Seedling Leaf Structure of New England Maples (*Acer*) in Relation to Light Environment

P. Mark S. Ashton, Hae Soon Yoon, Rajesh Thadani, and Graeme P. Berlyn

ABSTRACT. Seedling leaves of the genus *Acer* from southern New England were compared in relation to light. The species investigated were red maple (*A. rubrum* L.), a species tolerant of xeric and hydric sites; silver maple (*A. saccharinum* L.), a species restricted to riparian sites that are periodically flooded; and sugar maple (*A. saccharum* Marsh.), a mesic species of lower slopes and valleys. Germinating seedlings of all species were collected and grown within four shade treatments that had contrasting light quantity and quality: (1) approximately 100% of full sunlight, red:far-red ratio = 1.27; (2) 40% of full sunlight, ratio = 0.97; (3) 15% of full sunlight, ratio = 0.85; and (4) 4% of full sunlight, ratio = 0.46. Leaves, cuticles, and epidermal and palisade mesophyll cell layers were all thicker, and stomatal densities were higher for all three species in the full sun treatment. Dimensions of leaf structure (leaf thickness, palisade mesophyll thickness, lower epidermal thickness) were between 25 and 35% smaller for silver maple as compared to the other maples. Silver maple also allocated less biomass to roots (about 15% less) and more to stems. Its thin upper surface cuticle, thin leaves, and large leaf area predispose this species to desiccation. Phenotypic plasticity of leaf anatomical measures was greatest for red maple, suggesting it to be more of a generalist than its congeners. Red maple allocated greater biomass to roots in shade (17% and 27% more than sugar and silver maple respectively), with thicker leaves and cuticle, making it least prone to desiccation. Sugar maple had greater dry mass and total leaf area in the deepest shade than the other maples. Measures of leaf structure can provide useful insights into known ecological affinities of site and shade-tolerance among maples. For. Sci. 45(4):512–519.

Additional Key Words: *Acer rubrum*, *A. saccharinum*, *A. saccharum*, leaf anatomy, red:far red ratio.

The manipulation of a forest canopy for the purpose of altering light availability is an important silvicultural technique for favoring or excluding trees of a particular shade tolerance. Many studies have sought to differentiate light responses of temperate tree species in order to arrange them into different successional groups for the purpose of silviculture (Jackson 1967a, 1967b, Loach 1967, 1970, Carpenter and Smith 1975, Bazzaz 1979, Bazzaz and Carlson 1982, Walters and Reich 1996, Reich et al. 1998).

Researchers have focused on the regeneration stage of succession, because it is a period that determines future tree species composition for most forest types (Egler 1954, Grubb 1977, Ashton 1992). Therefore, this stage provides a critical window of time for understanding differences among species in anatomy, physiology, and ecology (Ashton and Berlyn 1992, 1994). By using more refined field and greenhouse studies, the regeneration stage can be used to examine differences in shade tolerance of closely related species.

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For maples (*Acer* spp.), field studies have focused on comparisons between canopy and understory species in relation to forest microenvironments (Lei and Lechowicz 1990, Sipe and Bazzaz 1994, 1995). Lei and Lechowicz (1990) studied saplings of co-occurring maple species, in a mesic northern hardwood forest in Quebec, Canada. The understory species in their study, striped maple (*A. pennsylvanicum* L.) and mountain maple (*A. spicatum* Lam.), had characteristics (large leaf area, planar architecture) that made them more shade-adapted than sugar maple (*A. saccharum* Marsh.). Among eight maple species from Asia and North America, Lei and Lechowicz (1997, 1998) found differences in photosynthesis and water-use between canopy and understory species and between those species known to be light-demanding versus those that are shade-tolerant. Similarly, Sipe and Bazzaz (1994, 1995) showed *A. rubrum* L. (red maple) survived in a wider range of light environments as compared to sugar and striped maple. All these studies have provided new insight into our understanding of linkages between the ecology and physiology of trees.

The objective of our study was to build on this work by examining relationships between attributes of leaf structure and the ecological site affinities of three maple species. Certain attributes of leaf structure (i.e., cuticle thickness, palisade mesophyll layer thickness, stomatal density) may provide insight into some of the underlying mechanisms affecting the shade tolerance and site affinities among red, sugar, and silver maple (*A. saccharinum*, L.). We hypothesize that species rankings of leaf structure attributes will change across the light treatments in a direction consistent with the known site affinity of the species. Based on the literature, we would also expect maple species that have been recorded to be more site generalist (i.e., red maple) to exhibit greater plasticity in leaf structure between the brightest and darkest shade treatments as compared to maple species that are considered site specialists (i.e., sugar maple).

The species selected for our study all occur within the mixed-deciduous forest of southern New England, but each species appears restricted more or less to different site conditions (Burns and Honkala 1990). Red maple is a light generalist that grows well in shade and sun. Red maple is also tolerant of shallow soils of uplands that are seasonally xeric, and also hydric soils that are seasonally anaerobic, although ecotypic differences in physiology and growth appear not to exist (Will et al. 1995). Sugar maple is a shade-tolerant species that occupies the lower toe-slopes with deep soils that are mesic year round. The third species, silver maple, is considered light-demanding and is restricted to flooded alluvial soils that are adjacent to moving waters. It can be planted and grown on well-drained sites, but seldom, if ever, reproduces there (Gabriel 1990).

Materials and Methods

Shade Treatments

Eight shelters were erected (1 × 1 × 2.5 m wooden frames) and placed on benches within a greenhouse at Yale University. We altered light quality (R:FR ratio) and photosynthetically active radiation (PAR, 400–700 nm) to create four treatments (two shelters per treatment) that simulated a range of forest light environments. The R:FR ratio was measured as the ratio of quantum irradiance between the wavebands 655–665 nm and 725–735 nm (Lee 1985, Lee et al. 1996). Treatments were based on measurements made across forest openings on ridge and valley sites at the Yale-Myers Forest in the northeastern uplands of Connecticut (41°57' N; 72°07' W) (Ashton and Larson 1996). This forest type is dominated by an oak-hickory canopy, with a subcanopy of red maple and sugar maple (Westveld et al. 1956). Compared with a mature northern hardwood forest, where maple species often dominate the canopy, understory R:FR ratios of the oak-hickory forest type are considerably higher (R:FR ratio of 0.47 versus 0.25), because of more open conditions with proportionately fewer stories of shade-tolerant trees below the forest canopy (Canham et al. 1990).

The quality and amount of light for three of the treatments was altered by spraying a ratio of selected paint pigments mixed in a varnish base onto clear plastic (Lee 1985, Ashton and Berlyn 1994). Quantum sensors and thermocouples (LI-190 SZ, 190 SA, LI-1000-16, LI-COR, Lincoln, Nebraska) were used to obtain an estimate of daily PAR and diurnal temperature variation. Measurements were made every 10 s on several sunny days at the beginning of the experiment (June 1994). Light and temperature sensors were positioned horizontally 15 cm above the greenhouse bench and within the shelters. Ten minute means for each shelter were calculated based on the 10 s readings. These means were simultaneously recorded from sunrise to sunset (approximately 15 hr) using a data logger (LiCor 1000).

Shade treatments provided seedlings with 4%, 15%, 40%, and 100% of sunlight, based on daily estimated totals of PAR received (see Table 1 for details). It should be noted that the 100% treatment is less than incident PAR because of some reflection from the greenhouse.

Germinating seed of red maple were collected during the last 2 wk of May 1994 from at least four different parent trees in separate locations within the Yale-Myers Forest, Union, Connecticut. All seedlings collected were at the earliest stages of cotyledon expansion, and none exhibited any observable leaf initiation. Germinating seedlings of silver maple of a similar developmental stage were collected at four separate locations along the banks of the Quinipiac river in

Table 1. Light treatments; PAR—photosynthetic active radiation as a measure of amount of light; R:FR—red to far red ratio of light as a measure of light quality.

	R:FR ratio	PAR (mols m^{-2} d^{-1})	Maximum PAR (μmols m^{-2} s^{-1})	Maximum temp (°C)
Full sun (100%)	1.27	36.62	1,600	32
Diffuse shade (40% sunlight)	0.97	14.65	700	30
Bright understory (15% sunlight)	0.85	5.31	300	30
Dark understory (4% sunlight)	0.46	1.50	60	29

Hamden, Connecticut, during the last week of May 1994. Sugar maple seeds that had been dispersed the previous fall were collected in the same manner as red maple at the Yale-Myers forest during the second week of May 1994. Each young germinant was immediately planted into a plastic pot (15 cm depth, 10 cm diameter) that contained Promix (a mixture of sphagnum moss, perlite, vermiculite, dolomite and calcite limestone, and wetting agent, Premier Brands, Inc., Quebec, Canada). A small amount of forest topsoil from the seedling collection sites was added to each pot at the beginning of the experiment to ensure a source of vesicular-arbuscular mycorrhizae (VAM) innoculum for the seedling roots. Five pots for each species were randomly assigned to positions within a shelter. All germinating seed was placed within the shelters before shoot extension to ensure proper exposure to the treatments during leaf expansion and development. To ensure against possible environmental variations in different parts of the greenhouse, the position of each shelter was rotated monthly and watering was regulated to maintain the Promix at field capacity. A standard strength of Miracle-Gro, an NPK fertilizer (15-30-15), was added at monthly intervals.

The seedlings were grown within these light treatments for at least four months (June 1–September 30, 1994). At the end of this period, all seedlings were measured for height, root collar diameter, and leaf area. Seedlings were then harvested and roots washed free of soil, then roots, stem, and leaves were separated and dried for 48 hr at 80°C for determination of dry weights. Leaf dry weights included both lamina and petiole.

Anatomy Measurements

For each species, a single leaf from five separate seedlings were randomly chosen within shelters for each shade treatment. Only undamaged, fully expanded leaves were selected.

To determine stomata density, stomata aperture length, and epidermal cell density leaf sections (1 × 1 cm) were taken from the sample leaf in the middle portion of the lamina. Each section was incubated in a 50°C oven in 5% sodium hydroxide to clear leaf pigments. Sections were then stained with 1–2 drops of 0.5% aqueous toluidine blue solution and mounted in Karo light corn syrup on a viewing slide. For each section, the total number of stomata and epidermal cells were counted and three stoma aperture lengths were measured for five fields of view on the abaxial side of the leaf. No stomata were observed on the adaxial leaf surfaces.

For cross-sections, another 1 × 0.5 cm piece was taken across the midrib, adjacent to the section used for stomata and epidermal measurement. This section was cut into three thin strips of about 1 × 0.2 cm and immediately fixed in cold FAA (formalin: acetic acid: alcohol). The strips were dehydrated in a tertiary butyl alcohol series and then embedded in separate wax blocks (Berlyn and Miksche 1976). Cross-sections were cut of each strip at 12 μm with a cryotome and mounted on a slide. The tissue was then stained with safranin and fast green (Berlyn and Miksche 1976). For each of the three slides, three measurements were made of leaf thickness, cuticle thickness of the upper leaf surface, upper and lower epidermal cell thickness, and spongy and palisade cell layer thickness. Each slide was measured in different positions that avoided the midrib region. Three leaf sections were measured for each single leaf selected from each of five seedlings per species and shade treatment. Measurements of cell dimensions were made using a 12.5 × filar micrometer eye-piece and suitable objectives for the various measurements.

Statistics

We investigated differences in leaf anatomy using an analysis of variance (SAS 1990) for a split-plot experimental design where shade treatments (two replicates) were the main plots and tree species were subplots. For each measure, we tested for main differences among shade treatments; subplot differences among species; and for interactions among species and shade treatments (Table 2). Where shade treatments were pooled, differences among species were analyzed at the 5% significance level using Fisher's PLSD post hoc test. Response curve analysis using orthogonal polynomial contrasts were carried out for each species across the shade treatments (4%, 15%, 40%, and 100% of full sunlight). Contrasts compared relationships between the various leaf structure measurements and light levels using both linear and quadratic constraints (Table 2). We used both linear and quadratic constraints to examine whether species exhibited different contrast relationships.

For a measure of the variation in anatomical attributes we used an index of phenotypic plasticity ($P = [1-(x/X)]$) that included comparisons between mean values for the darkest (x) and brightest (X) shade treatments. To obtain a relative comparison among species of the amount of stomata area per unit area of leaf we used the product of stomata aperture

Table 2. F-values for analyses of variance of various anatomical and growth measures using a split-plot design where shade treatments were the main treatments and the subplots were species. Variable codes are: LT—leaf thickness; CT—cuticle thickness; UE—upper epidermis; PM—palisade mesophyll; LE—lower epidermis; EP—upper epidermal density; SF—stomatal frequency; DW—dry weight; HT—height; RC—root collar diameter; SLA—specific leaf area; TLA—total leaf area; LMR—leaf mass ratio; SMR—stem mass ratio; RMR—root mass ratio. Levels of significance: $P < 0.05$, *; $P < 0.01$, **; $P < 0.001$, *.**

	df	LT	CT	UE	PM	LE	EP	SF	DW	HT	RC	SLA	TLA	LMR	SMR	RMR
Shade	3	10.1**	3.6*	6.5*	19.3***	4.8*	1.4ns	2.4ns	62.5***	75.1***	111.4***	4.2*	60.1***	1.6ns	1.3ns	2.0ns
Residual	4															
Species	2	23.8***	2.4ns	26.4***	30.8***	11.3**	5.74*	2.35ns	26.5***	83.8***	46.4***	3.4ns	15.6**	2.2ns	2.4ns	3.5*
Shade × species	6	0.9ns	0.9ns	0.9ns	1.9ns	0.9ns	0.6ns	1.1ns	5.8ns	10.2**	13.8***	3.1ns	4.5ns	0.9ns	1.0ns	1.0ns
Subplot residual	8															
Error	23															

length and stomata density, and called it stomata area index (SAI) (Ashton and Berlyn 1994). We also used Salisbury's stomatal index which is the ratio of guard cell number to total epidermal cell number (excluding guard cells) to better understand how stomata density is influenced by light during leaf development (Salisbury 1928).

Results

Leaf Anatomy

Analyses showed that differences among leaf samples taken from the same species and within the same light treatment were not significantly different from each other. Comparisons among species, and among light treatments, all showed highly significant F values ($P < 0.01$) (Table 2). Interestingly, interactions between species and light treatment were not significant, suggesting that species did not change rank in relation to each other across light levels for any of the anatomical measures.

Leaf thickness and upper epidermal, palisade mesophyll and lower epidermal layers were greatest for red maple. Cuticle thickness was an exception where red and sugar maple had the same thicknesses. Thicknesses for almost all attributes measured, except for the upper epidermal layer thickness, were least for silver maple (Table 3).

Phenotypic plasticity (P) of leaf blade, and upper and lower epidermal layer thicknesses, was greater for red maple as compared to the other maples (Table 3). Both red and sugar maple, however, had greater plasticities of cuticle and palisade mesophyll thicknesses than silver maple.

For all species, leaf thickness exhibited effects in contrast that were significant with increase in light levels. Contrasts showed approximately monotonic upward sloping relationships as leaf thickness increased with light

Table 3. F-values for response curve analyses comparing measures of the various leaf attributes across the four light treatments using linear and quadratic contrasts (levels of significance $P < 0.05$, *; $P < 0.01$). Means are given with standard errors in parentheses for the various leaf anatomical attributes for each light treatment and for all treatments combined. Fisher's PLSD test ($P < 0.05$) was used to compare pooled means of each species [letters denote differences among species ($A > B > C$)]. P = Plasticity [1 (x/X)] where x is the value in 4% sunlight and X is the value in 100% sunlight.**

	Contrasts		Comparisons among species (means over	Comparisons among treatments for each species				
	Linear	Quadratic	all treatments)	4%	15%	40%	100%	P
Leaf thickness (μm)								
A. rubrum	15.49**	26.12**	93.36 (2.68)A	77.39 (3.20)	84.98 (2.82)	98.83 (2.57)	108.92 (5.14)	0.29 (0.03)
A. saccharinum	25.47**	10.07*	67.83 (1.83)C	59.19 (2.48)	62.74 (2.89)	73.89 (2.79)	72.61 (3.72)	0.20 (0.02)
A. saccharum	8.56**	4.13ns	81.90 (2.88)B	71.79 (5.35)	71.81 (3.46)	87.63 (4.53)	90.76 (6.11)	0.20 (0.03)
Cuticle thickness (μm)								
A. rubrum	7.19*	62.49**	2.67 (0.09)A	2.56 (0.14)	2.33 (0.44)	2.63 (0.46)	3.10 (0.22)	0.24 (0.02)
A. saccharinum	1.71ns	0.34ns	2.41 (0.06)B	2.29 (0.06)	2.37 (0.14)	2.52 (0.13)	2.42 (0.17)	0.09 (0.01)
A. saccharum	0.31ns	5.77*	2.61 (0.12)AB	2.16 (0.10)	2.59 (0.13)	2.51 (0.24)	2.96 (0.30)	0.27 (0.03)
Upper epidermal layer thickness (μm)								
A. rubrum	16.14**	17.14**	15.33 (0.49)A	12.87 (0.74)	14.60 (0.98)	16.34 (0.69)	17.04 (0.99)	0.24 (0.05)
A. saccharinum	3.95*	0.95ns	11.77 (0.36)B	10.64 (0.95)	11.13 (0.62)	12.89 (0.70)	12.04 (0.61)	0.16 (0.02)
A. saccharum	1.10ns	0.10ns	11.71 (0.32)B	11.12 (0.29)	11.31 (0.45)	12.27 (0.70)	11.85 (0.73)	0.09 (0.02)
Palisade mesophyll layer thickness (μm)								
A. rubrum	11.76**	17.52**	40.65 (1.62)A	29.99 (1.34)	37.66 (1.31)	44.49 (2.04)	48.56 (2.04)	0.38 (0.06)
A. saccharinum	13.47**	6.72*	27.58 (1.02)C	22.27 (1.67)	24.87 (1.04)	30.53 (1.49)	30.88 (2.25)	0.27 (0.03)
A. saccharum	4.83ns	2.88ns	32.78 (1.85)B	25.64 (2.70)	25.17 (1.97)	36.58 (2.18)	39.70 (4.09)	0.37 (0.03)
Lower epidermal layer thickness (μm)								
A. rubrum	0.00ns	3.64ns	11.91 (0.40)A	12.16 (0.83)	10.30 (0.56)	11.41 (0.69)	13.69 (0.76)	0.25 (0.03)
A. saccharinum	11.30**	10.87**	9.63 (0.30)C	8.86 (0.34)	8.81 (0.40)	10.11 (0.80)	10.50 (0.49)	0.15 (0.02)
A. saccharum	0.04ns	0.00ns	10.25 (0.24)B	10.61 (0.42)	9.47 (0.40)	10.26 (0.43)	10.72 (0.54)	0.11 (0.03)
Epidermal cell density (number/mm^2)								
A. rubrum	0.47ns	0.28ns	3,092 (105)B	3,168 (72)	3,188 (131)	2,685 (114)	3,326 (103)	0.19 (0.03)
A. saccharinum	2.59ns	0.25ns	4,226 (101)A	3,996 (93)	4,628 (97)	4,025 (150)	4,254 (64)	0.13 (0.03)
A. saccharum	1.14ns	6.58*	3,999 (165)A	4,158 (148)	3,484 (175)	3,576 (146)	4,781 (192)	0.27 (0.04)
Stomata density (number/mm^2)								
A. rubrum	1.07ns	33.09***	460 (11.9)B	385 (11.6)	471 (10.0)	439 (13.0)	545 (13.1)	0.29 (0.03)
A. saccharinum	0.12ns	0.05ns	516 (12.3)A	420 (6.1)	573 (13.3)	576 (16.4)	498 (12.4)	0.26 (0.03)
A. saccharum	0.00ns	2.20ns	406 (16.4)C	378 (17.6)	339 (9.3)	364 (7.9)	543 (32.0)	0.38 (0.05)
Stoma aperture length (μm)								
A. rubrum	0.45ns	0.89ns	13.13 (0.23)A	12.5 (0.16)	12.5 (0.25)	13.0 (0.22)	14.4 (0.30)	0.12 (0.02)
A. saccharinum	0.00ns	1.13ns	9.30 (0.17)B	9.2 (0.09)	10.6 (0.22)	9.7 (0.26)	7.7 (0.11)	0.27 (0.03)
A. saccharum	3.02ns	3.63ns	10.13 (0.27)B	9.9 (0.12)	10.4 (0.27)	12.2 (0.44)	8.0 (0.26)	0.35 (0.04)
Stomata area index (number/mm^2 * aperture length)								
A. rubrum	1.91ns	18.83*	6,040 (131)A	4,812 (103)	5,887 (121)	5,707 (164)	7,848 (176)	0.39 (0.05)
A. saccharinum	2.24ns	0.76ns	4,799 (101)B	3,864 (44)	6,074 (130)	5,587 (153)	3,835 (75)	0.38 (0.06)
A. saccharum	0.49ns	0.07ns	4,113 (138)C	3,742 (108)	3,526 (100)	4,441 (128)	4,344 (198)	0.21 (0.06)
Stomatal index (guard cell number/epidermal cell number)								
A. rubrum	0.25ns	0.35ns	0.297 (0.030)A	0.243 (0.026)	0.295 (0.031)	0.327 (0.036)	0.328 (0.027)	0.26 (0.03)
A. saccharinum	5.52ns	0.01ns	0.244 (0.023)AB	0.210 (0.018)	0.276 (0.022)	0.286 (0.032)	0.234 (0.012)	0.27 (0.04)
A. saccharum	0.24ns	0.09ns	0.203 (0.034)B	0.182 (0.041)	0.195 (0.038)	0.203 (0.031)	0.227 (0.049)	0.20 (0.03)

level, with linear constraints showing levels of significance for all species, and quadratic constraints showing significance for red and silver maples only (Table 3).

Increases in cuticle thickness showed significant effects in quadratic contrast with an increase in light levels for red and sugar maple but not for silver maple. Linear contrast effects were only significant across light levels for red maple cuticle thickness. Upper epidermal layer thickness also increased with an increase in light levels but this contrast was only significant for red maple and to a lesser degree silver maple (linear contrast). Increases in palisade thickness showed significant contrast effects with increase in light levels for both red and silver maple. Only silver maple showed significant contrast effects for lower epidermis.

With the exception of the quadratic contrast for red maple, no significant effects were shown between stomata aperture length or stomata density and increasing light levels (Table 3). Stomata density was greatest for all treatments in silver maple, followed in declining order by red maple and sugar maple (Table 3). Stomata aperture length was greatest, but plasticity was lowest for all treatments in red maple as compared to silver and sugar maple. The smallest aperture lengths for silver and sugar maple were in the 100% treatment. However, red maple had the largest stomata aperture length at this light level. Sugar maple was the most plastic for stomata density and aperture length.

Stomata area index (SAI) indicated red maple to have the highest relative stomata area, followed in declining order by silver maple and sugar maple. SAI plasticity was also highest in red maple followed by silver maple and then sugar maple (Table 3). Salisbury's stomata index demonstrated similar trends (Table 3).

Whole-Plant Size

Contrasts using light levels against various measures of leaf size and area, and the various measures of plant size (height, root collar diameter, dry mass) all showed significant linear and quadratic effects, except for ratio between leaf area and leaf mass (specific leaf area; SLA) (Table 4). Specific leaf area was highest for all three species in the 4% treatment and lowest in the 100% treatment, but contrasts were only significant for silver maple (Table 4) Though the same general trend was evident for all species, relative differences between high and low light treatments were greatest for red maple. Total leaf area per seedling was greatest for the 100% treatment and lowest for the 4% treatment. In the 100 %treatment, the individual leaves are therefore smaller in area, but there were sufficiently more leaves so that the overall leaf area of the plants was greater Relative differences in total leaf area between the 4% and 100% treatments were greatest in both silver and red maple as compared to sugar maple. In addition, they had larger total leaf areas per seedling than sugar maple in all but the 4% treatment (Table 4).

All size measurements, namely height, diameter of root collar, and total dry mass of seedlings, were highest in the 100% treatment for all species, and were generally lowest in the 4% shade treatment. Measures of height and root collar showed significant interaction between species and light treatments indicating that species changed rank in relation to each other across the different light treatments. In all cases, greatest differences among treatments were shown in silver and red maple (Table 4). Silver maple allocated proportionately less amount of biomass to roots as compared to the other maple species (Figure 1).

Table 4. F-values for response curve analyses comparing measures of growth across the four light treatments using linear and quadratic contrasts (levels of significance P < 0.05, *; P < 0.01). Means are given with standard errors in parentheses for the various leaf anatomical attributes for each light treatment and for all treatments combined. Fisher's PLSD test ($P < 0.05$) was used to compare pooled means of each species [letters denote differences among species ($A > B > C$)]. P = Plasticity [1 – (x / X)] where x is the value in 4% sunlight and X is the value in 100% sunlight.**

	Contrasts		Comparisons among treatments for each species	Comparisons among species (means over all treatments)				
	Linear	Quadratic		4%	15%	40%	100%	*P*
Height (cm)								
A. rubrum	22.2**	32.1***	22.16 (1.26)B	9.99 (0.75)	19.36 (2.01)	26.94 (1.71)	30.46 (2.06)	0.67 (0.06)
A. saccharinum	63.5***	78.15***	28.97 (1.87)A	11.75 (1.51)	26.14 (2.25)	36.51 (2.56)	41.49 (2.38)	0.71 (0.06)
A. saccharum	4.41ns	5.86*	13.63 (0.81)C	10.53 (1.11)	10.51 (0.98)	14.82 (1.75)	17.47 (1.60)	0.41 (0.05)
Root collar diameter (mm)								
A. rubrum	75.9***	111.0***	4.46 (0.22)A	2.18 (0.15)	3.90 (0.26)	5.41 (0.27)	6.01 (0.38)	0.64 (0.06)
A. saccharinum	76.7***	57.0***	3.52 (0.20)B	1.61 (0.08)	3.17 (0.28)	4.52 (0.28)	4.80 (0.26)	0.66 (0.05)
A. saccharum	29.22**	73.8***	3.09 (0.15)B	2.73 (0.16)	2.28 (0.10)	3.16 (0.20)	3.94 (0.39)	0.32 (0.04)
Total dry mass (g)								
A. rubrum	19.1*	27.6**	2.49 (0.25)A	0.42 (0.07)	1.47 (0.24)	3.32 (0.32)	4.45 (0.50)	0.90 (0.08)
A. saccharinum	35.0***	34.5***	1.84 (0.23)B	0.13 (0.02)	1.15 (0.23)	2.68 (0.44)	3.41 (0.42)	0.97 (0.07)
A. saccharum	12.6**	22.1**	1.10 (0.12)C	0.69 (0.16)	0.52 (0.06)	1.24 (0.21)	1.78 (0.30)	0.61 (0.06)
Specific leaf area (SLA; cm^2/g)								
A. rubrum	2.94ns	3.82ns	3.96 (0.16)B	7.76 (0.46)	3.04 (0.09)	2.63 (0.09)	2.39 (0.13)	0.69 (0.06)
A. saccharinum	39.10**	42.16**	4.48 (0.26)A	6.21 (0.36)	4.81 (0.21)	3.75 (0.24)	3.17 (0.22)	0.49 (0.05)
A. saccharum	0.12ns	1.75ns	4.08 (0.37)B	6.80 (0.70)	3.63 (0.37)	3.38 (0.68)	2.53 (0.16)	0.64 (0.07)
Total leaf area (cm^2)								
A. rubrum	30.44*	14.65*	199.42 (19.09)B	38.6 (6.6)	175.4 (24.9)	281.1 (13.8)	302.7 (30.9)	0.87 (0.11)
A. saccharinum	29.21*	13.25*	250.01 (29.54)A	34.7 (6.1)	230.7 (30.2)	374.3 (51.7)	360.3 (30.3)	0.90 (0.08)
A. saccharum	27.05*	17.32*	103.17 (22.13)C	52.1 (12.4)	64.8 (15.0)	140.3 (33.0)	155.4 (28.1)	0.67 (0.07)

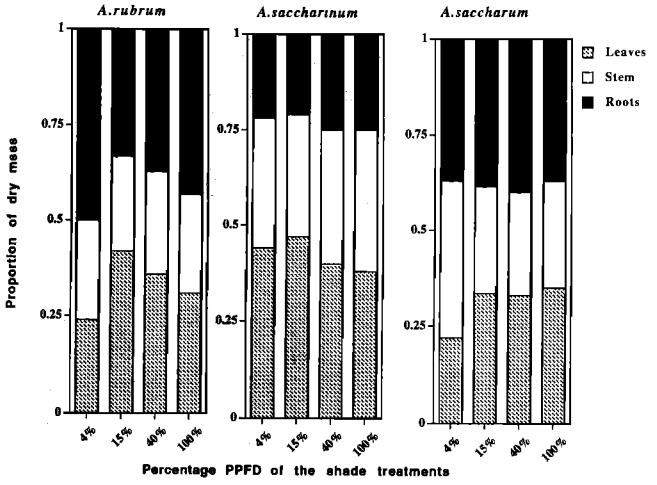

Figure 1. Proportional allocation of dry mass to roots, stem, and leaves among the light treatments for each of the species. Light treatment was measured as a percentage of the total photosynthetic photon flux density (PPFD) recorded in the full sun treatment.

Discussion

When leaf anatomical measures are taken separately, they did not change rank across light levels for the different species, suggesting that relationships among maple species remained consistent. However, comparing measures of leaf anatomy with gross measures of plant size, maple species showed separate relationships different from each other. Our study of leaf structure variation in relation to changes in whole-plant size supports evidence that maple species of the southern New England forests have site affinities that are both distinct yet overlapping in relation to each other with regard to availability of light and soil water. The study showed how leaf anatomical and plant size attributes of the three maple species are suited to the different habitats they occupy on the forest landscape from streamsides to hilltops. In the next few paragraphs, we discuss relationships between leaf structure, measures of plant size, and site affinity separately for each species.

Red maple grows in almost two-thirds of the 88 nontropical forest cover types in eastern North America (Walters and Yawney 1990). It forms red maple swamps and occurs on drought-prone hill tops in the region; it may grow in association with both sugar and silver maple. It is considered shade tolerant and grows from sea level to 2000 m (Walters and Yawney 1990, Harlow et al. 1991). Red maple had the thickest leaves under all light treatments. This thickness was manifested in all cell layers measured, viz. epidermis, palisade mesophyll, and cuticle. Red maple had the greatest root collar diameter for all light treatments as compared to the other species. In the low light treatment (4%) red maple had a high proportion of its dry mass allocated to roots (54% and 30% greater allocation as compared to sugar and silver maple respectively), supporting similar findings of studies done in shade houses (Gottschalk, 1994, Groninger et al. 1996) and the forest understory (Sipe and Bazzaz 1994, DeLucia et al. 1998). This morphological trait may provide structural bulk and considerable space for carbohydrate and water storage. All these attributes and their higher levels of responsiveness to differing light regimes are consistent with the broad distribution of red maple, as compared to the other maple species in this study. This may give red maple a competitive advantage where water is limiting and in more open, desiccating environments (Will et al. 1995, Canham et al. 1996).

Silver maple grows along streams, ponds, lakes and rivers. It grows largest in river valleys on wet, poorly drained soils. It is a medium-lived tree (125–150 yr) that is shade intolerant (Gabriel 1990), and it can withstand several weeks of flooding (Hosner 1960). Although it is widely planted in yards and parks, it seldom regenerates in these situations due to insufficient light and water (Gabriel 1990). The thinner dimensions in leaf structure, low proportional allocation to roots, greater height growth, and smaller root collar diameters indicate that silver maple would grow faster, relative to the other maples in high light environments where soil water was plentiful. In contrast to red maple, silver maple grows tall and thin seedlings, a growth habit that would be suitable for nutrient-rich and ever-moist soils of flood plains, and where competition for light, rather than soil water, is the most limiting factor. This is consistent with results from other studies that compared height growth of shade-intolerants with shade-tolerants; shade-intolerants have greater height growth than shade-tolerants even in low light conditions (Loach 1970—treatments providing 3 and 17% sunlight; Walters et al., 1993a,b—treatment providing 15–20% sunlight; Walters and Reich 1996–treatment providing 8%). However, in very low light conditions (e.g., 2%) Walters and Reich (1996) found shade-intolerant species grew slower than shade-tolerants; but we did not find this for our 4% light treatment. Also, the lower levels of responsiveness that we observed in silver maple, relative to red maple, in almost all dimensions of leaf structure (leaf thickness, cuticle, palisdae mesophyll, upper and lower epidermal layers) is also consistent with silver maple's restriction to certain sites. Our findings on the leaf structure of silver maple corroborate ecological observations that it is a light-demanding specialist requiring moist soils and the full sun of large canopy openings. Unlike red and sugar maple, there is no supporting literature on the functional properties of silver maple.

Sugar maple grows in 24 forest cover types of eastern North America and is a major component in 7 (Godman et al. 1990). It is fairly long-lived (250–400 yr) and one of the most shade tolerant maple species. It is restricted to well-drained soils and does not occur in swamps or sites that are periodically inundated. It also does not tolerate dry, shallow soils. It grows at high elevation in the southern Appalachians (2,000 m), whereas in New England it is seldom seen above 1,000 m (Godman et al. 1990). The leaf structure of sugar maple is, in most cases, intermediate in measured dimensions between red maple and silver maple. The high dimensional changes of sugar maple palisade mesophyll thicknesses across the light treatments suggests an ability of sugar maple to adapt to varied light environments. This is because the palisade mesophyll layer is an important site for light capture and photosynthesis. Sugar maple had a greater total dry mass (39% more), root collar diameter (20% more), and total leaf area (26% more) in the low light treatment as compared to red maple, the

next highest. The lower proportional allocation to roots of sugar maple (30% less) in low light suggests it might be more susceptible to soil moisture stress than red maple. Also, because measures of plant size across the light treatments were smaller for sugar maple (height–63% less; root collar diameter–50% less; total leaf area–22% less; and dry mass–32% less) than the other maples, we suggest that it is less responsive (Sipe and Bazzaz 1994, Canham et al. 1996, Lei and Lechowicz 1997). As concluded by Walters et al. (1993 a,b), sugar maple may be less responsive relative to other species because a greater concentration of resources may be allocated to production of protective compounds to resist herbivores and pathogens. However, our study showed that change in palisade mesophyll dimension is high, an indication of ability to photosynthesize in differing light levels. All these data support the ecological observations that sugar maple has a competitive advantage, relative to red and silver maple, in mesic shady conditions where it can invest in resource conservation for survival rather than height growth increase (Pacala et al. 1994, Kobe et al. 1995).

Findings in our study are consistent with those of Sipe and Bazzaz (1994, 1995) who showed that red maple survives better, overall, across canopy openings in a central New England forest as compared to sugar maple. This would fit the "generalist" growth of red maple. Our study also provides evidence for the ability of red maple to endure shade within the forest understory particularly on drier sites, as reported in studies by Lorimer (1984) and Kelty et al. (1988), and for its capacity to establish and grow well in the high light environments present in early seral stage forest (Oliver and Stephens 1977, Hibbs 1983).

Sipe and Bazzaz (1995) reported sugar maple to be the least responsive to differences in microenvironment. Ellsworth and Reich (1992) suggest sugar maple to be water-use efficient and conservative in growth allocation. Our study is consistent with their findings, but also demonstrates that sugar maple has the least overall responsiveness in whole-plant size (22–63% less in height, root collar diameter and dry mass) to different light treatments of the maples that we examined. However, anatomical measures (palisade mesophyll thickness, cuticle thickness) of sugar maple in our study demonstrated that, at the leaf level, it was responsive to differences in light. Similarly, Canham (1985, 1988) showed that, though sugar maple is shade tolerant and can establish in forest understory conditions, it grows best under higher light regimes of canopy edges and openings. Canham (1988) categorized sugar maple as a small gap specialist using Denslow's (1980) terminology.

There are limitations to using short-term greenhouse experiments (where competition has been excluded) for comparing growth of plants in differing light environments, and then using these results to relate to their known ecological affinities of site and shade-tolerance in the wild. For example, when grown under field conditions, shade intolerant species die in low light regimes, and shade tolerant species will survive, making measures of plasticity and performance different from those grown in the greenhouse (Pacala et al. 1994, Kobe et al. 1995). Further studies are therefore needed to investigate effects of competition under field situations.

In conclusion, this study is the first to provide insight into some of the linkages between leaf structure and ecological site affinities for maples. For example, in silver maple, a species known to be restricted to hydric sites, the thinner dimensions in leaf structure (an average of 17% less than red maple, the species with the thickest dimensions), and the overall lower level of leaf responsiveness to change in light (an average of 23% less than red maple, the most responsive species), make it the most susceptible to desiccation as compared to the other two maple species. Sugar maple exhibited changes in cuticle and palisade mesophyll thickness comparable to red maple suggesting an ability to adapt to varying light levels. Ecological studies have demonstrated sugar maple to be the most shade tolerant and in keeping with this it had the tallest height, and greatest root collar diameter and root biomass of the three species in the low light treatment. Red maple had the largest leaf structure dimensions across almost all light treatments, and also greatest allocation to root biomass under deep shade as compared to the other maples, supporting its known tolerance to varying light conditions (open to deep shade) and water-limiting environments. This study shows that determination of anatomical and morphological characteristics correlate well with the observed ecological distribution of these species. Determining where ecological performances do not agree with leaf structural and functional attributes permits exploration of the effects of competition on landscape patterns of distribution of these species.

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