How strong is intracanopy leaf plasticity in temperate deciduous trees?[1]

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Intracanopy plasticity in tree leaf form is a major determinant of whole-plant function and potentially of forest understory ecology. However, there exists little systematic information for the full extent of intracanopy plasticity, whether it is linked with height and exposure, or its variation across species. For arboretum-grown trees of six temperate deciduous species averaging 13–18 m in height, we quantified intracanopy plasticity for 11 leaf traits across three canopy locations (basal-interior, basal-exterior, and top). Plasticity was pronounced across the canopy, and maximum likelihood analyses indicated that plasticity was primarily linked with irradiance, regardless of height. Intracanopy plasticity (the quotient of values for top and basal-interior leaves) was often similar across species and statistically indistinguishable across species for several key traits. At canopy tops, the area of individual leaves was on average 0.5–0.6 times that at basal-interior, stomatal density 1.1–1.5 times higher, sapwood cross-sectional area up to 1.7 times higher, and leaf mass per area 1.5–2.2 times higher; guard cell and stomatal pore lengths were invariant across the canopy. Species differed in intracanopy plasticity for the mass of individual leaves, leaf margin dissection, ratio of leaf to sapwood areas, and stomatal pore area per leaf area; plasticity quotients ranged only up to ≈2. Across the six species, trait plasticities were uncorrelated and independent of the magnitude of the canopy gradient in irradiance or height and of the species' light requirements for regeneration. This convergence across species indicates general optimization or constraints in development, resulting in a bounded plasticity that improves canopy performance.

Key words: guard cells; hydraulic limitations; leaf size; leaf shape; plant intelligence; shade tolerance; stomata; succession.

Intracanopy plasticity has important impacts on many aspects of tree biology, potentially contributing to whole-canopy performance via effects on light penetration through the canopy and on the energy, carbon, and water balance of individual leaves (Meister et al., 1987; Gutschick and Wiegel, 1988; Niinemets and Tenhunen, 1997; Bond et al., 1999; Meir et al., 2002; Hikosaka, 2005). Many studies report strong intracanopy plasticity for temperate deciduous species, with the majority emphasizing the classic sun leaf vs. shade leaf dichotomy. Typically, shade leaves are larger, less deeply lobed, thinner, and have lower leaf mass per area (LMA) than sun leaves (e.g., Hanson, 1917; Wylie, 1949; Talbert and Holch, 1957; Bond et al., 1999). Shade leaves also typically have lower stomatal density than sun leaves but similar guard cell lengths (e.g., Bongers and Popma, 1988; Richardson et al., 2001; Sack et al., 2003b). These morphological differences and anatomical, biochemical, and physiological correlates are thought to enhance net carbon gain for inner canopy leaves (e.g., Hoflacher and Bauer, 1982; Lichtenthaler, 1985; Smith et al., 1997; Niinemets and Sack, 2006). Theoretical work has also suggested that species differences in the abundance and morphology of shade leaves influences forest understory ecology, playing a driving role in succession and regeneration (Horn, 1971; Canham et al., 1994; Grubb, 1998).

Despite the wealth of studies on intracanopy plasticity, our understanding remains fragmentary. Reports for a number of species even conflict with the general trends, including shade leaves smaller than sun leaves, lower-canopy leaves with higher LMA than upper-canopy leaves, and invariant stomatal density across the canopy (Wylie, 1949; Talbert and Holch, 1957; Niinemets and Kull, 1994; Carr, 2000; Richardson et al., 2000). In fact, in our literature review, we found that intracanopy plasticity has rarely been quantified systematically. Few studies compared leaves sampled across the entire canopy, from the basal-interior to the very top. Furthermore, non-systematic sampling procedures might have introduced bias in some studies, e.g., if expanding leaves were included, and/or if leaf appearance itself were used as a criterion in identifying sun and shade forms (e.g., Weyers et al., 1998). Systematic quantification of intracanopy plasticity, and its variability across species, is needed before whole plant implications can be assessed.

The basis for intracanopy leaf plasticity is also poorly understood. Recent studies argue that the plasticity might not be primarily related to canopy irradiance, but rather to hydraulic constraints that increase with tree height due to a stronger effect of gravity and a longer hydraulic path length (McDowell et al., 2002; Marshall and Monserud, 2003; Koch et al., 2004; Woodruff et al., 2004). Height-linked differences in leaf form have recently been described in conifers 30 to 100 m in height and might drive reduced growth at the tops of trees (Marshall and Monserud, 2003; Koch et al., 2004; Woodruff et al., 2004). However, the impacts of height might not be

Fig. 1. Schematic of canopy locations sampled with an aerial lift. Three shoots each were sampled from the basal-interior (circles), from the basal-exterior (squares), and from the canopy top (triangles).

general; in studies on trees up to 40 m tall, researchers have suggested that the gradient in the water supply to transpiring leaves from the base to top of the canopy can be partially mitigated by declines in leaf to sapwood area ratio with increasing height and by the hierarchical distribution of stem hydraulic resistances across branching orders (McDowell et al., 2002; Tyree and Zimmermann, 2002). In some cases water is in fact supplied to the upper, exposed leaves with higher conductance per leaf area than to the shaded, lower leaves (Cochard et al., 1999; Sellin and Kupper, 2004, 2005). A crucial question is whether intracanopy leaf plasticity is principally driven by height or exposure. In the first case, the variation would arise due to physical constraints, whereas in the second case it may indicate optimization during leaf development.

In this study we quantified intracanopy plasticity for temperate deciduous tree species, on average 13–18 m in height, diverse in leaf form and in phylogeny. Trees were studied in an arboretum in which open spacing minimized the influence of surrounding trees. Leaves were sampled from three extreme canopy locations: the basal-interior, basal-exterior, and top of the canopy (Fig. 1). Our system was designed to test whether intracanopy plasticity is linked most closely with differences in height or leaf exposure: basal-interior and basal-exterior leaves differed strongly in exposure but not in height, whereas basal-exterior and top leaves differed much less in exposure, but strongly in height. We hypothesized that leaf size and shape, LMA, and stomatal and sapwood characters would vary strongly across irradiances and heights in the canopy and that plasticity would differ among species according to the magnitude of the gradient across the canopy of irradiance and height, and potentially according to the species' light requirements for regeneration.

MATERIALS AND METHODS

Site, species, and sampling design—The study was conducted at the Arnold Arboretum of Harvard University, Jamaica Plain, Massachusetts ($42°17'55''$ N, $71°07'42''$ W). Six temperate deciduous tree species were selected for diversity in phylogeny, leaf form, and light requirement for regeneration: *Acer saccharum* Marshall (Sapindaceae), *Betula alleghaniensis* Britt. (Betulaceae), *Ginkgo biloba* L. (Ginkgoaceae), *Liriodendron tulipifera* L. (Magnoliaceae), *Quercus rubra* L. (Fagaceae), and *Sassafras albidum* (Nutt.) Nees (Lauraceae; nomenclature follows Flora of North America Editorial Committee, 2005 and Stevens, 2005). Based on their ability to persist as juveniles in shaded understorey, these species range from shade tolerant (*A. saccharum*), to intermediate (*B. alleghaniensis*, *Q. rubra*), to shade intolerant

(*G. biloba*, *L. tulipifera*, *S. albidum*; Burns and Honkala, 1990). For each species, five replicate trees were sampled; diameters at breast height ranged from 32 to 100 cm (mean 60 cm $\pm$ 4 SE).

Shoots with mature leaves were sampled in September 2001. For each crown position on each tree, three replicate shoots were sampled and sealed in bags to prevent leaf dehydration. For sampling, we used an aerial lift to reach basal-exteriors, basal-interiors and tops of the canopies, except for three individuals each of *A. saccharum* and *G. biloba*, for which the basal shoots were sampled with pole pruners 3 m above the ground. Shoots were sampled around the crowns from all orientations to average out the effects of differences in irradiance and light quality (Lichtenthaler, 1985). In the lab, the shoots were cut back to uniform length, 0.5 m of primary stem length from the tip (with attached branches), which included one to several years' growth (mean number of leaves: 44 $\pm$ 2 SE).

Trait measurements—Heights of canopy locations were measured with a measuring tape during sampling. For each sampled tree, the irradiance incident on leaves at the sampled canopy locations was measured (LI-250A light meter; LI-COR, Lincoln, Nebraska, USA), and measurements were made simultaneously in a clearing (LI-190 quantum sensor logging to a CRX-10 datalogger; Campbell Scientific, Logan, Utah, USA), on two overcast days, to estimate the percentage penetration of diffuse photosynthetically active radiation (diffuse site factor; Anderson, 1964).

For each shoot, total leaf area (LI-3100C Area Meter, LI-COR) and dry mass after oven-drying >48 h at over 70°C were determined. The mean mass of individual leaves was determined for each shoot by dividing total leaf mass by leaf number, and leaf mass per area (LMA) was determined as total leaf mass per total area. Individual leaf area, leaf shape, and stomatal characters were measured for one leaf randomly selected from each shoot, avoiding the terminal leaves which were often stunted. As an index of the degree of margin dissection independent of leaf size, leaves were scanned and the perimeter²/area (Sack et al., 2003b) was calculated for the digital images using the ImageJ freeware computer program (W. S. Rasband, U.S. National Institutes of Health, Bethesda, Maryland, USA; http://rsb.info.nih.gov/ij/). Nail varnish peels were made from the abaxial side of the leaf for measurement of stomatal traits (no stomata were found on the adaxial side for any of the species). One peel per leaf was made halfway along the leaf length, typically on the right side of the midrib. Using a light microscope (Olympus BH-2; Olympus America, Melville, New York, USA; and Nikon Eclipse E800; Nikon Instech Co., Kanagawa, Japan) at 200× or 400× total magnification, we counted the stomata in two fields of view to calculate stomatal density, and measured guard cell lengths for four stomata. An index of total stomatal pore area per leaf area was quantified as stomatal density × the square of the guard cell length (SPI_{gcl}; Sack et al., 2003b, 2005). For *L. tulipifera* and *S. albidum*, stomatal pore length was also measured, and a second index of stomatal pore area per leaf area was calculated as stomatal density × the square of the pore length (SPI_{pl}) to confirm that this index would parallel that based on guard cell length.

Two shoot traits were also determined. Shoot sapwood cross-sectional area (SCA) was determined by measuring cross-sectional radius r, excluding the phloem, estimating the disk area as πr^2, and then subtracting the pith disk area as estimated by πr^2 using the radius of the pith as r. The leaf to sapwood area ratio (LA : SA) was determined as the total leaf area of the shoot divided by SCA. We assumed all the sapwood to have potential functional importance in hydraulic conductance and/or capacitance, as well as biomechanical support (Melcher et al., 2003).

For *S. albidum*, the three leaf types—entire, one-lobed, and two-lobed—were all included when pooling leaves from each shoot to determine mean individual leaf mass, LMA and LA : SA. However, one-lobed and two-lobed leaves were rare, as previously reported (Ghent, 1973); respectively they constituted on average 4.0 and 2.9% of leaves on basal-interior shoots, 3.8 and 0.8% on basal-interior shoots, and 2.6 and 1.0% on top shoots (three shoots from each canopy location were sampled from each of the five trees, 15 shoots in total from each location). Because the lobed leaf types were so infrequent, they were never selected as the one random leaf sampled per shoot for determination of individual leaf size, perimeter²/area, and stomatal characters.

Statistics—For each trait, values were averaged for the three shoots at each of the three canopy locations for each tree. The values for the three canopy locations for the five replicate trees were analyzed for all species together using a two-way, repeated-measures ANOVA, blocked for given trees, with species and canopy location as factors. Next, for each species, data were analyzed using a one-way, repeated-measures ANOVA, blocked by tree, for the effects of canopy location. Orthogonal contrasts were used to compare canopy locations,

testing for differences between basal-interior and basal-exterior, and between basal-exterior and top (Gilligan, 1986; Sokal and Rohlf, 1995) using the computer program Genstat, 8th ed. (Lawes Agricultural Trust, Rothamsted, UK). Prior to running ANOVAs, data were log-transformed to increase homoscedasticity and to model for multiplicative effects.

Maximum likelihood analyses were performed to test whether intracanopy plasticity in each trait was linked with differences in irradiance, height, or both. For each trait that showed significant intracanopy plasticity for the six species, the mean values for each canopy location for each tree were divided by the value for the canopy top leaves, such that all values were expressed as percentages of the values for top leaves (for leaf area and LA : SA, the ratio was inverted, such that relative values always increased with greater height and irradiance); values for all species were treated together. Then, three models were tested with these data (Hilborn and Mangel, 1997), the irradiance model (TRAIT = $a \times \ln(\text{IRRADIANCE}) + b + \varepsilon$), height model (TRAIT = $a \times \text{HEIGHT} + b + \varepsilon$), and irradiance × height model (TRAIT = $a \times \ln(\text{IRRADIANCE}) + b \times \ln(\text{IRRADIANCE}) \times \text{HEIGHT} + c \times \text{HEIGHT} + d + \varepsilon$), where the capitalized variables represent measured data; a, b, c and d fitted parameters; and the error ε is normally distributed. A log-linear response was considered for the irradiance model because impacts on plant function tend to level off at increasing irradiance (e.g., Bond et al., 1999; Sellin, 2001), whereas a linear response was considered for the height model because the impacts of height would increase linearly with gravity or hydraulic pathlength. The irradiance × height model is the product of the irradiance and height models, with simplified parameters. Maximum likelihood parameters were determined for the three models applied to the data for each trait; the R^2 and slope of expected vs. observed values were used as indices of goodness of fit. For each trait, the models were compared using the Akaike information criterion (AIC), corrected for low n; the model with the lowest AIC value has best support, and differences >2 in AIC values are meaningful (Burnham and Anderson, 2002, 2004). These procedures were run using the "optim" function in the R language (RDCT, 2005; code for the analyses in this study is available on request). Parameters were estimated using the Simulated Annealing procedure for global optimization, then used as the initial values in Nelder–Mead simplex search procedure for local optimization; standard errors for the parameters were generated from the Hessian matrix.

For each trait, an index of maximum intracanopy plasticity was calculated as the quotient of the value for top leaves divided by that for basal-interior leaves (for leaf area and LA : SA, the inverse was used, so that for all traits a higher index would correspond to higher plasticity). For each trait, this index was determined for each tree and average values determined for each species; the data for all trees were then analyzed for species-differences using ANOVA (Sokal and Rohlf, 1995; using Minitab Release 14.1; Minitab Inc., State College, Pennsylvania, USA).

Tests were also made for correlation across species between trait values (for top leaves), trait plasticity values, and species' light requirements for regeneration using Pearson correlations (r_p) and Spearman rank correlations (r_s). Relationships were considered significant when both r_s and r_p were significant (Sokal and Rohlf, 1995; correlations were calculated using Minitab Release 14.1). Species' light requirements for regeneration were assigned values of 1 for shade-tolerant, 2 for intermediate, and 3 for light-demanding (see *Site, species, and sampling design* earlier; Burns and Honkala, 1990; Coomes and Grubb, 2000).

RESULTS

Differences in height and irradiance among canopy positions—The canopy locations sampled—from base to canopy top—differed strongly in their height (Table 1). For all species, the mean sampling height for basal-interior shoots was within 10 cm of that for basal-exterior shoots, and ranged from 4.4 ± 0.9 m (mean ± 1 SE) for *G. biloba* to 8.4 ± 1.2 m for *S. albidum* (mean for all species 6.5 ± 0.6 m), whereas top leaves ranged from 13.3 ± 0.6 m for *G. biloba* to 18.1 ± 0.2 for *A. saccharum* (mean for all species 15.5 ± 0.9 m). On average, the top leaves ranged from 7.9 ± 0.7 m above basal

leaves for *Q. rubra* to 12.3 ± 1.1 m for *A. saccharum* (mean for all species 9.6 ± 0.7 m).

The sampled canopy locations differed strongly in irradiance incident on the leaf (Table 1). Basal-interior leaves were the most shaded, ranging in diffuse site factor (dsf) from 2.8 ± 0.5% for *A. saccharum* to 9.7 ± 0.7% for *B. alleghaniensis* (mean for all species 6.5 ± 1.2%). Basal-exterior leaves were moderately shaded, with dsf ranging from 24.2 ± 6.4% for *A. saccharum* to 57.6 ± 3.0% for *S. albidum* (mean for all species 41.9 ± 5.2%). The basal-exterior leaves ranged from 3.5 times more exposed than basal-interior leaves for *G. biloba*, to 18 times for *S. albidum* (mean for all species 8.0 ± 3.3), and top leaves ranged from 1.7 times more exposed than the basal-exterior leaves for *S. albidum* to 4.1 times for *A. saccharum* (mean for all species 2.6 ± 1.1). The top leaves ranged from 10 times more exposed than basal-interior leaves for *B. alleghaniensis* to 36 times for *A. saccharum* (mean for all species 19.5 ± 8.0).

Differences in leaf and sapwood structure across canopy positions—Shoots varied strongly in leaf and sapwood structure across canopy locations for nine of the 11 traits measured (Tables 1–3). The general trends of intracanopy plasticity for leaf area, LMA, stomatal density, and guard cell length confirmed those in previous studies; we saw novel trends for individual leaf mass, perimeter^2/area, stomatal pore length, SPI$_{gcl}$, SPI$_{pl}$, and LA : SA. For all species together, following the transition from the canopy basal-interior to basal-exterior to top, leaves became smaller in area and tended to increase in mass (significantly for *B. alleghaniensis*), leading to a higher LMA (Table 1). Leaf perimeter^2/area changed in different ways across species, decreasing from the basal-interior to basal-exterior to top leaves for *A. saccharum*, *B. alleghaniensis*, and *L. tulipifera*, reflecting reduced lobing for leaves of a given size and reduced toothing for *B. alleghaniensis*. The opposite trend held empirically for *Q. rubra* and *S. albidum*, which had greater lobing in the exposed and top leaves, but because of high variability, the trend was nonsignificant for *Q. rubra* (Table 1). Trends in stomatal characters were also strong. For all species considered together, and in some cases individually, stomatal density increased from basal-interior to basal-exterior leaves (Table 2). However, guard cell length was invariant across canopy locations, as was stomatal pore length for the two species examined, *L. tulipifera* and *S. albidum*. Consequently, for all the species considered together, there were significant increases in SPI$_{gcl}$ from the basal-interior to basal-exterior to top leaves. For both species for which SPI$_{pl}$ was measured, *L. tulipifera* and *S. albidum*, there were likewise from basal-interior to basal-exterior leaves (Table 2). The SCA also increased from the basal-interior to basal-exterior to top leaves, and LA : SA declined (Table 3). For leaf area and LMA, the trends that held for all species together were also significant for each species individually, while for other traits, trends were generally consistent for each species, but not always significant due to high variability at a given canopy position.

One major finding of this study is a similarity across the six species in their intracanopy plasticity for many traits. There

AMERICAN JOURNAL OF BOTANY

TABLE 1. Height, irradiance, and leaf traits related to gross form at three canopy positions (mean ± SE) for six tree species; plasticity quotients for traits; and results of analyses of variance for individual species in tests for effect of canopy location and for all species together in tests for species differences, for effect of canopy location, and for an interaction.

Trait / Species	Mean ± SE for canopy position			Canopy position effect	Quotient for given species		For all species together, effect of			Quotient for all species	
	Basal-interior	Basal-exterior	Top		BE/BI	T/BE	Species	Canopy position	Species × position	BE/BI	T/BE
Height (m)											
A. saccharum	6.8 ± 1.8	6.8 ± 1.8	18.1 ± 0.2	**	1.00^{ns}	2.66**	ns	***	*	1.0^{ns}	2.49***
B. alleghaniensis	5.7 ± 0.6	5.7 ± 0.6	14.4 ± 0.6	***	1.00^{ns}	2.53***					
G. biloba	4.4 ± 0.9	4.5 ± 0.9	13.3 ± 0.6	***	1.03^{ns}	2.95***					
L. tulipifera	5.5 ± 0.0	5.5 ± 0.0	17.8 ± 1.1	***	1.00^{ns}	3.24***					
Q. rubra	8.0 ± 0.9	8.1 ± 1.1	16.0 ± 0.8	***	1.01^{ns}	1.97***					
S. albidum	8.4 ± 1.2‡	8.4 ± 1.2‡	13.6 ± 1.2	***	1.00^{ns}	1.61***					
Irradiance (% diffuse)											
A. saccharum	2.8 ± 0.5	24.2 ± 6.4	100 ± 0.0	***	8.72***	4.13***	***	***	***	6.49***	2.38***
B. alleghaniensis	9.7 ± 0.7	49.9 ± 7.2	100 ± 0.0	***	5.13***	2.00***					
G. biloba	8.2 ± 2.6	28.9 ± 5.3	100 ± 0.0	***	3.54**	3.46**					
L. tulipifera	6.7 ± 2.3	45.0 ± 4.3	100 ± 0.0	***	6.75***	2.22*					
Q. rubra	8.3 ± 0.6‡	46.1 ± 16.1†	100 ± 0.0	**	5.58**	2.17*					
S. albidum	3.2 ± 0.4§	57.6 ± 3.0§	100 ± 0.0	***	18.2***	1.74**					
Leaf area (cm²)											
A. saccharum	83.7 ± 9.4	47.6 ± 1.4	44.1 ± 1.8	**	0.57**	0.93^{ns}	***	***	ns	0.70***	0.80***
B. alleghaniensis	49.8 ± 13.5	32.1 ± 3.7	25.9 ± 1.9	*	0.64^{ns}	0.81^{ns}					
G. biloba	16.2 ± 1.4	16.0 ± 2.4	9.2 ± 0.7	*	0.99^{ns}	0.58*					
L. tulipifera	135 ± 9.2	117 ± 3.1	86.4 ± 10.4	**	0.87^{ns}	0.74*					
Q. rubra	103 ± 12.6	75.1 ± 7.2	58.8 ± 2.3	**	0.73*	0.78^{ns}					
S. albidum	101 ± 12.9	52.4 ± 4.0	48.5 ± 2.6	***	0.52***	0.93^{ns}					
Leaf dry mass (g)											
A. saccharum	0.28 ± 0.05	0.34 ± 0.05	0.33 ± 0.04	ns	1.23^{ns}	0.95^{ns}	***	**	ns	1.24**	0.94^{ns}
B. alleghaniensis	0.14 ± 0.03	0.23 ± 0.06	0.23 ± 0.07	*	1.63*	1.02^{ns}					
G. biloba	0.15 ± 0.01	0.16 ± 0.02	0.12 ± 0.01	ns	1.11^{ns}	0.74^{ns}					
L. tulipifera	0.48 ± 0.06	0.66 ± 0.12	0.60 ± 0.08	ns	1.39^{ns}	0.91^{ns}					
Q. rubra	0.45 ± 0.04	0.46 ± 0.05	0.44 ± 0.06	ns	1.02^{ns}	0.96^{ns}					
S. albidum	0.20 ± 0.02	0.24 ± 0.01	0.24 ± 0.02	ns	1.19^{ns}	0.99^{ns}					
Leaf mass per area (g · m⁻²)											
A. saccharum	43.8 ± 7.7	67.9 ± 10.1	87.0 ± 10.9	**	1.55**	1.28^{ns}	**	***	*	1.49***	1.20***
B. alleghaniensis	55.4 ± 1.6	88.4 ± 11.0	110 ± 7.8	***	1.59**	1.24*					
G. biloba	91.5 ± 5.5	105 ± 11.2	136 ± 8.0	*	1.14^{ns}	1.30*					
L. tulipifera	50.0 ± 3.5	81.2 ± 5.6	91.2 ± 7.0	**	1.62**	1.12^{ns}					
Q. rubra	69.4 ± 4.2	97.2 ± 8.8	120 ± 9.9	***	1.40**	1.24*					
S. albidum	36.0 ± 1.4	77.6 ± 4.4	78.8 ± 4.1	***	2.16***	1.01^{ns}					
Leaf perimeter²/area											
A. saccharum	44.3 ± 3.1	36.9 ± 1.8	36.5 ± 1.4	*	0.83**	0.99^{ns}	***	**	***	0.93***	1.05^{ns}
B. alleghaniensis	22.2 ± 0.7	19.5 ± 0.4	18.5 ± 0.8	*	0.88*	0.95^{ns}					
G. biloba	19.5 ± 0.8	17.7 ± 0.4	17.8 ± 0.4	ns	0.91^{ns}	1.00^{ns}					
L. tulipifera	23.6 ± 1.4	19.0 ± 1.6	19.8 ± 1.0	ns	0.81*	1.04^{ns}					
Q. rubra	65.3 ± 7.4	67.6 ± 5.1	75.5 ± 6.8	ns	1.03^{ns}	1.12^{ns}					
S. albidum	15.7 ± 0.2	16.4 ± 0.3	17.1 ± 0.4	**	1.04^{ns}	1.04^{ns}					

Note: Values are means for $N = 5$ trees, except †$N = 2$ trees; ‡$N = 3$ trees; §$N = 4$ trees. Plasticity quotients are given for the means at the basal-exterior positions divided by means at the basal-interior positions (BE/BI), and for canopy top/basal-exterior (T/BE), with significance of these effects determined by orthogonal contrasts (ns > 0.05; *$P \leq 0.05$; **$P \leq 0.01$; ***$P < 0.001$).

was no significant interaction between species and canopy position for six of the nine traits measured for the six species, indicating that proportional changes among leaf positions were similar across species (Tables 1–3). Species differed significantly in the intracanopy plasticities of LMA; leaf perimeter²/area, which showed different trends across species as described; and LA : SA, which did not significantly differ across canopy positions for *G. biloba* or *S. albidum*, but was strongly plastic for the other species. The consistency in intracanopy variation across species was analyzed more closely with quotients for maximum intracanopy plasticity, calculated

as the value for top/basal-interior leaves (Figs. 2 and 3). Notably, intracanopy plasticity was constrained; mean plasticity ranged up to ≈2 (Figs. 2 and 3). The maximum intracanopy plasticity quotients did not vary significantly for five of the nine traits measured for the six species (one-way ANOVA testing species differences; *P* values ranged from 0.07 to 0.85; Fig. 2), including guard cell length (mean quotients ranged from 0.96 ± 0.008 to 1.1 ± 0.07), stomatal density (1.1 ± 0.07 to 1.5 ± 0.2), SCA (1.3 ± 0.2 to 1.7 ± 0.2), leaf area (1.6 ± 0.2 to 2.1 ± 0.2), and LMA (1.5 ± 0.1 to 2.2 ± 0.2). Quotients also did not vary for stomatal pore length, measured

TABLE 2.   Stomatal traits (mean ± SE) for leaves at three canopy positions for six tree species; plasticity quotients for traits; and results of analyses of variance for individual species in tests for effect of canopy location and for all species together in tests for species differences, for effect of canopy location, and for an interaction.

Trait	Mean ± SE for canopy position			Canopy position	Quotients for given species		For all species together, effect of			Quotient for all species	
Species	Basal-interior	Basal-exterior	Top	effect	BE/BI	T/BE	Species	Canopy position	Species × position	BE/BI	T/BE
Stomatal density (mm^{-2})											
A. saccharum	100 ± 5.8	118 ± 6.0	115 ± 13.1	ns	1.17*	0.98^{ns}	***	***	ns	1.14**	1.08^{ns}
B. alleghaniensis	109 ± 8.8	124 ± 5.1	157 ± 6.4§	**	1.14^{ns}	1.27*					
G. biloba	126 ± 15.6	122 ± 12.6	141 ± 11.1	ns	0.97^{ns}	1.16^{ns}					
L. tulipifera	189 ± 17.5	260 ± 17.3	266 ± 26.2	ns	1.38^{ns}	1.02^{ns}					
Q. rubra	400 ± 19.4	431 ± 10.5	454 ± 21.0	*	1.08^{ns}	1.05^{ns}					
S. albidum	302 ± 38.7	338 ± 35.4	377 ± 37.2	ns	1.12^{ns}	1.12^{ns}					
Guard cell length (μm)											
A. saccharum	10.7 ± 0.3	11.0 ± 0.3	11.1 ± 0.3	ns	1.03^{ns}	1.01^{ns}	***	ns	ns	1.04^{ns}	1.00^{ns}
B. alleghaniensis	19.0 ± 0.5	19.0 ± 0.4	18.7 ± 0.2§	ns	1.00^{ns}	0.99^{ns}					
G. biloba	19.7 ± 1.8	21.0 ± 0.8	20.4 ± 0.6	ns	1.06^{ns}	0.98^{ns}					
L. tulipifera	25.5 ± 0.8	27.5 ± 1.0	28.8 ± 1.1	ns	1.08^{ns}	1.05^{ns}					
Q. rubra	13.4 ± 0.5	14.1 ± 0.4	13.8 ± 0.4	ns	1.06*	0.98^{ns}					
S. albidum	18.8 ± 0.6	18.5 ± 0.7	18.0 ± 0.6	ns	0.98^{ns}	0.97^{ns}					
SPI$_{gcl}$ (×10^{-2})											
A. saccharum	1.16 ± 0.12	1.42 ± 0.08	1.43 ± 0.17	ns	1.22*	1.00^{ns}	***	***	ns	1.24***	1.10*
B. alleghaniensis	3.95 ± 0.47	4.54 ± 0.23	5.57 ± 0.21§	*	1.15^{ns}	1.23^{ns}					
G. biloba	4.70 ± 0.35	5.39 ± 0.65	6.07 ± 0.55	ns	1.15^{ns}	1.13^{ns}					
L. tulipifera	12.4 ± 1.29	19.6 ± 0.40	21.8 ± 0.81	***	1.59***	1.11^{ns}					
Q. rubra	7.88 ± 0.44	7.83 ± 0.43	8.53 ± 0.15	ns	0.99^{ns}	1.09^{ns}					
S. albidum	10.6 ± 1.74	11.8 ± 1.08	12.5 ± 1.29	ns	1.12^{ns}	1.06^{ns}					
Stomatal pore length (μm)											
L. tulipifera	17.5 ± 0.8	18.3 ± 1.1	18.5 ± 0.9	ns	1.04^{ns}	1.01^{ns}	ns	ns	ns	1.01^{ns}	0.99^{ns}
S. albidum	14.7 ± 1.4	14.2 ± 1.5	13.7 ± 1.4	ns	0.97^{ns}	0.97^{ns}					
SPI$_{pl}$ (×10^{-2})											
L. tulipifera	5.8 ± 0.4	8.6 ± 0.6	9.1 ± 0.5	***	1.50***	1.06^{ns}	ns	**	*	1.33**	1.02^{ns}
S. albidum	6.5 ± 1.0	7.6 ± 1.9	7.5 ± 1.5	ns	1.17^{ns}	0.99^{ns}					

Note: Values are means for $N = 5$ trees, except §$N = 4$ trees. Plasticity quotients are given for the means at the basal-exterior positions divided by means at the basal-interior positions (BE/BI), and for canopy top/basal-exterior (T/BE), with significance of these effects determined by orthogonal contrasts (ns > 0.05; *$P \leq 0.05$; **$P \leq 0.01$; ***$P < 0.001$). SPI$_{gcl}$ is an index of total stomatal pore area per leaf area, calculated as stomatal density × the square of the guard cell length; SPI$_{pl}$ is a second index, calculated as stomatal density × the square of stomatal pore length.

TABLE 3.   Means (±SE) for sapwood cross-sectional area and leaf to sapwood area ratio for shoots at three canopy positions for six tree species; plasticity quotients for traits; and results of analyses of variance for individual species in tests for effect of canopy location and for all species together in tests for species differences, for effect of canopy location, and for an interaction.

Trait	Mean ± SE for canopy position			Canopy position	Quotients for given species		For all species together, effect of			Quotient for all species	
Species	Basal-interior	Basal-exterior	Top	effect	BE/BI	T/BE	Species	Canopy position	Species × position	BE/BI	T/BE
Sapwood cross-sectional area (mm^2)											
A. saccharum	11.8 ± 1.5	14.2 ± 2.3	18.3 ± 3.1	ns	1.21^{ns}	1.29^{ns}	ns	***	ns	1.23**	1.09^{ns}
B. alleghaniensis	12.5 ± 1.5	18.4 ± 2.5	19.7 ± 2.3	***	1.48***	1.07^{ns}					
G. biloba	14.2 ± 1.7	18.8 ± 4.2	17.5 ± 3.7	ns	1.33^{ns}	0.93^{ns}					
L. tulipifera	16.5 ± 1.5	21.0 ± 2.3	22.3 ± 2.2	ns	1.27^{ns}	1.07^{ns}					
Q. rubra	13.5 ± 1.2	16.1 ± 1.4	21.4 ± 1.6	*	1.19^{ns}	1.33*					
S. albidum	20.5 ± 2.1	20.6 ± 2.3	19.5 ± 2.2	ns	1.01^{ns}	0.95^{ns}					
Leaf to sapwood area ratio (cm^2 / mm^{-2})											
A. saccharum	144 ± 10.5	127 ± 18.2	97.2 ± 17.2	*	0.89^{ns}	0.76^{ns}	***	***	*	0.82***	0.82**
B. alleghaniensis	94.4 ± 6.0	66.5 ± 3.4	58.4 ± 2.2	**	0.71**	0.88^{ns}					
G. biloba	54.2 ± 10.6	54.3 ± 5.0	46.2 ± 8.0	ns	1.00^{ns}	0.85^{ns}					
L. tulipifera	123 ± 7.2	101 ± 5.2	77.2 ± 4.9	**	0.82*	0.76**					
Q. rubra	114 ± 15.9	75.5 ± 7.1	51.2 ± 7.5	***	0.66*	0.68**					
S. albidum	108 ± 16.9	95.8 ± 6.0	95.7 ± 9.3	ns	0.88^{ns}	1.00^{ns}					

Note: Values are means for $N = 5$ trees. Plasticity quotients are given for the means at the basal-exterior positions divided by means at the basal-interior positions (BE/BI), and for canopy top/basal-exterior (T/BE), with significance of these effects determined by orthogonal contrasts (ns > 0.05; *$P \leq 0.05$; **$P \leq 0.01$; ***$P < 0.001$).
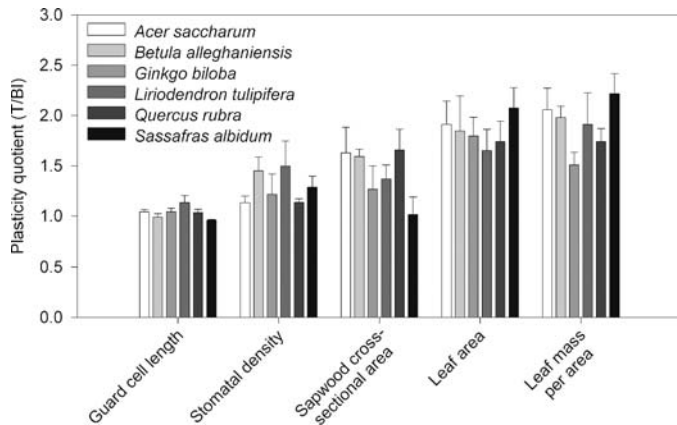
Fig. 2. Maximum intracanopy plasticity for leaf traits that had similar plasticity between the top and basal-interior positions for six temperate deciduous tree species. Traits did not differ significantly in one-way ANOVAs ($P > 0.05$; $N = 5$ trees per species, except $N = 4$ for stomatal density for *B. alleghaniensis*). Plasticity was calculated as the quotient of the means for the top leaves (T) divided by the means for the basal-interior leaves (BI) for all traits but leaf area, for which plasticity was calculated as the inverse.
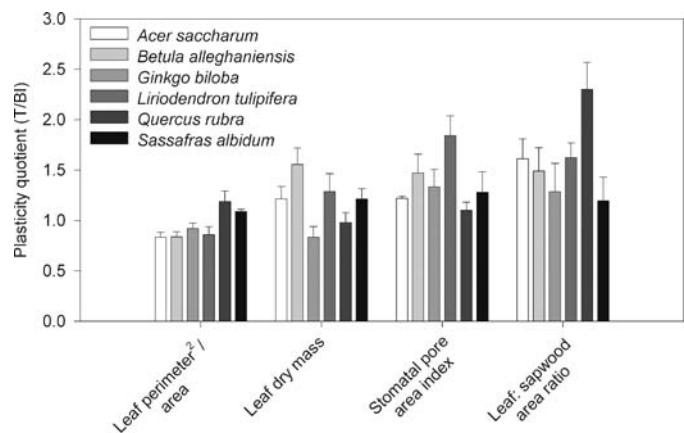

Fig. 3. Maximum intracanopy plasticity for leaf traits that had significantly different platisticities between the top and basal-interior positions for six temperate deciduous tree species ($P < 0.05$, one-way ANOVAs, $N = 5$ trees per species except $N = 4$ for stomatal pore area index for *B. alleghaniensis*). Plasticity was calculated as the quotient of the means for the top leaves (T) divided by the means for the basal-interior leaves (BI) for all traits but leaf area to sapwood area ratio, for which plasticity was calculated as the inverse.

for *L. tulipifera* and *S. albidum* (1.1 ± 0.09 and 0.9 ± 0.01, respectively). Species differed significantly in maximum intracanopy plasticity for leaf perimeter²/area (ranging from 0.83 ± 0.05 for *A. saccharum* to 1.2 ± 0.10 for *Q. rubra*; $P = 0.002$; Fig. 3), leaf dry mass (0.83 ± 0.10 for *G. biloba* to 1.6 ± 0.16 for *B. alleghaniensis*; $P = 0.014$), SPI_{gcl} (1.1 ± 0.08 for *Q. rubra* to 1.8 ± 0.20 for *L. tulipifera*; $P = 0.041$), and LA : SA (1.2 ± 0.24 for *S. albidum* to 2.3 ± 0.27 for *Q. rubra*; $P = 0.037$); quotients also differed significantly for SPI_{pl}, measured for *L. tulipifera* and *S. albidum* (1.6 ± 0.10 and 1.1 ± 0.11, respectively; $P = 0.010$).

Was this variation best linked with differences in leaf height or irradiance incident on the leaves? Differences between basal-interior and basal-exterior leaves were pronounced for every trait in which intracanopy plasticity occurred, indicating irradiance-linked effects separate from height-linked effects. Differences between basal-exterior and top leaves were of course linked with moderate differences in irradiance as well as height. The differences between basal-interior and basal-exterior leaves were typically stronger than the differences between basal-exterior and top leaves considering all species together (Tables 1–3, right columns). For leaf dry mass, perimeter²/area, stomatal density and SCA (SPI_{pl}, for the two species measured), significant differences were only found comparing basal-interior with basal-exterior leaves (for all species, mean differences were 24, 7, 14, 23, and 33%, respectively), while the basal-exterior did not differ significantly from the top leaves. For leaf area, LMA, and SPI_{gcl}, the difference between basal-interior and basal-exterior was empirically stronger than the difference between basal-exterior and top leaves; on average for all species, mean differences were, respectively, 30 vs. 20% for leaf area, 49 vs. 20% for LMA, and 24 vs. 10% for SPI_{gcl}. Only for LA : SA were the differences empirically the same, 18%. The same pattern was found for the majority of cases when considering species

individually for the nine traits with significant overall intracanopy plasticity. Of the 50 cases, differences were empirically stronger between the basal-interior and basal-exterior than between basal-exterior and top leaves for 35 cases (70%), the same for two cases (5%); and weaker for 13 cases (25%; (Tables 1–3).

The maximum likelihood analyses also indicated that intracanopy leaf plasticity was primarily linked with irradiance differences within the canopy (Table 4). One or more of the models—irradiance, height, and irradiance × height—fit the data with a statistically significant R^2 (from 0.13 for stomatal density to 0.65 for LMA; $N = 77$–79; $P < 0.001$) and little bias (slope of observed vs. expected values ≈1) for seven of the eight traits that had significant intracanopy plasticity (all except leaf dry mass, for which R^2 for the three models ranged from 0.001 to 0.05). For each of these seven traits, the AIC values for the irradiance model and the irradiance × height model were typically within 2 units of each other, and thus were both well supported by the data. By contrast, the height model fell far behind for five of seven traits. Only for stomatal density and LA : SA were all models equally effective. Thus, the irradiance model alone had approximately the same support or better than the other models (Table 4). Notably, when the irradiance × height model was fitted, the height-related parameters (parameters *b* and *c*) tended to have very large standard errors, and were thus not distinguishable from zero, confirming the redundancy of these parameters (Table 4).

Correlations across species between trait values, plasticity, and regeneration light requirement—The measured traits were typically uncorrelated across the six species for the canopy top leaves. Individual leaf area was positively correlated with individual leaf dry mass ($r_s = 0.94$; $r_p = 0.95$; $P < 0.05$), and two correlations were found between leaf- and shoot-level traits: individual leaf area was positively correlated with SCA ($r_s = 0.83$; $r_p = 0.87$; $P < 0.05$), and LMA was

Table 4. Maximum likelihood parameters for three models applied to the data for each trait.

Trait			Parameters							
Model	N	a	b	c	d	SD	R^2	Slope	AIC
Leaf area	79								
Irradiance model		12.8 ± 1.40	37.8 ± 5.06			16.0 ± 1.28	0.52	0.96	**668.9**
Height model		2.49 ± 0.413	57.0 ± 4.53			19.0 ± 1.51	0.32	0.95	692.9
Irradiance vs. Height model		6.57 ± 3.73	0.564 ± 0.426	-1.54 ± 1.79	53.1 ± 13.0	15.6 ± 1.24	0.54	0.96	**666.6**
Leaf mass per area	79								
Irradiance model		14.1 ± 1.17	33.7 ± 4.23			13.4 ± 1.06	0.65	0.98	**640.4**
Height model		2.61 ± 0.392	56.0 ± 4.30			18.0 ± 1.44	0.36	0.95	684.3
Irradiance $\times$ Height model		13.0 ± 3.15	-0.0518 ± 0.360	0.815 ± 1.51	31.7 ± 11.0	13.2 ± 1.05	0.66	0.98	**640.1**
Leaf perimeter²/area	79								
Irradiance model		-4.48 ± 1.06	119 ± 3.84			12.2 ± 0.967	0.18	0.99	**625.3**
Height model		-0.548 ± 0.285	109 ± 3.13			13.2 ± 1.05	0.04	0.98	634.5
Irradiance $\times$ Height model		-8.11 ± 2.88	0.376 ± 0.329	-1.24 ± 1.38	129 ± 10.1	12.0 ± 2.19	0.20	0.99	**625.5**
Stomatal density	77								
Irradiance model		5.06 ± 1.49	75.8 ± 5.40			17.0 ± 1.37	0.13	0.97	**660.9**
Height model		0.905 ± 0.383	84.1 ± 4.25			17.5 ± 1.41	0.07	0.96	**663.0**
Irradiance $\times$ Height model		4.01 ± 4.10	0.080 ± 0.465	-0.160 ± 1.95	77.9 ± 14.2	17.0 ± 1.37	0.13	0.97	663.0
Stomatal pore area index	77								
Irradiance model		6.90 ± 1.36	67.5 ± 4.91			15.4 ± 1.24	0.25	0.97	**646.4**
Height model		1.37 ± 0.359	77.4 ± 3.96			16.4 ± 1.32	0.16	0.97	652.1
Irradiance $\times$ Height model		8.75 ± 3.69	-0.988 ± 0.419	2.01 ± 1.75	56.1 ± 12.8	15.2 ± 1.23	0.27	0.97	**646.8**
Sapwood Cross-sectional area	79								
Irradiance model		7.08 ± 1.99	67.4 ± 7.17			22.7 ± 1.81	0.14	0.94	**723.9**
Height model		0.850 ± 0.522	83.0 ± 5.74			24.1 ± 192	0.03	0.94	730.0
Irradiance $\times$ Height model		5.48 ± 5.41	0.375 ± 0.617	-2.02 ± 2.59	78.4 ± 18.9	22.6 ± 1.80	0.15	0.94	**725.2**
Leaf : sapwood area ratio	79								
Irradiance model		8.32 ± 2.10	58.2 ± 7.58			24.0 ± 1.91	0.17	0.93	**732.6**
Height model		1.74 ± 0.54	69.4 ± 5.89			24.7 ± 1.97	0.12	0.92	**734.0**
Irradiance $\times$ Height model		1.94 ± 5.68	0.599 ± 0.649	-1.76 ± 2.72	74.6 ± 19.9	23.7 ± 1.89	0.18	0.93	**733.0**

Note: Irradiance model (TRAIT $= a \times \ln(\text{IRRADIANCE}) + b + \varepsilon$), height model (TRAIT $= a \times \text{HEIGHT} + b + \varepsilon$), irradiance $\times$ height model (TRAIT $= a \times \ln(\text{IRRADIANCE}) + b \times \ln(\text{IRRADIANCE}) \times \text{HEIGHT} + c \times \text{HEIGHT} + d + \varepsilon$), where the capitalized variables represent measured data; a, b, c and d fitted parameters; and the error ε is normally distributed with standard deviation SD. In analysis for each trait, the means for canopy locations for each tree were expressed as a percentage of the value for the top leaves to generate TRAIT values, with data for all species considered together (for leaf area and leaf tosapwood area ratio, values were inverted, so TRAIT values always increased with greater height and irradiance). The R^2 and slope are for values predicted by the model plotted against observed values; AIC = Akaike information criterion, corrected for low N (values lowest by more than 2, in boldface type, indicate best-supported models).

negatively correlated with *LA : SA* ($r_s = -0.94$; $r_p = -0.95$; $P < 0.05$). For the other 33/36 pairwise trait comparisons, r_s or r_p was not significant at $P < 0.05$.

In general, across the six species, the 11 traits varied independently in their intracanopy plasticity. The maximum plasticity quotients for given traits were correlated only for stomatal density and SPI_{gcl} ($r_s = 0.88$; $r_p = 0.89$; $P < 0.05$). For the other 35/36 pairwise relationships among trait plasticity quotients, r_s or r_p was not significant at $P < 0.05$. Thus, high intracanopy plasticity in a given trait does not predict high plasticity in another trait for that species; species shifted ranking in their intracanopy plasticity quotients for the different traits (Figs. 2 and 3).

We tested each of the traits having significant intracanopy plasticity for a correlation between plasticity and gradients in irradiance and height across the crown across the six species. In no case was such a linkage found (r_s or r_p not significant at $P < 0.05$). Further, the absolute trait values for top leaves and the intracanopy plasticity of given traits were uncorrelated with the

six species' regeneration light requirements (r_s or r_p not significant at $P < 0.05$).

DISCUSSION

Substantial intracanopy plasticity was observed for nine of the 11 traits studied, indicating the potential for major impacts on whole plant function. Our systematic sampling across canopies supported the qualitative trends highlighted by the classic sun–shade leaf dichotomy: basal-exterior and canopy top leaves were smaller than basal-interior leaves and had higher LMA and higher stomatal densities (see references for Introduction). Our findings also included novel differences; sun shoots tended to have larger individual leaf mass values, higher stomatal pore area per leaf area, and higher SCA, but lower LA : SA. However, guard cell and stomatal pore lengths did not vary across the canopy, and the direction of intracanopy plasticity in leaf perimeter²/area differed across species. Our findings thus did not support the occasional observations in the literature of species with sun leaves larger than shade leaves or upper-canopy leaves with lower LMA than lower-canopy leaves (Wylie, 1949; Talbert and Holch, 1957; Niinemets and

Kull, 1994; Richardson et al., 2000). It is possible that in those previous studies the particular species, conditions, or sampling protocols led to findings that did not represent the general patterns of intracanopy plasticity shown here for the six species. Our findings are consistent with the observation of an invariant stomatal density with leaf insertion height in small trees reported for *Eucalyptus crenulata* and *Quercus robur* (Carr, 2000). In that study only exposed leaves were apparently sampled. In our study stomatal density was invariant with insertion height per se, but plastic throughout the canopy according to differences in leaf exposure.

Strikingly, intracanopy plasticity was conservative across species for several traits. The finding of no interaction between canopy position and species for several traits suggests that intracanopy plasticity was similarly constrained across species. This likeness in the direction and degree of plasticity occurred despite strong differences across species in the magnitude of gradients in height and irradiance across the crowns; indeed, across the six species the plasticity of each trait was unrelated to the magnitude of these gradients. The maximum intracanopy plasticity in all traits (from basal-interior to canopy top) was similar and statistically indistinguishable across species for several traits, ranging from nonplastic for the lengths of guard cells and stomatal pores to 1.1–1.5 on average for stomatal density, 1.3–1.7 for SCA, 1.6–2.1 for leaf area, and 1.5–2.2 for LMA. Intracanopy plasticity in leaf shape differed significantly across species; the intracanopy plasticty in leaf perimeter^2/area ranged from 0.8 to 1.2. Pinnately lobed *Q. rubra* and *S. albidum* were more lobed at exposed locations, while palmately lobed *A. saccharum* and *L. tulipifera* tended to be more lobed in the shaded part of the crown. Species also differed significantly in the intracanopy plasticity of leaf mass (0.8–1.6), LA : SA (1.2–2.3), and SPI$_{gcl}$ (1.1–1.8); whether these differences scale up to species differences in whole canopy performance requires further study. Our findings are consistent with previous reports of invariance in guard cell length (Bongers and Popma, 1988; Carr, 2000; Richardson et al., 2000, 2001) and of a maximum intracanopy plasticity in LMA of ≈1.5–2 times as reported for *Populus tremula* and *Tilia cordata* (Niinemets et al., 2004) and for three species of conifers (Bond et al., 1999). The observed boundedness of intracanopy plasticity for all the traits, up to ≈2 times the maximum, implies a general convergence across species due to general optimization and/or to similar developmental constraints operating during primordia formation and leaf expansion, as discussed in the following section.

We expect that for trees growing in forests the extent of intracanopy plasticity may be somewhat larger given the deeper shade cast on basal leaves from surrounding trees. The additional plasticity is likely to be limited, however; in previous studies, forest-grown trees up to ≈30 m had a plasticity in individual leaf area and LMA of 2–3 times (Sack et al., 2003a; Aranda et al., 2004; Niinemets et al., 2004). Further, even the limited plasticity in our study was high in relation to that reported for evergreen broadleaved trees from Mediterranean forests and tropical rainforest for which intracanopy plasticity was on average 1.5 or lower in leaf area and LMA and 1.1 in stomatal density (Bongers and Popma, 1988; Sack et al., 2003a). Notably, in those studies the full intracanopy plasticity

may not have been sampled because top leaves were not accessed. Species with long-lived leaves may typically have lower intracanopy plasticity than temperate deciduous species because their leaves are subject to longer-term dynamics of canopy growth and disturbance. For instance, leaves may expand in sun and during their expansion become deeply shaded (Kitajima et al., 2005); conversely, leaves may expand in shade and then be exposed to sun by branch breakage.

The plasticities of given traits were typically uncorrelated across the six species, indicating independent control of the plasticity of individual traits. This complexity contrasts with what is known of plastic responses for seedlings across different environments; fast-growing species tend to be more plastic for most or all leaf morphological traits (e.g., Valladares et al., 2000; Grime, 2001). We note that this difference may be due to the expressed plasticity in many seedling traits arising from ontogenetic trajectories (e.g., mean LMA increases as seedlings grow; Sack et al., 2003a) as well as size-independent environmental plasticity. That difference would also explain why plasticity in leaf traits for seedlings grown across low and high irradiances can be many-fold higher than found here for canopies.

Intracanopy plasticity in the trees in this study was linked primarily with leaf exposure. The plasticity may be linked with the gradient across the canopy in temperature, vapor pressure deficit, and wind speed, all of which would be lower in the canopy interior (Hanson, 1917); however, in previous work, leaf plasticity correlated best with irradiance quantity and quality (Holbrook and Lund, 1995; Frak et al., 2002; Niinemets et al., 2004). The smaller differences between basal-exterior and canopy top leaves relative to basal-interior and basal-exterior leaves would be accounted for by the smaller difference in irradiance and also by less sensitivity at the higher range of irradiances (as found for seedlings; Montgomery, 2004). Our findings do not support the idea that plasticity in leaf form for these trees is primarily determined by hydraulic effects linked with height differences, whether mediated by gravity or increased path length. Notably, in tall or very tall trees, hydraulic constraints on leaf form may be much more consequential (Marshall and Monserud, 2003; Koch et al., 2004; Woodruff et al., 2004), even driving up to 5-fold variation in LMA from base to top of 112-m giant redwoods (Koch et al., 2004). We hypothesize that the bounded, ≈2 times plasticity observed in this study is the typical maximum plasticity associated with optimization to intracanopy variation in irradiance for trees of 13–18 m, and possibly up to ≈20–30 m height, while taller trees will develop a stronger plasticity, potentially linked with much deeper shading of the basal-interior leaves, with height effects driving a passive response in developing upper leaves, and with ontogenetic effects. Intracanopy leaf plasticity was reported to increase between *Tsuga heterophylla* stands of 55 and 145 years of age (Richardson et al., 2001).

We note that the irradiance-driven plasticity in this study may arise from both internal cues and environmental responses. For temperate deciduous trees, the induction of intracanopy differences between shaded and exposed parts of the canopy has begun by the time the leaf is in the bud (Lichtenthaler, 1985; Uemura et al., 2000), and leaves are even more sensitive to irradiance quantity and quality experienced during expansion; LMA and pigment concentrations correlate with irradiance during leaf expansion, whether natural or manipulated (Uemura et al., 2000; Frak et al., 2002; Niinemets et al.,

2004). The exposed leaves may also be directly influenced ("stunted") by the irradiance and/or by the associated higher temperature and vapor pressure deficits, which might reduce leaf water status (Zwieniecki et al., 2004). Notably, leaf form may be optimized at not only the local but also the whole canopy scale; developing leaves receive signals from elsewhere in the plant. For instance, the irradiance incident on other leaves in the crown can influence stomatal density in expanding leaves (Lake et al., 2001; Miyazawa et al., 2006). The developmental mechanisms for intracanopy leaf plasticity are thus potentially layered and complex and invite further research.

Whatever the developmental mechanism, intracanopy leaf plasticity can confer functional benefits. There are at least three hypotheses for why sun and shade leaves would be optimal in their respective irradiances. The classic explanation is related to light harvesting and carbon balance (Horn, 1971; Ellsworth and Reich, 1993; Holbrook and Lund, 1995; Smith et al., 1997; Meir et al., 2002; Niinemets et al., 2004; Hirose, 2005). A shade leaf, which spreads its mass more thinly over a wider area (i.e., a larger leaf with lower LMA), captures more diffuse irradiance. Shade leaves are typically also rich in spongy mesophyll, that scatters irradiance internally, and given their lower LMA and biochemical adjustments, they favor light harvesting over Calvin cycle reactions (e.g., via higher chlorophyll concentration per mass, higher chlorophyll to N and lower chlorophyll a to b ratios). Thus, shade leaves have higher net CO_2 assimilation rates at low irradiance as well as lower construction and maintenance costs due to their lower dark respiration rates and lower investment in many aspects of leaf composition: tissue density, N concentration, xylem conduit size, minor vein density, hydraulic conductance, and mechanical support tissue per given leaf area (due to having larger leaves; Wylie, 1949, 1951; Carpenter and Smith, 1981; Hoflacher and Bauer, 1982; Lichtenthaler, 1985; Givnish, 1987; Frak et al., 2002; Larcher, 2003; Sack et al., 2003b; Niinemets and Sack, 2006). Conversely, sun leaves have higher stomatal densities and stomatal pore areas to maximize CO_2 absorption rates and are thicker, with greater internal surface area, higher LMA, and higher N per area. In higher irradiance these leaves repay the higher construction cost for their thicker cell walls, the higher investment in N and vascular tissue, and their higher respiration rates. An additional advantage of smaller, lobed sun leaves is that they may allow more light to penetrate inside the crown, given that they are spaced widely enough apart to avoid local self-shading, and thus they would improve whole-canopy light capture (Horn, 1971; Cardelus and Chazdon, 2005; Pearcy et al., 2005).

A second hypothesis holds that sun leaf traits assist with heat dissipation by minimizing boundary layer conductance. Smaller and/or more deeply lobed sun leaves give rise to a thinner boundary layer, which may assist with heat convection (Vogel, 1968). These leaves would also have a higher stomatal density for cooling.

A third hypothesis is that sun and shade leaves are structurally divergent for optimal water relations. According to this idea, sun leaves require a greater hydraulic supply for their higher transpiration rates. A lower LA : SA, consistent with a higher leaf-specific branch conductivity, is achieved by developing higher SCA and by expanding a smaller leaf surface for a given vasculature (cf. Cochard et al., 1999, 2005; Lemoine et al., 2002). Further, to minimize transpirational demand, sun leaves would also have a lower surface area to volume ratio associated with their greater thickness and LMA.

The intracanopy plasticity in this study may thus confer all these advantages in each leaf's individual environment, contributing to the simultaneous optimization of light capture, carbon assimilation, energy balance, and water relations. To determine the precise advantages of intracanopy plasticity at the leaf and whole canopy levels require further research, as does the question of why intracanopy leaf plasticity is so limited, in this study up to ≈ 2 times. Constrained plasticity may indicate 'bet-hedging' in a relatively unpredictable environment (cf. Wong and Ackerly, 2005). For example, if a shade leaf were to become too large or have a too-low LMA, a sudden increase in exposure might endanger the leaf. We note that plasticity of the order found here can have strong benefits for canopy resource use. In mathematical models, a plasticity of 2 times in LMA alone corresponded with a 5–20% increase in canopy photosynthesis per area relative to invariant canopies, as well as a major increase in water use efficiency and a greater shading of the understory (Meister et al., 1987; Gutschick and Wiegel, 1988; Bond et al., 1999).

Intracanopy plasticity may also play a wider ecological role. Shade leaves may function in casting shade to exclude understory plants, thus reducing competition for soil resources. This benefit has been hypothesized to be strongest for canopy species that are shade-tolerant as juveniles; these species would filter out invading light-demanding species beneath their crowns. Canopy species that are shade-tolerant as juveniles have thus been hypothesized to develop greater intracanopy plasticity in leaf area and LMA (Grubb, 1998). However, in this study, plasticity was conserved across the six species and did not correlate with a regeneration light requirement. We recognize that our index of shade tolerance is coarse; a more comprehensive test would seek correlation between intracanopy tree leaf plasticity and several components of shade tolerance, including the abilities of juveniles to establish, persist for years, and/or grow to later ontogenetic stages in deep shade (Coomes and Grubb, 2000; Poorter et al., 2005). Further work is needed on other species sets. Our finding that intracanopy plasticity was not linked with seedling light requirement for these six diverse species is the first evidence that the two are not intrinsically linked in a general way.

Intracanopy leaf plasticity in many traits is general and substantial, and its complete elucidation will clarify many aspects of tree biology. Our study found that in addition to the substantial plasticity observed across extreme canopy locations there was also strong variation in leaf traits even within canopy locations, which may partly arise from heterophylly, and also may represent acclimation to smaller-scale gradients in microclimate within shoots. In temperate deciduous trees the leaf-to-leaf variation develops each season via adaptive adjustment to speculated future conditions, bounded by developmental constraints. This process involves the plant integrating several forms of perceived and inherent information for problem-solving, and thus meets the criteria for plant intelligence (Trewavas, 2005). Indeed, intracanopy plasticity may be the most accessible developmental model for new studies of this topic. Finally, we note that across temperate deciduous tree species, intracanopy plasticity can drive as much variation as species differences for given traits (e.g., LMA). Thus, accounting for intracanopy plasticity in sampling design will increase resolution in comparative studies of tree leaves, such as those determining differences in leaf function

across vegetation types (Wright et al., 2004) or those extrapolating past climates from fossil leaves based on the leaf properties of extant species (Royer et al., 2005).

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