig. 2). For most plants, leaf-margin photosynthesis and transpiration are positively coupled (PA: $r = 0.40$, $P = 0.07$; NC: $r = 0.55$, $P = 0.0002$; Spearman's coefficient of rank correlation). Because the marginal areas in these young leaves constitute a large fraction of the total leaf area ($24\% \pm 9\%$ [1 SD]; sites pooled), leaf-margin gas exchange in many species represents an important, and sometimes dominant, component of gas exchange early in the growing season. By the third round of measurements, when leaves are approximately 30 d old, the percentage of species with leaf margins that are more active than their whole leaves drops significantly (9% and 13% for Pennsylvania and North Carolina, respectively; $F_{2,245} = 4.00$, $P = 0.05$ based on a repeated-measures ANOVA with sites and margin types pooled; see also fig. 3). The same overall seasonal pattern of leaf-margin gas exchange, with high values early in the growing season followed by low values for the remainder of the season, is also evident within individual species; see figure 3 for examples of species with the highest early-season values of leaf-margin gas exchange.

Importantly, the large fraction of active leaf margins in young leaves is lost in subsequent cohorts; leaf-margin gas exchange is significantly lower in the second than the first cohort for the first round of measurements, even though both sets of leaves are identical in age ($F_{1,231} = 8.35$, $P = 0.004$; sites and margin types pooled; see fig. 2).

Margin Effects (Toothed vs. Untoothed)

Toothed species have leaf margins that are more active with respect to photosynthesis and transpiration than those of untoothed species. For the North Carolina sample (fig. 2G, 2H; fig. 4), the difference is statistically significant for the first round of measurements ($F_{1,83} = 26.9$, $P < 0.0001$) as well as the full growing season ($F_{1,78} = 62.2$, $P < 0.0001$). This same pattern is apparent within the subset of evergreen species, where the seasonal means of toothed species are higher than those of untoothed species ($F_{1,11} = 11.5$, $P = 0.006$, $n = 6$ species). For the Pennsylvania sample, the most active leaf margins are typically toothed, particularly early in the growing season (fig. 2A–2F), but the differences are not
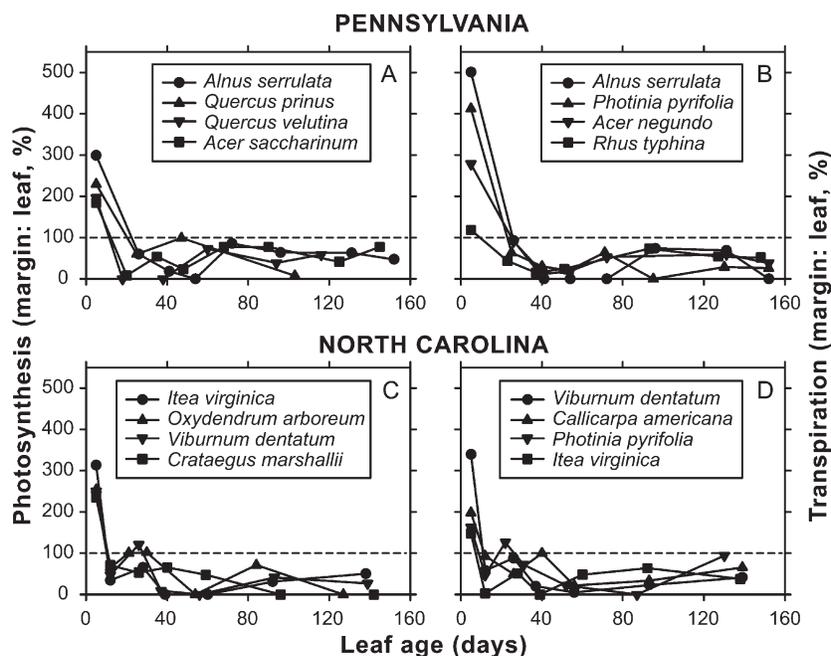
Fig. 3 Seasonal patterns of leaf-margin gas exchange (A, C, photosynthesis; B, D, transpiration) for a subset of the most responsive species from the Pennsylvania (A, B) and North Carolina (C, D) samples. In each panel, the four species with the most active leaf margins during the first round of measurements (leaf age = 5 d) are plotted (cf. fig. 2). All data are normalized to their respective leaf rates (see "Material and Methods") and expressed as percentages. Horizontal dashed lines at 100% represent parity, above which rates of leaf-margin gas exchange are higher per unit area than their associated whole-leaf rates.
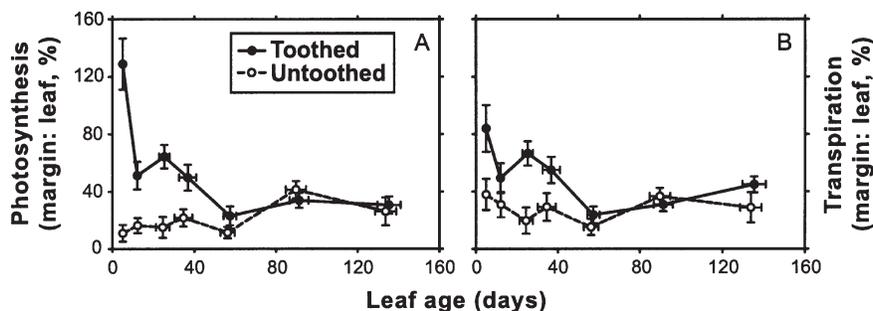
Fig. 4 Mean response of leaf-margin photosynthesis (A) and leaf-margin transpiration (B) for the first cohort of plants from North Carolina (means from Pennsylvania sample are not plotted because of the low number of untoothed species; see fig. 2). Each data point represents the mean of all species (toothed or untoothed) for a given round of measurement (cf. species data in fig. 2G, 2H). All data are normalized to their respective leaf rates (see "Material and Methods") and expressed as percentages. Plotted errors for gas-exchange data are standard errors of the mean; errors in time axis resulting from staggered measurements represent ± 1 SD.

significant ($P > 0.05$); this is likely because of the low number of untoothed species in this sample ($n = 5$).

Leaf habit (deciduous vs. evergreen; see tables 1, 2) did not significantly affect leaf-margin activity in the North Carolina sample ($F_{1,22} = 2.90$, $P = 0.10$ for toothed species; $F_{1,15} = 0.051$, $P = 0.82$ for untoothed species; analyses based on the full growing season); a similar test for the Pennsylvania sample could not be performed because of a lack of evergreens ($n = 1$). Growth habit (shrub vs. tree at maturity) also did not affect leaf-margin gas exchange ($P > 0.11$ for toothed and untoothed species in both North Carolina and Pennsylvania samples); because some species can be either shrubs or trees (see tables 1, 2), this result incorporates three iterations of tests: (1) exclusion of species that display both growth habits; (2) categorization of species that display both growth habits as shrubs; (3) trees.

Climate Effects

The rate of leaf-margin gas exchange is higher, on average, for plants native to colder Pennsylvania. For untoothed species, the difference is significant for the first round of measurements ($F_{1,37} = 5.99$, $P = 0.02$) and the full growing season ($F_{1,40} = 59.2$, $P < 0.0001$). A similar but nonsignificant pattern is observed in toothed species ($F_{1,84} = 2.12$, $P = 0.15$ for full growing season; $F_{1,86} = 0.018$, $P = 0.90$ for first round). Six of our plant species are native to both Pennsylvania and North Carolina; this overlap facilitates testing the influence of climate on leaf-margin gas exchange within species. The seasonal means of all six species are consistent with the patterns observed among species: leaf-margin gas exchange in plants from Pennsylvania is higher than in conspecifics from North Carolina, particularly for untoothed species ($F_{1,6} = 44.8$, $P = 0.0005$ for untoothed species; $F_{1,14} = 2.80$, $P = 0.12$ for toothed species).

Variation

The gas-exchange data form a spectrum; even early in the growing season, not all toothed species exhibit active leaf margins (fig. 2). Our analysis is based on one binary character state, the presence versus absence of teeth, which does not fully capture this spectrum. To explore why some

toothed species may have more active leaf margins than others, we correlated a series of continuous variables that describe the size and number of teeth (see "Material and Methods" for list of variables measured) with their rates of leaf-margin gas exchange at time of measurement. The most significant correlations are for end-of-season leaves from Pennsylvania, where leaf-margin photosynthesis is positively correlated with number of teeth ($r^2 = 0.34$, $P = 0.02$) and tooth area/leaf perimeter ($r^2 = 0.38$, $P = 0.01$).

Discussion

These results, derived from plants native to two temperate regions, support the gas-exchange hypothesis: leaf margins are most physiologically active early in the growing season, toothed margins are more active than untoothed margins, and plants native to the cooler climate have the most active leaf margins. In contrast to previous suggestions (Baker-Brosh and Peet 1997), these patterns are not significantly influenced by leaf habit or growth habit. Our results may apply broadly to seasonally cold, mesic temperate forests and may be useful in predicting the roles of teeth in other forest types. We expect toothed species from cool, mesic climates to exhibit the highest rates of leaf-margin gas exchange early in the growing season, optimizing photosynthate production against temperature, and teeth may enhance gas exchange throughout the year in cold climates with little temperature variation. In warm climates, where the potential for plant growth is greater, there are proportionally fewer toothed species, and teeth are smaller (e.g., Royer et al. 2005) because any pulse in carbon production is outweighed by attendant water losses. This trade-off between carbon uptake and water loss can also explain why toothed species are proportionally less abundant in water-stressed environments (e.g., Wolfe 1993), although this moisture effect is secondary to the influence of MAT (Wolfe 1993). Leaf-margin percentage is probably most closely linked with indices of heat accumulation such as growing-season degree days, but these variables correlate significantly with MAT (Royer et al. 2005), accounting for the strong correlation between leaf-margin percentage and MAT.

Feild et al. (2005) measured photosynthesis for the herb *Chloranthus japonicus* in a manner similar to that of this study and found that the teeth contribute a small, nonsignificant component to leaf photosynthesis. They instead consider the hydathodal teeth in this species to function as a release valve for positive root pressure, which they suggest may be adaptive in wet or cold environments (see "Introduction"). The lack of photosynthetic function apparently contrasts with our study, but not all species in our data set exhibited enhanced leaf-margin gas exchange early in the growing season (fig. 2). We emphasize that Feild et al. (2005) made a detailed study of a single herbaceous species, whereas we investigated 60 woody species native to two climates. Woody species are conventionally used in leaf-margin analysis, whereas herbaceous species are excluded because of their relatively low preservation potential (Bailey and Sinnott 1915). Although more work is needed to clarify the multiple roles of leaf teeth, the functions proposed by Feild et al. (2005) and in this study both involve the movement of xylem sap and the resultant carbon economy of the plant, and both can be linked to temperature. Importantly, hydathodes are probably responsible for a considerable fraction of the water lost by leaves early in the growing season (Mauseth 1988); in many species, wax plugs form in hydathodes by the time leaves are fully developed (Mortlock 1952; Stevens 1956; Takeda et al. 1991), which is consistent with both the mechanism proposed by Feild et al. (2005) and our observed drop over time in leaf-margin transpiration (figs. 2–4).

This study provides new experimental support for the importance of leaf-margin gas exchange in the long-observed pattern that woody angiosperm species (exclusive of monocots) with sufficient access to water and nutrient resources are more likely to have toothed leaves in colder climates. This mechanism explains empirical negative correlations of leaf margins and temperature to the extent that the selective advantage of teeth with regard to gas exchange increases with decreasing temperature. Further, variables such as number of teeth and tooth area/leaf perimeter may also correlate with leaf-margin gas exchange. Although further work is needed to clarify these preliminary links, these two variables have already been shown to correlate significantly with MAT in eastern North American forests (Royer et al. 2005) and may therefore also be linked to MAT from a mechanistic standpoint. Our results provide a new context for paleoclimate methods such as leaf-margin analysis that have been used with little biological understanding of the link between leaf teeth and MAT.

Acknowledgments

Literature Cited

Ash AW, B Ellis, LJ Hickey, KR Johnson, P Wilf, SL Wing 1999 Manual of leaf architecture: morphological descriptions and categorization of dicotyledonous and net-veined monocotyledonous angiosperms. Smithsonian Institution, Washington, DC. http://www.peabody.yale.edu/collections/pb/MLA/.

Bailey IW, EW Sinnott 1915 A botanical index of Cretaceous and Tertiary climates. Science 41:831–834.

——— 1916 The climatic distribution of certain types of angiosperm leaves. Am J Bot 3:24–29.

Baker-Brosh KF, RK Peet 1997 The ecological significance of lobed and toothed leaves in temperate forest trees. Ecology 78:1250–1255.

Brown VK, JH Lawton 1991 Herbivory and the evolution of leaf size and shape. Philos Trans R Soc Lond B 333:265–272.

Canny M 1990 What becomes of the transpiration stream? New Phytol 114:341–368.

Dolph GE, DL Dilcher 1979 Foliar physiognomy as an aid in determining paleoclimate. Palaeontogr Abt B Palaeophytol 170:151–172.

Feild TS, TL Sage, C Czerniak, WJD Iles 2005 Hydathodal leaf teeth of *Chloranthus japonicus* (Chloranthaceae) prevent guttation-induced flooding of the mesophyll. Plant Cell Environ 28:1179–1190.

Fricke HC, SL Wing 2004 Oxygen isotope and paleobotanical estimates of temperature and $\delta^{18}O$-latitude gradients over North America during the early Eocene. Am J Sci 304:612–635.

Givnish T 1979 On the adaptive significance of leaf form. Pages 375–407 *in* OT Solbrig, S Jain, GB Johnson, PH Raven, eds. Topics in plant population biology. Columbia University Press, New York.

Givnish TJ 1978 Ecological aspects of plant morphology: leaf form in relation to environment. Bibl Biotheor 27:83–142.

Greenwood DR, P Wilf, SL Wing, DC Christophel 2004 Paleotemperature estimation using leaf-margin analysis: is Australia different? Palaios 19:129–142.

Greenwood DR, SL Wing 1995 Eocene continental climates and latitudinal temperature gradients. Geology 23:1044–1048.

Gregory-Wodzicki KM 2000 Relationships between leaf morphology and climate, Bolivia: implications for estimating paleoclimate from fossil floras. Paleobiology 26:668–688.

Hickey LJ, JA Wolfe 1975 The bases of angiosperm phylogeny: vegetative morphology. Ann Mo Bot Gard 62:538–589.

Huff PM, P Wilf, EJ Azumah 2003 Digital future for paleoclimate estimation from fossil leaves? preliminary results. Palaios 18:266–274.

Jacobs BF 2002 Estimation of low-latitude paleoclimates using fossil angiosperm leaves: examples from the Miocene Tugen Hills, Kenya. Paleobiology 28:399–421.

Lewis MC 1972 The physiological significance of variation in leaf structure. Sci Prog 60:25–51.

Liang M-M, A Bruch, M Collinson, V Mosbrugger, C-S Li, Q-G Sun, J Hilton 2003 Testing the climatic estimates from different palaeobotanical methods: an example from the Middle Miocene Shanwang flora of China. Palaeogeogr Palaeoclimatol Palaeoecol 198:279–301.

Mauseth JD 1988 Plant anatomy. Benjamin/Cummings, Menlo Park, CA.

Mortlock C 1952 The structure and development of the hydathodes of *Ranunculus fluitans* Lam. New Phytol 51:129–138.

Parkhurst DF, OL Loucks 1972 Optimal leaf size in relation to environment. J Ecol 60:505–537.

Parrish JT, RA Spicer 1988 Late Cretaceous terrestrial vegetation: a near-polar temperature curve. Geology 16:22–25.

Radford AE, HE Ahles, CR Bell 1968 Manual of the vascular flora of the Carolinas. University of North Carolina Press, Chapel Hill.

Rivero-Lynch AP, VK Brown, JH Lawton 1996 The impact of leaf shape on the feeding preference of insect herbivores: experimental and field studies with *Capsella* and *Phyllotreta*. Philos Trans R Soc Lond B 351:1671–1677.

Roth A, V Mosbrugger, G Belz, H Neugebauer 1995 Hydrodynamic modelling study of angiosperm leaf venation types. Bot Acta 108: 121–126.

Roth-Nebelsick A, T Utescher, V Mosbrugger, L Diester-Haass, H Walther 2004 Changes in atmospheric CO_2 concentrations and climate from the Late Eocene to Early Miocene: palaeobotanical reconstruction based on fossil floras from Saxony, Germany. Palaeogeogr Palaeoclimatol Palaeoecol 205:43–67.

Royer DL, P Wilf, JA Janesko, EA Kowalski, DL Dilcher 2005 Correlating climate and plant ecology with leaf size and shape: potential proxies for the fossil record. Am J Bot 92:1141–1151.

Schuepp PH 1993 Leaf boundary layers. New Phytol 125:477–507.

Spicer RA, JT Parrish 1986 Paleobotanical evidence for cool North Polar climates in middle Cretaceous (Albian-Cenomanian) time. Geology 14:703–706.

Stevens ABP 1956 The structure and development of the hydathodes of *Catha palustris* L. New Phytol 55:339–345.

Takeda F, ME Wisniewski, DM Glenn 1991 Occlusion of water pores prevents guttation in older strawberry leaves. J Am Soc Hortic Sci 116:1122–1125.

Uhl D, V Mosbrugger, A Bruch, T Utescher 2003 Reconstructing palaeotemperatures using leaf floras: case studies for a comparison of leaf margin analysis and the coexistence approach. Rev Palaeobot Palynol 126:49–64.

USDA, NRCS 2004 The PLANTS database. Version 3.5. National Plant Data Center, Baton Rouge, LA. http://plants.usda.gov.

Utescher T, V Mosbrugger, AR Ashraf 2000 Terrestrial climate evolution in northwest Germany over the last 25 million years. Palaios 15:430–449.

Webb LJ 1968 Environmental relationships of the structural types of Australian rain forest vegetation. Ecology 49:296–311.

Wilf P 1997 When are leaves good thermometers? a new case for leaf margin analysis. Paleobiology 23:373–390.

Wilf P, KR Johnson, BT Huber 2003 Correlated terrestrial and marine evidence for global climate changes before mass extinction at the Cretaceous-Paleogene boundary. Proc Natl Acad Sci USA 100:599–604.

Wilson T, M Canny, M McCully 1991 Leaf teeth, transpiration and the retrieval of apoplastic solutes in balsam poplar. Physiol Plant 83: 225–232.

Wing SL, H Bao, PL Koch 2000 An early Eocene cool period? evidence for continental cooling during the warmest part of the Cenozoic. Pages 197–237 *in* BT Huber, KG MacLeod, SL Wing, eds. Warm climates in Earth history. Cambridge University Press, Cambridge.

Wolfe JA 1979 Temperature parameters of humid to mesic forests of eastern Asia and relation to forests of other regions of the Northern Hemisphere and Australasia. US Geol Surv Prof Pap 1106:1–37.

——— 1993 A method of obtaining climatic parameters from leaf assemblages. US Geol Surv Bull 2040:1–71.

Wong SC, IR Cowan, GD Farquhar 1979 Stomatal conductance correlated with photosynthetic capacity. Nature 282:424–426.