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 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 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 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 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 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 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 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 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).
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.

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

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 ($\Psi_w$) and stable carbon isotope discrimination ($\Delta$) ($n = 6$). 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 were 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 ($A_{\text{net}}$) and leaf conductance ($g_c$) 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 $\mu$mol 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 $\delta$ values ($^\%e$) were calculated as:

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

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 ($\Delta$), which currently has a deviation ($\delta_a$) 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 ($D^2 H$).

**Data analysis** Gas exchange data were normalized by log transformation. Treatment effects on growth, gas exchange, ABA concentration and $\Psi_o$ were evaluated by analysis of variance (ANOVA) with a univariate general linear model. Provenance variation in the $\Psi_o$ 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 provenresses 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 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 ($\Psi_{sw}$), which was determined to be small (= 0.1 MPa) in our experiments. Osmotic adjustment was calculated as the difference in $\Psi_{sw}$ 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 ($V_{\text{max}}$) and maximum rate of coupled photosynthetic electron transport ($J_{\text{max}}$) 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 ($A_{\text{sat}}$: 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 Flanagan and Jefferies 1988; Geber and Dawson 1997). To estimate $V_{\text{max}}$ and $J_{\text{max}}$ 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 CO₂ concentration, leaf temperature and VPD were set identical to those in the A–C 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 (Rd) was calculated with the modeled parameter, which was determined by curve fitting (φ), and the light compensation point (Ic) estimated from the intersection of the regression line with the x-axis. Apparent quantum yield (Qapp) 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 Jmax from the A–C curve analysis, we wanted to verify our findings by arbitrarily holding Jmax constant. By calculating an estimate of J from the independent light curve data set and making Jmax the same for wet and dry sites, we avoided biasing our results:

\[
J = Q_{app}I + J_{max} - \sqrt{((Q_{app}I + J_{max})^2 - 4\Theta Q_{app}I J_{max})/2\Theta}
\]

where I is a light-saturated rate of photosynthesis for red maple (900 µmol m⁻² s⁻¹), Jmax 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 Aₙₑₜ 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 Aₙₑₜ was similar among the eight provenances.

The univariate general linear model analysis of variance for Aₙₑₜ and stomatal conductance (gs) indicated a significant difference (P < 0.001) between wet- and dry-site provenances. The repeated measures analysis of variance for ABA and predawn Ψw indicated that there was a significant (P < 0.01) difference between wet- and dry-site provenances. Under well-watered conditions, mean Aₙₑₜ 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 gs 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 (W¹ and WII, respectively; Figure 1B). Abscisic acid concentration and Ψw did not differ among provenances under well-watered conditions (W¹ and WII; Figures 1C and 1D). Under drought conditions, both wet- and dry-site Aₙₑₜ, gs and Ψw declined, whereas ABA concentration increased. At the peak of the first drought (D¹), Aₙₑₜ and gs declined to 5.3 µmol m⁻² s⁻¹ and 0.06 mol m⁻² s⁻¹, re-

Figure 1. Net photosynthesis (Aₙₑₜ), stomatal conductance to water vapor (gₛ), abscisic acid concentration (ABA) and predawn leaf water potential (Ψw) subsetted four consecutive times in order to pair ABA and Ψw 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: W¹ = well-watered conditions at the beginning of the study; D¹ = the first peak drought (= 35% less than container capacity); WII = time point at the end of a 10-day post-drought recovery period (plants were well watered for 10 days after D¹ was reached); and DII represents the second drought peak.
respectively, 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 Ψw values were similar in both provenance groups (−1.29 and −1.34 MPa for encantences. Differences in osmotic potential with no interactions between treatment and site in either year’s analysis.

Experiment 2: water availability and tissue water relations
There were no drought or provenance effects on leaf relative water content at zero turgor (Ψπ) or average bulk modulus of elasticity (εavg) (Table 2).

Under well-watered conditions, Ψπ and Ψπl of wet-provenance seedlings were not significantly different from values for dry-provenance seedlings (Table 2). Furthermore, there were no treatment effects on Ψπ, or Ψπl of seedlings of wet-site provenances (Table 2). However, drought significantly lowered Ψπ and Ψπl of seedlings of dry-site provenances (Table 2), whereas mean Ψπ and Ψπl of seedlings of wet-site provenances increased slightly in the drought treatment (Table 2). Seasonal changes in Ψπ, Ψπl, Rπ and εavg showed no significant differences in well-watered seedlings of wet- or dry-site provenances.

Measurement of osmotic potential with the vapor pressure osmometer (Ψπvp) supported the conclusion that there was no real difference in Ψπ 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 Ψπvp 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 Ci 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 Ci curves. Seedlings of wet-site provenances had slightly greater carboxylation rates than seedlings of dry-site provenances, as indicated by slightly higher mean Vcmax estimates, initial slopes derived from regression analyses of the A versus Ci curves, and a scatter plot with trend lines of Ci values below 200 µmol mol⁻¹ (Table 3 and Figure 3). Estimates of maximum rate of ribulose-1,5-bisphosphate regeneration (Jmax) 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, CO₂ assimilation was always higher in wet-site provenances than in dry-site provenances; light-saturated photosynthesis, Amax, gS, Qapp and Rq 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

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.
provenances than in wet-site provenances, and $A_{\text{max}}$ 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 $Q_{\text{app}}$ 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 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: $\Psi_o$ = osmotic potential at 100% relative water content (RWC); $\Psi_{\text{osvp}}$ = psychrometric osmotic water potential at 100% RWC; $\Psi_{\text{tlp}}$ = osmotic potential at the turgor loss point; $R_{\text{tlp}}$ = RWC at the turgor loss point; and $\varepsilon_{\text{avg}}$ = mean modulus of elasticity.

<table>
<thead>
<tr>
<th>Provenance</th>
<th>Weight loss (%)</th>
<th>$\Psi_o$ (MPa)</th>
<th>$\Psi_{\text{osvp}}$ (MPa)</th>
<th>$\Psi_{\text{tlp}}$ (MPa)</th>
<th>$R_{\text{tlp}}$ (%)</th>
<th>$\varepsilon_{\text{avg}}$</th>
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<tr>
<td><strong>Well-watered treatment</strong></td>
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<td>−1.21 b*</td>
<td>−1.02 a*</td>
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<td>−1.18 a</td>
<td>−1.00 a</td>
<td>92.7 a</td>
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<td>40</td>
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<td>−1.18 a</td>
<td>−0.95 a</td>
<td>92.4 a</td>
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<td>−1.01 b *</td>
<td>−1.25 b*</td>
<td>−1.07 b*</td>
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<td>15.82 a</td>
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<td>−1.31 b*</td>
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<td>92.5 a</td>
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<td></td>
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<td>−1.30 b*</td>
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</tr>
<tr>
<td></td>
<td>40</td>
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<td>−1.37 b*</td>
<td>−1.27 b*</td>
<td>90.4 a</td>
<td>13.27 a</td>
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Table 3. The CO$_2$ response gas exchange parameters of well-watered red maple seedlings of wet- and dry-site provenances. Maximum carboxylation rate ($V_{\text{cmax}}$), estimates of the maximum rate of ribulose-1,5-bisphosphate regeneration ($J_{\text{max}}$) 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 ($\Gamma^*$) was calculated for each individual curve.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wet-site</th>
<th>Dry-site</th>
<th>$P$-Value$^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{cmax}}$ (µmol m$^{-2}$ s$^{-1}$)</td>
<td>77.3 ± 2.9</td>
<td>60.9 ± 2.6</td>
<td>*</td>
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<tr>
<td>$J_{\text{max}}$ (µmol m$^{-2}$ s$^{-1}$)</td>
<td>189.1 ± 5.0</td>
<td>109.4 ± 5.5</td>
<td>**</td>
</tr>
<tr>
<td>$\Gamma^*$ (µmol mol$^{-1}$)</td>
<td>132.5 ± 2.4</td>
<td>122.2 ± 2.6</td>
<td>ns</td>
</tr>
<tr>
<td>$S_l$ (%)</td>
<td>37 ± 0.0</td>
<td>43 ± 0.0</td>
<td>*</td>
</tr>
</tbody>
</table>

$^1$ 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; µmol 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 K"uppers and Schulze (1985).

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>Dry-site</th>
<th>P-Value¹</th>
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</thead>
<tbody>
<tr>
<td>A_{max}</td>
<td>9.60 ± 0.47</td>
<td>6.79 ± 0.50</td>
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<td>A_{max} (model)</td>
<td>9.66 ± 0.48</td>
<td>6.91 ± 0.49</td>
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<td>R_d</td>
<td>-1.86 ± 0.07</td>
<td>-1.44 ± 0.09</td>
<td>**</td>
</tr>
<tr>
<td>R_d (model)</td>
<td>-1.74 ± 0.07</td>
<td>-1.24 ± 0.06</td>
<td>***</td>
</tr>
<tr>
<td>g_s</td>
<td>0.14 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>***</td>
</tr>
<tr>
<td>Q_{app} (10⁻²)</td>
<td>3.63 ± 0.12</td>
<td>2.79 ± 0.14</td>
<td>**</td>
</tr>
<tr>
<td>I_c (model)</td>
<td>39.14 ± 2.05</td>
<td>36.64 ± 2.73</td>
<td>ns</td>
</tr>
<tr>
<td>J</td>
<td>27.48</td>
<td>21.99</td>
<td>na</td>
</tr>
</tbody>
</table>

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 (1998a, 1998b) 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 Ψ_w, g_s, and Ψ_n, 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 adaptive 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 (Ψ_m) and zero turgor (Ψ_min) have been reported for red maple (Roberts and Knorr 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 Ψ_w and Ψ_min. 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, The diffusion resistance created by stomatal patchiness could limit
the CO₂ response (Cornic and Briantais 1991, Brestic et al. 1995, Lal and Edwards 1996), yet be indistinguishable from metabolic regulation (Graun 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–C₅ 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, CO₂ assimilation is regulated by the intrinsic photosynthetic capacity of the mesophyll and by CO₂ 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 provenances 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ concentration and PPFD. As indicated by the A–C₅ analysis, wet- and dry-site progeny appear to be limited by both electron transport capacity and carboxylation capacity at the transition from CO₂ 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 CO₂ 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**


