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Ecophysiology of *Acer rubrum* seedlings from contrasting hydrologic habitats: growth, gas exchange, tissue water relations, abscisic acid and carbon isotope discrimination

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Summary Eight red maple (*Acer rubrum* L.) provenances, four each from wet and dry sites, were grown under the same conditions and their physiological responses to soil water availability investigated. Under well-watered conditions, seedlings of wet-site provenances grew faster and had consistently higher net photosynthesis, leaf conductance, maximum carboxylation rate, maximum rate of coupled photosynthetic electron transport, apparent quantum use efficiency, light-saturated photosynthesis and dark respiration than seedlings of dry-site provenances. Under conditions of low soil water availability, only dry-site provenances responded with decreased osmotic potential at full hydration and at the turgor loss point; however, provenances from wet sites showed a smaller reduction in absolute growth rate, a greater reduction in gas exchange and a greater increase in abscisic acid concentrations than dry-site provenances.

Keywords: ecotypic variation, leaf conductance, net photosynthesis, red maple, water-use efficiency.

Introduction

To understand the physiological basis of intraspecific adaptive variation at the landscape level, it is useful to examine variation among populations in a suite of physiological parameters. In this study, our objective was to investigate physiological responses of red maple seedlings from eight provenances to soil water availability. Six provenances represented the wet and dry hydrologic extremes in New York State. The other provenances, from Virginia, are from among the wettest and driest North American sites occupied by red maple. We investigated the following five questions. Is there in red maple: (1) intraspecific variation in water-use efficiency; (2) an effect of hydrological habitat extremes on carbon assimilation; (3) a similar abscisic acid (ABA) stress response among provenances; (4) ecotypic variation in leaf ABA synthesis; and (5) variation in turgor maintenance capacity under drought conditions? Our findings support the conclusion that red maple has two physiologically distinct ecotypes that differ in their adaptation to soil water availability.

Materials and methods

Plant material

Red maple seeds were collected at six sites or provenances within a 100 km radius of Ithaca, NY and from two sites in Virginia. The geographic origin of parent trees for wet and dry sites are as described in Anella and Whitlow (2000) and are summarized in Table 1. Sites were selected to represent the extremes of red maple's hydrologic range: wet sites have poorly drained soils that are saturated in the spring, and dry sites have well-drained upland soils. Seeds from wet sites were sown immediately following collection in flats containing a mixture of coarse sand, peat moss and silt loam (1:2:1, v/v). To overcome dormancy, dry-site seeds were cold stratified at 4 °C for 40 days before planting (Anella and Whitlow 1998). Seedlings were transplanted to 0.35-l plastic pots containing a mixture of sand, peat and silt loam (1:2:1, v/v) in a greenhouse, and fertilized weekly with 5 g l^{-1} of 5:11:26 N,P,K fertilizer. Seedlings were overwintered in a walk-in cooler at 3 °C, then transplanted into 3.7-l plastic pots set outdoors on a gravel pad. Sowings were made each year from 1995 through 1998. Trees were transplanted to 7.6-l pots after the second winter and 11-l pots after the third winter. Trees more than 1 year old were overwintered in an outdoor cold frame.

Experiment 1: soil water and gas exchange, leaf ABA, stable carbon isotope discrimination and growth

In May 1998, 24 plants (from seed sown in 1995 and 1996) of

Table 1. Description and location of ecotype collection sites. Designation indicates the site hydrologic condition. Abbreviations for drainage classes: ED = excessively drained; WD = well-drained; MWD = moderately well-drained; and VPD = very poorly drained.

Location	Designation	Soil series	Drainage class	Elevation (m)	Latitude, longitude
Spencer-Van Etten Swamp Chemung County, NY	Wet	Papakating	VPD	308	42°12′15″ N, 76°32′35″ W
Montezuma National Wildlife Refuge, Seneca County, NY	Wet	Muck	VPD	119	42°57′30″ N, 76°45′00″ W
Cayuta Lake, Schuyler County, NY	Wet	Carlisle Muck	VPD	402	42°22′35″ N, 76°44′00″ W
Presquile National Wildlife Refuge, Chesterfield County, VA	Wet	Hydraquent	VPD	2	37°21′40″ N, 77°15′20″ W
Taughannock Falls, Tompkins County, NY	Dry	Howard	WD	244	42°32′10″ N, 76°36′40″ W
Dodge Road, Tioga County, NY	Dry	Lordstown	WD	488	42°11′20″ N, 76°29′40″ W
Bald Hill, Tompkins County, NY	Dry	Mardin	MWD	518	42°21′45″ N, 76°22′50″ W
George Washington National Forest, Page County, VA	Dry	Drall-Wallen Complex	ED	503	38°43′15″ N, 78°33′34″ W

each of the eight provenances were randomly assigned to each of two watering treatments and placed outdoors on a gravel pad in a completely randomized block design. Pots were spaced 0.45 m center-to-center and surrounded by a three-plant-deep buffer strip. Half the plants of each provenance and treatment combination were randomly selected and sampled for repeated measurements of growth, gas exchange, ABA concentration, leaf water potential (Ψ_w) and stable carbon isotope discrimination (Δ) ($n = 6$). The remaining plants were randomly dispersed among the sampled plants to act as buffers. For gas exchange measurements, plants were moved to a greenhouse where supplemental light was provided by high-pressure sodium lamps for 0.5 h before, and during measurements.

Plants were either well-watered (control) or drought-treated. Initially, all pots were watered to saturation and allowed to drain for 18 h. Control plants were then watered daily to container capacity, whereas drought-treated plants were not watered except during the recovery phase, when they were watered daily to container capacity. Evaporation from the soil surface and penetration of rain were prevented by white plastic film, which was sealed to the stem of each plant with Parafilm at a point 7 cm above the soil surface, and extended below the pot rim.

After drainage and at each gas exchange measurement (once every fourth day), containers was weighed. When four of the six replicates of a provenance weighed ≤ 35% of container capacity, all replicates of that provenance were rewatered the following morning and kept well watered for 10 days (recovery phase). Predawn leaf water potential was measured with a pressure chamber (Soil Moisture, Santa Barbara, CA) immediately before rewatering and at the end of the drought recovery phase. After the drought recovery phase, water was again withheld from plants in the drought treatment until container capacity gravimetric loss = 35%.

Gas exchange To avoid accumulation of dew on leaves, plants were moved to a greenhouse the evening before gas exchange measurements. On 4 days each week, before noon, net photosynthesis (A_{net}) and leaf conductance (g_s) of the first fully expanded leaf were measured with a portable steady state gas-exchange system (LI-6400, Li-Cor, Lincoln, NE). Leaves were tagged and used repeatedly throughout the experiment. Plants were measured in random order, and the experiment was blocked over time so that on any given day, only half of the replicates were measured (48 plants per day^{-1}). During measurements, leaf temperature was 25 °C, photosynthetic photon flux density (PPFD) from a high-pressure sodium lamp was 1000 μmol m^{-2} s^{-1}, and humidity in the cuvette was 1.3 ± 0.3 kPa.

Abscisic acid concentration Between 1200 and 1300 h, leaf disks were cut from the first to the fifth fully expanded leaf of each tree with a cork borer. Two punches per leaf were taken on four occasions: after the initial watering; at the peak of each drought; and after the 10-day recovery period. The sampling, processing and analysis protocols were modifications of those described by Alves and Setter (2000). Abscisic acid concentration was determined by enzyme-linked immunosorbent assay as described by Alves and Setter (2000).

Stable carbon isotope discrimination On Days 0 and 56 (the first and last days of the experiment), 10 disks (1 cm diameter) were harvested from the first, third, fifth and seventh fully expanded leaves. Leaf disks were oven-dried at 70 °C for a minimum of 72 h, ground and analyzed for $\delta^{13}C$ by the Cornell

Boyce Thompson Stable Isotope Laboratory, Ithaca, NY (AncaSL-Europa 20/20 EA IRMS, PDZ Europa, Crewe, England). We also collected and analyzed ambient air samples from the greenhouse in which photosynthesis was measured and from above the outdoor gravel pad. Plant samples were analyzed for stable isotope composition ($^{13}C/^{12}C$) and δ values (‰) were calculated as:

$$\delta = \frac{R_{sample} - R_{standard}}{R_{sample}} 1000$$

where R is the $^{13}C/^{12}C$ ratio. The values were then converted to carbon isotope discrimination of the plant tissue based on free atmospheric CO_2 concentration (Δ), which currently has a deviation (δ_a) of approximately –8‰ (Farquhar et al. 1989).

Growth Tree heights (H) and diameters (D) were measured on six plants per treatment and ecotype, and a cylindrical volume index was calculated (D^2H).

Data analysis Gas exchange data were normalized by log transformation. Treatment effects on growth, gas exchange, ABA concentration and Ψ_w were evaluated by analysis of variance (ANOVA) with a univariate general linear model. Provenance variation in A_{net} was assessed by Fisher's least significant difference test ($P < 0.05$). Carbon isotope discrimination data were analyzed by two-way ANOVA with provenance and treatment as factors and a preplanned Student-Newman-Keuls post-hoc test for multiple pairwise comparisons.

Experiment 2: water availability and tissue water relations

Unless otherwise indicated, plant material, growing conditions and treatments were as for Experiment 1, except that plants were from the seed sown in 1997 and the experiment was initiated in May 1999. A randomly selected plant from each provenance and treatment combination was chosen for repeated investigations of osmotic adjustment. An additional replicate from each of two wet-site and two dry-site provenances was randomly selected and included in the investigation of osmotic adjustment as an independent block. We used a randomized incomplete block design with day as a blocking factor, and the experiment was repeated twice. Individual pressure–volume (P–V) parameters revealed no significant block difference. Therefore, we eliminated the block parameter from the analysis of variance model and pooled the data.

Pots were weighed 18 h after the initial watering and daily thereafter. When a pot weighed 0, 15, 30 and 40% less than container capacity, a randomly selected leaf was excised at 2000 h from one of the first four fully expanded sun-leaves (second and third nodes from the terminal). The leaf was enclosed in a plastic bag containing moist paper and immediately transported to the laboratory, where the petiole was recut under distilled water and the leaf left to rehydrate overnight. The next morning, samples were blotted dry and weighed to obtain saturated weight, and then enclosed in plastic bags containing moist paper and sealed in a pressure chamber (Plant Moisture Status Console, Soil Moisture Equipment, Santa Barbara, CA) with the petiole protruding. We used up to four pressure chambers simultaneously to measure balance pressure by methods similar to those of Robichaux (1984).

In another experiment with five randomly selected plants from each provenance and treatment combination, the experimental protocol was as described above until the point at which leaves had been rehydrated and weighed. Then, leaf samples were placed in 3-cm^3 syringes and frozen at –18 °C for 24 h. After thawing for 10–15 min, the samples were squeezed with the plunger of the syringe and an 8 µl aliquot of sap expelled onto a filter paper disk. The disk was placed in a vapor pressure osmometer (Model 5100c, Wescor, Logan, UT) and the osmolality of the expressed sap determined and normalized to 20 °C. Data were not corrected for apoplastic dilution of symplast osmotic potential at 100% relative water content ($\Psi_{\pi o}$), which was determined to be small (= 0.1 MPa) in our experiments. Osmotic adjustment was calculated as the difference in $\Psi_{\pi o}$ between the control and drought treatments.

Data analysis Pressure–volume data were fit by segmented regression. Water relations parameters were evaluated with a repeated measures analysis of variance.

Experiment 3: gas exchange of well-watered plants

Except as otherwise indicated, plant material and growing conditions and treatments were as for Experiment 2. There were six half-sib seedlings of each provenance and all were watered to pot capacity daily.

Gas exchange Gas exchange of each plant was measured with an open system (model MPH-1000, Campbell Scientific) described by Geber and Dawson (1997). Calculations of gas exchange rates and C_i were performed according to Farquhar et al. (1980). Cuvette temperature was 25 °C and VPD was 1.6 ± 0.2 kPa during determination of A–C_i curves. Photosynthetic photon flux density (from a sodium vapor lamp) at the leaf surface was 1000 µmol m^{-2} s^{-1}. Measurements were taken at an initial cuvette CO_2 concentration of 360 µmol mol^{-1} and then at 200, 100 and 50 µmol mol^{-1}; subsequently, the cuvette CO_2 concentration was returned to 360 µmol mol^{-1} and measurements were taken as the concentration was sequentially increased to 600, 800, 1000 and 1200 µmol mol^{-1}. The models of Farquhar and von Caemmerer (1982) and Kirschbaum and Farquhar (1984) were used to estimate maximum carboxylation rate (V_{cmax}) and maximum rate of coupled photosynthetic electron transport (J_{max}) simultaneously by fitting the biochemical model to the A–C_i values with a maximum of 1000 iterations. An estimate of the rate of photosynthesis (A_o; µmol m^{-2} s^{-1}) in the absence of stomatal limitation was obtained from the A–C_i curve when the internal C_i was equal to 360 µmol mol^{-1} (see also Flannagan and Jefferies 1988; Geber and Dawson 1997). To estimate V_{cmax} and J_{max} from A–C_i curves, we used Equations 2–6 of Geber and Dawson (1997). For each plant, the nonlinear regression curve explained more than 90% of the variation in the A–C_i data.

Light response curves Light response curves were generated by measurements on the leaves used to construct A–C_i curves.

The ambient CO_2 concentration, leaf temperature and VPD were set identical to those in the A–C_i experiment. Before measurement, plant leaves were illuminated at approximately 1000–1200 μmol m^{-2} s^{-1} for 5–7 min. Photosynthetic photon flux density was monitored with a quantum sensor (LI-189, Li-Cor). Neutral density filters of different opacity were combined to obtain the following sequence of PPFD: 1200, 900, 600, 425, 300, 200, 100, 50 and 0 μmol m^{-2} s^{-1}. Carbon uptake rate was then measured 5 min after exchange rates stabilized (approximately 10–20 min), which amply exceeded time constants for the photosynthesis system as determined by Barradas and Jones (1996). Photosynthesis versus irradiance data were fit to the empirical model of Küppers and Schulze (1985). The nonlinear regression coefficients of determination for each curve explained over 95% of the variation in A versus PPFD. Dark respiration (R_d) was calculated with the modeled parameter, which was determined by curve fitting (ϕ), and the light compensation point (I_c) estimated from the intersection of the regression line with the x-axis. Apparent quantum yield (Q_{app}) was calculated independently by linear regression analysis as the slope of the first four points of individual curves (PPFD = 0–200 μmol m^{-2} s^{-1}).

An estimate of the rate of electron transport (J) was calculated from the light curve data according to Lambers et al. (1998; p 27). Rather than use our estimates of J_{max} from the A–C_i curve analysis, we wanted to verify our findings by arbitrarily holding J_{max} constant. By calculating an estimate of J from the independent light curve data set and making J_{max} the same for wet and dry sites, we avoided biasing our results:

$$J = Q_{app}I + J_{max} - \sqrt{((Q_{app}I + J_{max})^2 - 4\Theta Q_{app}IJ_{max})/2\Theta} \quad (1)$$

where I is a light-saturated rate of photosynthesis for red maple (900 μmol m^{-2} s^{-1}), J_{max} is held constant for both ecotypes and set at an arbitrarily determined value of 100 μmol m^{-2} s^{-1}, and Θ is the curvature factor, which can vary between 0 and 1 (set at a constant value of 0.5 for calculations of J).

Results

Experiment 1: soil water and gas exchange, leaf ABA, stable carbon isotope discrimination and growth

No significant differences in A_{net} were found between annual cohorts, both within and between Blocks 1 and 2 over time. The block and annual cohort effects were therefore removed from the model. Within-provenance variation in A_{net} was similar among the eight provenances.

The univariate general linear model analysis of variance for A_{net} and stomatal conductance (g_s) indicated a significant difference ($P < 0.001$) between wet- and dry-site provenances. The repeated measures analysis of variance for ABA and predawn Ψ_w indicated that there was a significant ($P < 0.01$) difference between wet- and dry-site provenances. Under well-watered conditions, mean A_{net} of the four wet-site provenances was 16% higher than that of the four dry-site provenances (11.6 and 9.9 μmol m^{-2} s^{-1} for wet- and dry-site provenances, respectively) (Figure 1A). Mean g_s of wet- and dry-site provenances was 0.18 and 0.16 mol m^{-2} s^{-1}, respectively, for well-watered plants measured either before the imposition of drought or after recovery from drought (W^I and W^{II}, respectively; Figure 1B). Abscisic acid concentration and Ψ_w did not differ among provenances under well-watered conditions (W^I and W^{II}; Figures 1C and 1D). Under drought conditions, both wet- and dry-site A_{net}, g_s and Ψ_w declined, whereas ABA concentration increased. At the peak of the first drought (D^I), A_{net} and g_s declined to 5.3 μmol m^{-2} s^{-1} and 0.06 mol m^{-2} s^{-1}, re-

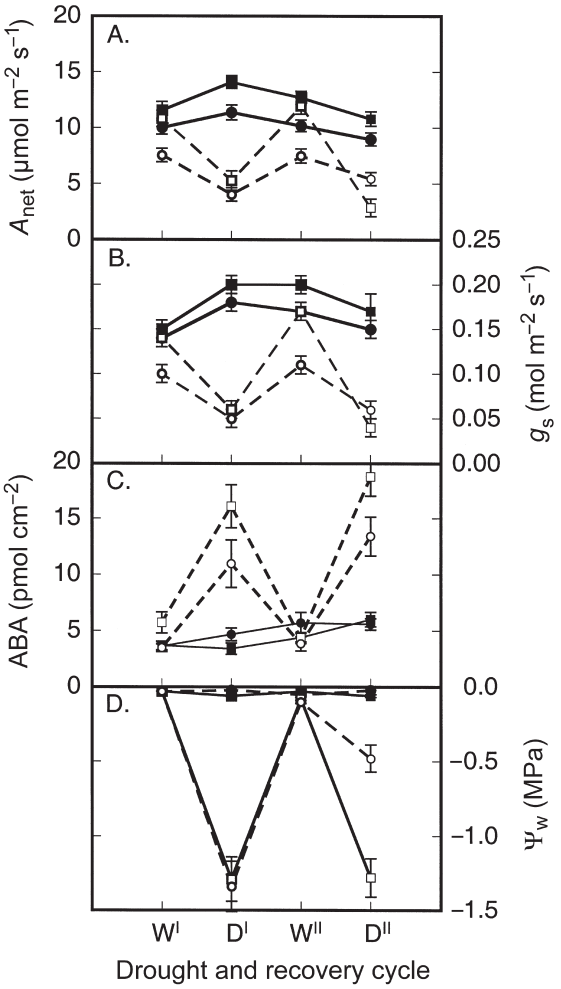

Figure 1. Net photosynthesis (A_{net}), stomatal conductance to water vapor (g_s), abscisic acid concentration (ABA) and predawn leaf water potential (Ψ_w) subsetted four consecutive times in order to pair ABA and Ψ_w measurements to gas exchange on well-watered (solid symbols) and drought-treated (open symbols) red maple seedlings. Provenances are from wet (squares) and dry sites (circles). Each value represents the mean ± SE (n = 6). Well-watered seedlings were watered every day, whereas drought-treated seedlings were watered only during a 10-day recovery cycle. The data are not a true series; however, they are plotted as a line graph for purposes of clarity. Abbreviations: W^I = well-watered conditions at the beginning of the study; D^I = the first peak drought (= 35% less than container capacity); W^{II} = time point at the end of a 10-day post-drought recovery period (plants were well watered for 10 days after D^I was reached); and D^{II} represents the second drought peak.

spectively, for wet-site provenances, and to 4.0 μmol m^{-2} s^{-1} and 0.05 mol m^{-2} s^{-1}, respectively, for dry-site provenances. During the first drought period, bulk leaf ABA concentration was higher in wet-site provenances than in dry-site provenances (16.1 versus 11.0 pmol cm^{-2}). Mean Ψ_w values were similar in both provenance groups (–1.29 and –1.34 MPa for wet- and dry-site provenances, respectively). Ten days after drought relief (W^{II}), A_{net}, g_s, ABA concentration and Ψ_w returned to near-initial values in both the wet- and dry-site provenances.

At the peak of the second imposed drought (D^{II}), there were differences in A_{net}, g_s, ABA concentration and Ψ_w among provenances (Figure 1). However, Ψ_w of the dry-site provenances declined less during the second drought period than during the first, whereas ABA concentrations increased more during the second drought period than during the first. Mean A_{net} and g_s in wet-site provenances were lower at the end of the second drought period than the first.

Although there was a significant ($P < 0.001$) drought treatment effect on leaf Δ, which is an indicator of water-use efficiency over the course of the study, there were no differences in Δ between wet- and dry-site provenances. Initial mean Δ values were similar in wet- and dry-site provenances (20.5 ± 0.2‰ and 20.3 ± 0.2‰, respectively). In response to drought stress, mean wet-site Δ decreased to 19.3 ± 0.7‰, whereas dry-site mean Δ decreased to 19.0 ± 0.6‰.

Differences in growth rate between wet- and dry-site provenances were similar among years. Figures 2A and 2B illustrate the individual growth patterns of two annual cohorts of plants (1995 and 1996). Under well-watered conditions, plants of dry-site provenances grew less than plants of wet-site provenances in both years ($P < 0.001$ for 1995; $P < 0.001$ for 1996), with no interactions between treatment and site in either year's analysis.

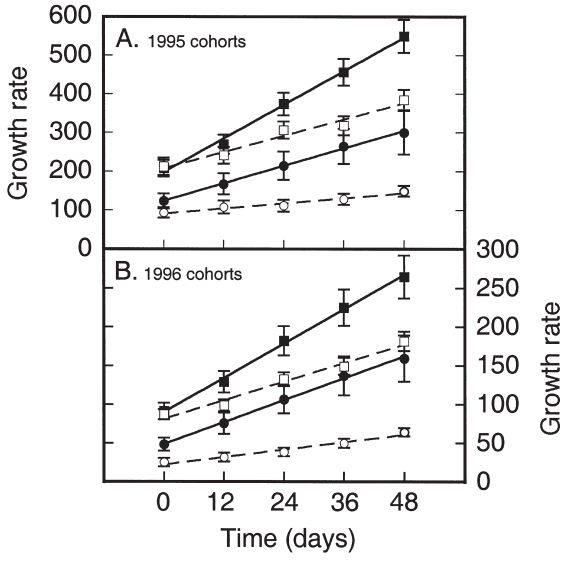

Figure 2. Absolute growth rate expressed as cylindrical volume index (the diameter squared multiplied by height) measured at five 12-day intervals for well-watered (solid symbols) and water-stressed (open symbols) red maple seedlings. Provenances are from wet (squares) and dry sites (circles). Each value is a mean ± SE ($n = 6$). Well-watered seedlings were watered every day, whereas water-stressed seedlings were watered only during a 10-day post-drought recovery cycle.

Experiment 2: water availability and tissue water relations

There were no drought or provenance effects on leaf relative water content at zero turgor (R_{tlp}) or average bulk modulus of elasticity (ε_{avg}) (Table 2).

Under well-watered conditions, $\Psi_{\pi o}$ and $\Psi_{\pi tlp}$ of wet-provenance seedlings were not significantly different from values for dry-provenance seedlings (Table 2). Furthermore, there were no treatment effects on $\Psi_{\pi o}$ or $\Psi_{\pi tlp}$ of seedlings of wet-site provenances (Table 2). However, drought significantly lowered $\Psi_{\pi o}$ and $\Psi_{\pi tlp}$ of seedlings of dry-site provenances (Table 2), whereas mean $\Psi_{\pi o}$ and $\Psi_{\pi tlp}$ of seedlings of wet-site provenances increased slightly in the drought treatment (Table 2). Seasonal changes in $\Psi_{\pi o}$, $\Psi_{\pi tlp}$, R_{tlp} and ε_{avg} showed no significant differences in well-watered seedlings of wet- or dry-site provenances.

Measurement of osmotic potential with the vapor pressure osmometer ($\Psi_{\pi o(vp)}$) supported the conclusion that there was no real difference in $\Psi_{\pi o}$ between wet- and dry-site seedlings (Table 2). Moreover, both techniques indicated that the osmotic potential of dry-site progeny became more negative during drought treatment, indicating solute accumulation and osmotic adjustment (Table 2). The $\Psi_{\pi o(vp)}$ significance level was $P = 0.08$. The magnitude of the observed osmotic adjustment was in the range reported by Tschaplinski et al. (1998) for red maple seedlings under severe drought conditions.

Experiment 3: gas exchange of well-watered plants

A versus C_i relationships Under well-watered conditions, seedlings of wet-site provenances had greater photosynthetic capacities than seedlings of dry-site provenances. Table 3 shows mean values of gas exchange parameters obtained from A versus C_i curves. Seedlings of wet-site provenances had slightly greater carboxylation rates than seedlings of dry-site provenances, as indicated by slightly higher mean V_{cmax} estimates, initial slopes derived from regression analyses of the A versus C_i curves, and a scatter plot with trend lines of C_i values below 200 μmol mol^{-1} (Table 3 and Figure 3). Estimates of maximum rate of ribulose-1,5-bisphosphate regeneration (J_{max}) and rate of electron transport (J) were considerably higher for wet-site provenances than for dry-site provenances (Tables 3 and 4). Stomatal limitation, on the other hand, was significantly lower for wet-site provenances than for dry-site provenances (Table 3). Carbon dioxide compensation points did not differ significantly between wet- and dry-site provenances.

Light response curves At irradiances greater than or equal to 50 μmol m^{-2} s^{-1}, CO_2 assimilation was always higher in wet-site provenances than in dry-site provenances; light-saturated photosynthesis, A_{max}, g_s, Q_{app} and R_d were always higher in wet-site provenances than in dry-site provenances (Table 4). In general, A–PPFD curves saturated more abruptly in dry-site

Table 2. Leaf tissue water relations for well-watered and drought-stressed red maple seedlings. Weight loss is expressed as percent loss of container capacity. Statistically significant habitat differences ($P < 0.05$) within a treatment are indicated by different letters. Significant differences between treatments within a provenance type are indicated by asterisks: * = $P < 0.05$. Abbreviations: $\Psi_{\pi o}$ = osmotic potential at 100% relative water content (RWC); $\Psi_{\pi o(vp)}$ = psychrometric osmotic water potential at 100% RWC; $\Psi_{\pi tlp}$ = osmotic potential at the turgor loss point; R_{tlp} = RWC at the turgor loss point; and ε_{avg} = mean modulus of elasticity.

Provenance	Weight loss (%)	$\Psi_{\pi o}$ (MPa)	$\Psi_{\pi o(vp)}$ (MPa)	$\Psi_{\pi tlp}$ (MPa)	R_{tlp} (%)	ε_{avg}
Well-watered treatment						
Wet-site	0	–0.99 a	–1.24 a	–0.99 a	92.5 a	18.44 a
	15	–1.07 a	–1.27 a	–1.13 a	92.3 a	12.65 a
	30	–0.91 a	–1.19 a	–0.97 a	92.1 a	12.34 a
	40	–0.97 a	–1.22 a	–1.03 a	91.5 a	12.09 a
Dry-site	0	–0.93 a*	–1.36 b*	–0.99 a*	92.7 a	15.59 a
	15	–0.97 a*	–1.37 b*	–1.05 a*	92.6 a	11.33 a
	30	–0.95 a*	–1.20 b*	–1.00 a*	92.1 a	12.76 a
	40	–1.00 a*	–1.21 b*	–1.02 a*	91.0 a	12.62 a
Drought treatment						
Wet-site	0	–0.94 a	–1.18 a	–1.00 a	92.7 a	12.04 a
	15	–0.94 a	–1.22 a	–0.99 a	92.7 a	12.45 a
	30	–0.92 a	–1.18 a	–0.97 a	92.4 a	14.06 a
	40	–0.90 a	–1.18 a	–0.95 a	92.4 a	13.73 a
Dry-site	0	–1.01 b *	–1.25 b*	–1.07 b*	92.7 a	15.82 a
	15	–0.99 b *	–1.31 b*	–1.06 b*	92.5 a	13.74 a
	30	–1.03 b *	–1.30 b*	–1.10 b*	91.3 a	12.26 a
	40	–1.20 b *	–1.37 b*	–1.27 b*	90.4 a	13.27 a

provenances than in wet-site provenances, and A_{max} and R_d were significantly greater for wet-site provenances than for dry-site provenances in both the modeled and original data sets (Table 4). Mean I_c, however, did not differ significantly between provenances grouped by source habitat hydrology (Table 4). Electron transport rate, calculated from apparent quantum-use efficiency, differed between wet- and dry-site provenances (Table 4). Net photosynthetic rates of dry-site provenances were lower than those of wet-site provenances at irradiances greater than 200 µmol m^{-2} s^{-1}. Provenances from both wet and dry sites reached light saturation at about 600 µmol m^{-2} s^{-1}, with signs of photoinhibition at 1200 µmol m^{-2} s^{-1}. Stomatal conductances of wet-site provenances were higher than those of dry-site provenances at all PPFDs. At light saturation, g_s was about 36% higher in wet-site provenances than in dry-site provenances. The Q_{app} was about 23% greater in wet-site provenances than in dry-site provenances. At PPFDs above and below 200 µmol m^{-2} s^{-1}, g_s of wet-site progeny was 34 and 33% higher, respectively, than g_s of dry-site progeny.

Table 3. The CO_2 response gas exchange parameters of well-watered red maple seedlings of wet- and dry-site provenances. Maximum carboxylation rate (V_{cmax}), estimates of the maximum rate of ribulose-1,5-bisphosphate regeneration (J_{max}) and relative stomatal limitation to photosynthesis (S_l) were calculated from the biochemical model of von Caemmerer and Farquhar (1981), following the parameter estimates of Kirschbaum and Farquhar (1984). The CO_2 compensation point (Γ*) was calculated for each individual curve.

Parameter	Wet-site	Dry-site	P-Value[1]
V_{cmax} (µmol m^{-2} s^{-1})	77.3 ± 2.9	60.9 ± 2.6	*
J_{max} (µmol m^{-2} s^{-1})	189.1 ± 5.0	109.4 ± 5.5	**
Γ* (µmol mol^{-1})	132.5 ± 2.4	122.2 ± 2.6	ns
S_l (%)	37 ± 0.0	43 ± 0.0	*

[1] Significant differences between habitats are indicated by asterisks: * = $P < 0.05$; ** = $P < 0.01$; and ns = nonsignificant.

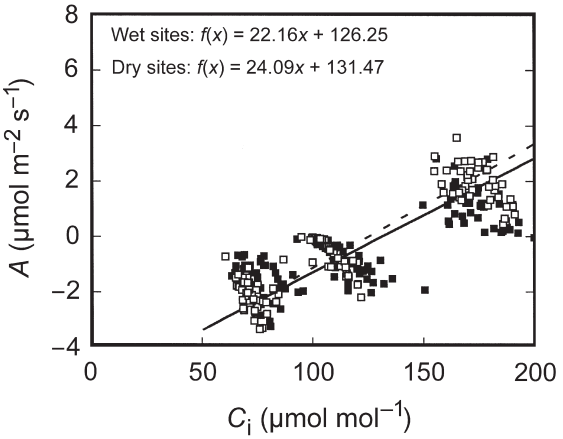

Figure 3. Scatter plot of the CO_2-limited portion of A–C_i curves at C_i < 200 µmol mol^{-1}. A linear regression line is fit to all values on the curve at C_i < 200 µmol mol^{-1} separately for dry- (■, solid line) and wet-site provenances (□, dashed line).

Table 4. Light response gas exchange parameters of red maple seedlings of wet- and dry-site provenances at 25 °C and VPD = 1.6 ± 0.2 kPa: maximum net photosynthetic rate (A_{max}; μmol m^{-2} s^{-1}), stomatal conductance to water vapor (g_s; mol m^{-2} s^{-1}) and dark respiration (R_d; μmol m^{-2} s^{-1}). Apparent quantum yield (Q_{app}), R_d (model), light compensation point (I_c; μmol m^{-2} s^{-1}) and rate of electron transport (J; μmol m^{-2} s^{-1}) were calculated from the linear portion of the initial part of the light-response curve. Modeled A_{max} and I_c were obtained from curve fitting, following the model used by Küppers and Schulze (1985).

Parameter	Wet-site	Dry-site	P-Value[1]
A_{max}	9.60 ± 0.47	6.79 ± 0.50	***
A_{max} (model)	9.66 ± 0.48	6.91 ± 0.49	***
R_d	–1.86 ± 0.07	–1.44 ± 0.09	**
R_d (model)	–1.74 ± 0.07	–1.24 ± 0.06	***
g_s	0.14 ± 0.01	0.09 ± 0.01	***
Q_{app} (10^{-2})	3.63 ± 0.12	2.79 ± 0.14	**
I_c (model)	39.14 ± 2.05	36.64 ± 2.73	ns
J	27.48	21.99	na

[1] Significant differences between habitats are indicated by asterisks: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; ns = nonsignificant; and na = not applicable.

Discussion

Experiment 1: soil water and gas exchange, leaf ABA, stable carbon isotope discrimination and growth

Predawn Ψ_w values were consistently less negative in dry-site provenances than in wet-site provenances, which is consistent with greater stomatal control of water loss by dry-site provenances, although it might be a consequence of osmotic adjustment. Loewenstein and Pallardy (1998*a*, 1998*b*) found a positive relationship between xylem sap ABA concentration and g_s. We observed that, under well-watered conditions, A_{net} and g_s were consistently higher in wet-site provenances, but ABA concentrations did not differ among provenances. However, at the peak of drought, we observed higher ABA concentrations in wet-site provenances than in dry-site provenances, and a greater decrease in their A_{net} and g_s. This supports the view that changes in ABA concentration, A_{net} and g_s enable red maple to adapt to wet- and dry-site conditions. The higher ABA concentrations observed in leaf tissue of wet-site provenances indicate that these provenances have a greater ability than dry-site provenances to synthesize ABA and optimize performance under well-watered conditions with periodic stress episodes. Seedlings of dry-site provenances, conversely, may avoid, rather than tolerate, drought stress by conserving water through reduced stomatal conductance.

Wet-site provenances responded to the second drought episode differently from dry-site provenances, having lower A_{net}, g_s and Ψ_w, and higher ABA concentrations. Higher concentrations of ABA in leaves of wet-site provenances may reflect a lack of osmotic adjustment, which may be a key adaptative mechanism of dry-site provenances.

Plants may respond to drought by modulating stomatal aperture in such a way as to keep the ratio of assimilation to evaporation constant (Cowan and Farquhar 1977), thereby maximizing water-use efficiency, and hence Δ (Sun et al. 1996). In our study, however, we detected no difference in Δ between dry- and wet-site red maple provenances. Our data do not support the hypothesis that variation in drought tolerance among red maple provenances is explained by variation in WUE. Similar conclusions were reported by Zhang et al. (1993), who found no significant differences in Δ between coastal and interior varieties of Douglas-fir in a common garden experiment.

Experiment 2: water availability and tissue water relations

Different values of osmotic potential at full ($\Psi_{\pi o}$) and zero turgor ($\Psi_{\pi tlp}$) have been reported for red maple (Roberts and Knoerr 1977, Abrams 1988, Abrams and Kubiske 1990, Nash and Graves 1993, Bauerle 2001). Zwack et al. (1998) found that cultivars of Freeman maple, which are hybrids of red and silver maples, had a uniform capacity for osmotic adjustment. In contrast, Townsend and Roberts (1973) observed ecotypic differences in transpiration and growth rate between seedlings from wet and dry sites; wet-site ecotypes had higher transpirational water loss than dry-site ecotypes. Their study, however, provided no independent estimate of drought and no measure of osmotic adjustment, and used elevation as a surrogate for soil water availability (A.M. Townsend, USDA, ARS, Beltsville, MD, personal communication).

In our study, the osmotic potential at full turgor of drought-stressed seedlings of dry-site provenances was slightly more negative than that of control seedlings in both the P–V and vapor pressure osmometer experiments (Table 2), which suggests that there is some ecotypic variation in the response of red maple to water availability. This finding is in contrast to that of Abrams and Kubiske (1990), who used incipient wilting to identify peak drought. Furthermore, the conclusion of Abrams and Kubiske (1990) that red maple seedlings native to boggy habitats were able to adjust osmotically, whereas seedlings native to upland sites were not, was based on a study without replication of provenances.

Under well-watered control conditions, seedlings of wet- and dry-site provenances maintained similar $\Psi_{\pi o}$ and $\Psi_{\pi tlp}$. Under conditions of drought, however, dry-site seedlings underwent slight osmotic adjustment, whereas wet-site seedlings did not. This further supports the idea that red maple shows ecotypic variation in adaptation to drought.

Our results are consistent with studies reporting that xeric species undergo osmotic adjustment during drought to a greater extent than mesic species (Parker et al. 1982, Abrams and Knapp 1986). Furthermore, because six of the eight red maple populations in our study were within 100 km of Ithaca, NY, it is unlikely that the observed differences in osmotic adjustment are a result of regional variation independent of soil water.

Experiment 3: gas exchange of well-watered plants

Terashima et al. 1988 demonstrated that short-term water stress can lead to patchy stomatal closure, which can in turn lead to underestimation of A and overestimation of C_i. The diffusion resistance created by stomatal patchiness could limit

the CO_2 response (Cornic and Briantais 1991, Brestic et al. 1995, Lal and Edwards 1996), yet be indistinguishable from metabolic regulation (Graan and Boyer 1990, Lauer and Boyer 1992, Tezara and Lawlor 1995, Kanechi et al. 1995, 1996). On the other hand, dynamic and uncoordinated patchy stomatal closure could balance out over the whole leaf and produce a steady gas-exchange rate (Cardon et al. 1994). It is also important to consider the shape of the response curve (Prioul and Chartier 1977, Leverenz and Jarvis 1979, Terashima 1989, Leverenz et al. 1990). To reduce the likelihood of stomatal closure due to water stress, and thus the likelihood of falsely interpreting the A–C_i relationship, our experiments were conducted under conditions of uniformly high soil water availability and moderate vapor pressure deficit (VPD). Moreover, we observed no dissimilarity in the shape of the response curve between individuals, either within or between populations.

The J value was calculated from an independent light curve data set and supports the use of J_{max} for comparison of wet- and dry-site ecotypes. The J_{max} and J values were higher in wet-site provenances than in dry-site provenances. Our observations of significantly different gas exchange parameters among red maple populations from different hydrologic habitats are consistent with the general observation of Pearcy et al. (1987) that plant species on resource-rich sites have much higher photosynthetic capacities than species on resource-poor sites. When environmental conditions are non-limiting, CO_2 assimilation is regulated by the intrinsic photosynthetic capacity of the mesophyll and by CO_2 conductance to sites of carboxylation at the level of the chloroplast (Syvertsen et al. 1995). The higher assimilation rates in wet-site progeny could represent greater investment in the biochemical components of the photosynthetic machinery. In the case of dry-site progeny, photosynthesis may be down-regulated as a result of limitations imposed by the low water availability. The physiological characteristics of red maple provenancess are likely to have a complex genetic basis, involving multiple genes. To understand why red maple populations flourish where they do, we characterized the response of leaf gas exchange to differences in CO_2 concentration and PPFD. Raven et al. (1992) states that a population of plants tends to respond to selection as an integrated unit. Our use of multiple half-sib families in gas exchange experiments was intended to address this observation. The hydrologic conditions of the sites may have placed a selection pressure on the population. The gas exchange responses to CO_2 and PPFD observed in the half-sib seedlings, therefore, may indicate the results of past selection pressure on the provenance.

We found intraspecific differences in photosynthetic responses to CO_2 and PPFD between red maple seedlings from contrasting hydrologic habitats. Our results show that multiple populations of wet-site red maple provenances growing under well-watered conditions have higher rates of net assimilation at saturating CO_2 concentration and PPFD. As indicated by the A–C_i analysis, wet- and dry-site progeny appear to be limited by both electron transport capacity and carboxylation capacity at the transition from CO_2 to RuBP limitation. Wet-site progeny, however, have higher maximum assimilation and carboxylation rates than dry-site progeny. Therefore, it is likely that intraspecific variation between contrasting hydrologic habitats of red maple results from differences in the intrinsic photosynthetic capacity of the mesophyll and stomata and internal CO_2 transfer conductances within the mesophyll.

Integrated physiological mechanisms

In conclusion, several physiological stress tolerance and resource capture mechanisms were present in response to both drought and well-watered conditions, indicating that red maple has evolved into wet- and dry-site ecotypes. The study as a whole indicates that evolution of a suite of traits has occurred in response to environmental stress and supports Chapin's (1991) concept of a centralized stress response syndrome in an ecologically distinct woody species. The heritable hormonal control of the stress response system may be related to ABA and could cause a change in suites of traits (Chapin et al. 1993). More fundamental research is needed to further elucidate the role of ABA in the control of stress tolerance and whether the ecotype divergence is hormonally mediated.

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References

Abrams, M.D. 1988. Comparative water relations of three successional hardwood species in central Wisconsin. Tree Physiol. 4: 263–273.

Abrams, M.D. and A.K. Knapp. 1986. Seasonal water relations of three gallery forest hardwood species in northeast Kansas. For. Sci. 32:687–696.

Abrams, M.D. and M.E. Kubiske. 1990. Photosynthesis and water relations during drought in *Acer rubrum* L. genotypes from contrasting sites in central Pennsylvania. Funct. Ecol. 4:727–733.

Alves, A.C. and T.L. Setter. 2000. Response of Cassava to water deficit: leaf area growth and abscisic acid. Crop Sci. 40:131–137.

Anella, L.B. and T.H. Whitlow. 1998. Germination of *Acer rubrum* seeds collected from wet and dry habitats. Seed Sci. Technol. 26: 755–762.

Anella, L.B. and T.H. Whitlow. 2000. Photosynthetic response to flooding of *Acer rubrum* seedlings from wet and dry sites. Am. Mid. Nat. 143:330–341.

Barradas, V.L. and H.G. Jones. 1996. Responses of CO_2 assimilation to changes in irradiance: laboratory and field data and a model for beans (*Phaseolus vulgaris* L.). J. Exp. Bot. 47:639–645.

Bauerle, W.L. 2001. The water relations of *Acer rubrum* L. ecotypes from contrasting hydrologic habitats. Ph.D. Diss., Cornell Univ., Ithaca, NY, 152 p.

Brestic, M., G. Cornic, M. Fryer and N. Baker. 1995. Does photorespiration protect the photosynthetic apparatus in French bean leaves from photoinhibition during drought stress? Planta 196:450–457.

Cardon, Z.G., K.A. Mott and J.A. Berry. 1994. Dynamics of patchy stomatal movements, and their contribution to steady-state and oscillating stomatal conductance calculated using gas-exchange techniques. Plant Cell Environ. 17:995–1007.

Chapin, F.S., III. 1991. Integrated responses of plants to stress. Bioscience 41:29–36.

Chapin, F.S., III, K. Autumn and F. Pugnaire. 1993. Evolution of suites of traits in response to environmental stress. Am. Nat. 142: 578–592.

Cornic, G. and J. Briantais. 1991. Partitioning of photosynthetic electron flow between CO_2 and O_2 reduction in a C_3 leaf (*Phaseolus vulgaris* L.) at different CO_2 concentrations and during drought stress. Planta 183:178–184.

Cowan, I.R. and G.D. Farquhar. 1977. Stomatal function in relation to leaf metabolism and environment. Symp. Soc. Exp. Biol. 31: 471–505.

Farquhar, G.D. and S. von Caemmerer. 1982. Modelling of photosynthetic response to environmental conditions. *In* Encyclopedia of Plant Physiology. II. Physiological Plant Ecology. Eds. O.L. Lange, P.S. Nobel, C.B. Osmond and H. Ziegler. Springer-Verlag, Berlin, pp 549–588.

Farquhar, G.D., S. von Caemmerer and J.A. Berry. 1980. A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta 149:78–90.

Farquhar, G.D., J.R. Ehleringer and K.T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. Mol. Biol. 40:503–537.

Flannagan, L.B. and R.L. Jefferies. 1988. Stomatal limitation of photosynthesis and reduced growth of the hylophyte, *Plantago maritime* L., at high salinity. Plant Cell Environ. 11:239–245.

Geber, M.A. and T.E. Dawson. 1997. Genetic variation in stomatal and biochemical limitations to photosynthesis in the annual plant, *Polygonum arenastrum*. Oecologia 109:535–546.

Graan, T. and J. Boyer. 1990. Very high CO_2 partially restores photosynthesis in sunflower at low leaf water potentials. Planta 181: 378–384.

Kanechi, M., E. Kunitomo, N. Inagaki and S. Maekawa. 1995. Water stress effects on ribulose-1,5-bisphosphate carboxylase and its relationship to photosynthesis in sunflower leaves. *In* Photosynthesis from Light to Biosphere. Ed. P. Mathis. Kluwer Academic Publishers, The Hague, pp 597–600.

Kanechi, M., N. Uchida, T. Yasuda and T. Yamaguchi. 1996. Non-stomatal inhibition associated with inactivation of rubisco in dehydrated coffee leaves under unshaded and shaded conditions. Plant Cell Physiol. 37:455–460.

Kirschbaum, M.U.F. and G.D. Farquhar. 1984. Temperature dependance of whole-leaf photosynthesis in *Eucalyptus pauciflora* Sieb. ex Spreng. Aust. J. Plant Physiol. 11:519–538.

Küppers, M. and E.-D. Schulze. 1985. An empirical model of net photosynthesis and leaf conductance for the simulation of diurnal courses of CO_2 and H_2O exchange. Aust. J. Plant Physiol. 12: 513–526.

Lal, A. and G. Edwards. 1996. Analysis of inhibition of photosynthesis under water stress in the C_4 species *Amaranthus cruentus* and *Zea mays*: electron transport, CO_2 fixation and carboxylation capacity. Aust. J. Plant Physiol. 23:403–412.

Lambers, H., F.S. Chapin, III and T.J. Pons. 1998. Plant physiological ecology. Springer-Verlag, New York, 540 p.

Lauer, M.J. and J.S. Boyer. 1992. Internal CO_2 measured directly in leaves. Abscisic acid and low leaf water potential cause opposing effects. Plant Physiol. 98:1310–1316.

Leverenz, J. and P. Jarvis. 1979. Photosynthesis in Sitka spruce. VIII. The effects of light flux density and direction on the rate of net photosynthesis and the stomatal conductance of needles. J. Appl. Ecol. 16:919–932.

Leverenz, J., S.F.C.-M. Pilstrom and G. Sammuelsson. 1990. The effects of photoinhibition on the photosynthetic light response curve of green plant cells (*Chlamydomonas reinhardtii*). Planta 182: 161–168.

Loewenstein, N.J. and S.G. Pallardy. 1998*a*. Drought tolerance, xylem sap abscisic acid and stomatal conductance during soil drying: a comparison of young plants of four temperate deciduous angiosperms. Tree Physiol. 18:421–430.

Loewenstein, N.J. and S.G. Pallardy. 1998*b*. Drought tolerance, xylem sap abscisic acid and stomatal conductance during soil drying: a comparison of canopy trees of three temperate deciduous angiosperms. Tree Physiol. 18:431–439.

Nash, L.J. and W.R. Graves. 1993. Drought and flood stress effects on plant development and leaf water relations of five taxa of trees native to bottomland habitats. J. Am. Soc. Hortic. Sci. 118:845–850.

Parker, W.C., S.G. Pallardy, T.M. Hinckley and R.O. Teskey. 1982. Seasonal changes in tissue water relations of three woody species of the *Quercus–Carya* forest type. Ecology 63:1259–1267.

Pearcy, R., O. Björkman, M.M. Caldwell, E. Keeley, R.K. Monson and B.R. Strain. 1987. Carbon gain by plants in natural environments. Bioscience 37:21–29.

Prioul, J. and P. Chartier. 1977. Partitioning of transfer and carboxylation components of intracellular resistance to photosynthetic CO_2 fixation: a critical analysis of the methods used. Ann. Bot. 41: 789–800.

Raven, P.H., R.F. Evert and S.E. Eichhorn. 1992. Biology of plants. Worth Publishers, New York, 791 p.

Roberts, S.W. and K.R. Knoerr. 1977. Components of water potential estimated from xylem pressure measurements in five tree species. Oecologia 28:191–202.

Robichaux, R.H. 1984. Variation in the tissue water relations of two sympatric Hawaiian Dubautia species and their natural hybrid. Oecologia 65:75–81.

Sun, Z.J., N.J. Livingston, R.D. Guy and G.J. Ethier. 1996. Stable carbon isotopes as indicators of increased water use efficiency and productivity in white spruce (*Picea glauca* (Moench) Voss) seedlings. Plant Cell Environ. 19:887–894.

Syvertsen, J.P., J. Lloyd, C. McConchie, P.E. Kriedemann and G.D. Farquhar. 1995. On the site of biophysical constraints to CO_2 diffusion through the mesophyll of thick hypostomatous leaves. Plant Cell Environ. 18:149–157.

Terashima, I. 1989. Productive structure of a leaf. *In* Photosynthesis. Ed. W.R. Briggs. Alan R. Liss, New York, pp 207–226.

Terashima, I., S.C. Wong, C.B. Osmond and G.D. Farquhar. 1988. Characterization of non-uniform photosynthesis induced by abscisic acid in leaves having different mesophyll anatomies. Plant Cell Physiol. 29:385–394.

Tezara, W. and D. Lawlor. 1995. Effects of water stress on the biochemistry and physiology in sunflower. *In* Photosynthesis: From Light to Biosphere. Ed. P. Mathis. Kluwer Academic Publishers, The Hague, 625 p.

Townsend, A.M. and B.R. Roberts. 1973. Effect of moisture stress on red maple seedlings from different seed sources. Can. J. Bot. 51: 1989–1995.

Tschaplinski, T.J., G.M. Gebre and T.L. Shirshac. 1998. Osmotic potential of several hardwood species as affected by manipulation of throughfall precipitation in an upland oak forest during a dry year. Tree Physiol. 18:291–298.

von Caemmerer, S. and G.D. Farquhar. 1981. Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. Planta 153:376–387.

Zhang, J.W., J.D. Marshall and B.C. Jaquish. 1993. Genetic differentiation in carbon isotope discrimination and gas exchange in *Pseudotsuga menziesii*. A common garden experiment. Oecologia 93:80–87.

Zwack, J.A., W.R. Graves and A.M. Townsend. 1998. Leaf water relations and plant development of three freeman maple cultivars subjected to drought. J. Am. Soc. Hortic. Sci. 123:371–375.