Genetic Variation in Leaf Morphology and Plant and Tissue Water Relations During Drought in Cercis canadensis L.

Marc D. Abrams

ABSTRACT. Genetic variation in plant and tissue water relations in response to drought, and in leaf morphology, were examined in greenhouse-grown Cercis canadensis L. (eastern redbud) seedlings originating from seed obtained in three contrasting habitats: a relatively xeric Kansas prairie, a Kansas gallery forest understory, and a relatively mesic Indiana understory. Kansas prairie redbud maintained significantly greater leaf conductance ($g_{\text{w}}$) during a 13-day drought than did Kansas and Indiana understory redbud, despite similar decreases in leaf water potential ($\psi_{l}$) in all three sources. Moreover, Kansas prairie redbud had significantly lower osmotic potentials at full and zero turgor compared to Indiana redbud at both the early and later stages of drought. Kansas understory redbud was the only source to undergo a significant decrease in relative water content at zero turgor and the bulk modulus of elasticity during drought. Kansas prairie redbud leaves were smaller and thicker and had higher specific leaf mass (i.e., more xerophytic) than understory redbud leaves. Kansas understory redbud had intermediate characteristics in terms of $g_{\text{w}}$ at the early to middle stages of drought, osmotic potentials at high and low $\psi_{l}$, and leaf area and thickness compared to Kansas prairie and Indiana understory redbud. These results suggest that genetically controlled physiological and morphological adaptation has occurred in redbud that should facilitate its survival in greatly contrasting habitats. For. Sci. 34(1):200–207.

ADDITIONAL KEY WORDS. Ecophysiology, Kansas prairie, gallery forest, Indiana understory, leaf morphology.

Species with extensive geographic ranges often exhibit genotypes that are adapted to local environmental conditions (Berry and Bjorkman 1980). Environmental gradients involving temperature, light, relative humidity, and precipitation have been related to distinct physiological differentiation among populations (Slatyer and Ferrar 1977, Ledig and Koroboro 1983, Gurevitch et al. 1986). Many species also are capable of physiological plasticity in response to varying environmental conditions, a phenomenon that does not involve genetic differences (Wallace and Dunn 1980, Buzzaz and Carlson 1982, Roy and Mooney 1982).

Eastern redbud (Cercis canadensis L.) is widely distributed in the Eastern Deciduous Forest, and it is generally described as a small, shade-tolerant tree that grows in the understory of closed forests on moist, rich sites (Rochow 1972, Rogers and Anderson 1979, Elias 1980). In eastern Kansas, redbud is common in oak-dominated gallery forest understories, but it also frequently invades unburned prairie, growing in full sunlight (Abrams 1986a,b). A study of redbud in that region reported a degree of physiological plasticity in water relations and leaf morphology for saplings growing in the prairie compared with those located in the understory (Abrams

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In addition, prairie redbud had the capacity to maintain high leaf conductances at relatively low leaf water potentials. That study, however, did not investigate possible genetic differences in understory and prairie-grown redbud populations. This study was undertaken to examine genetic variation in water relations in response to drought, and leaf morphology for redbud seedlings from an eastern Kansas prairie and gallery forest understory and from a southwestern Indiana forest understory.

**METHODS AND MATERIALS**

**STUDY SITE DESCRIPTION**

Kansas seeds were collected during September 1986 from understory redbud in bur oak (Quercus macrocarpa) and chinquapin oak (Q. muehlenbergii) dominated gallery forests and from open-grown redbud in Andropogon—Panicum—Sorghastrum dominated tallgrass prairie on Konza Prairie near Manhattan in northeast Kansas (39°05'N, 96°35'W). Indiana seeds were collected during September 1986 from understory redbud in Hemmer Woods near Princeton in southwestern Indiana (38°20'N, 87°35'W), which is dominated by mixed oak species (Q. alba, Q. rubra, and Q. shumardii).

The climates of the study areas are predominantly continental, but Kansas is more xeric and has greater seasonal temperature extremes than Indiana (Court 1974). Compared to southwestern Indiana, northeastern Kansas experiences 23% less annual precipitation (1091 mm versus 835 mm), 27% higher pan evaporation (870 mm versus 1200 mm), and 11% greater annual total sunshine (2700 h versus 3000 h; Court 1974).

**SEED GERMINATION**

Redbud seeds have hard, impermeable seed coats in addition to internal dormancy (Schopmeyer 1974). The pregermination treatment used in this study was scarification by nicking the radicle end of the seed, followed by 7–8 wk of cold, moist stratification at 2–3°C. Pretreated seeds were then planted in a potting mixture of peat, loam, sand, vermiculite and perlite (2:2:1.5:1:1) in late November to early December 1986. Germination ranged from 25–35% for all three sources.

**PLANT WATER RELATIONS**

Seedlings were grown in an artificially lighted greenhouse under Sylvania "Growlux" and Westinghouse "Agro-Lite" fluorescent bulbs. Daily photoperiod was 15 h and photosynthetic photon flux density (PPFD) was approximately 100 μmol m⁻² s⁻¹ at the seedling canopies. Diurnal temperature and relative humidity ranged from 18–23°C and 25–33%, respectively. The seedlings were planted in 16 × 18 cm (3620 cm³) plastic pots one month prior to the experiment, kept well watered, and were periodically rotated to minimize possible positional effects associated with environmental gradients in the greenhouse.

Five, 4-month old seedlings from each of the three sources were selected for the water relations study and were randomly arranged on the greenhouse bench. On April 6, 1987, the seedlings were watered to soil capacity, after which time water was withheld for the duration of the 13-day study. At 1–2 day intervals, leaf conductance to water vapor diffusion (g₅₅) was measured on the abaxial surface (redbud is hypostomatic) of 3–4 mature, fully expanded leaves at the midcanopy on each of the 5 seedlings (15–17 leaves per source) with a transient diffusion porometer (MK3, Delta-T Devices, Cambridge, England). The porometer was calibrated in the greenhouse prior to each set of measurements. At 4-day intervals, starting at the first day after cessation of watering (Day 1), predawn leaf water potential (Ψₑₒᵥ) was estimated with a pressure chamber (Model 600, PMS Instrument Co., Corvallis, Oregon) on 3–5 leaves from different plants for each source (Scholander et al. 1965). Soil moisture content was measured gravimetrically from a sample taken at the pot center for each seedling at Day 13.
Tissue Water Relations
On the first and thirteenth days after cessation of watering, fully expanded leaves from each source were collected from the greenhouse, rehydrated in the laboratory overnight, and subjected to pressure-volume analysis. Three leaves from different plants, randomly chosen from several collected for each source, were used at each sampling date. Methods employed for constructing the curves were similar to those of Robichaux (1984), in which the weight and water potential of leaves were periodically measured as they dried under ambient conditions on the laboratory bench. From the curves, the following parameters were derived: the osmotic potential at full turgor (ϕ₀₀) and zero turgor (ϕ₀), relative water content at zero turgor (RWC₀) and the bulk modulus of elasticity (e), calculated according to Fanjul and Rosher (1984).

Leaf Morphology
Leaf area and thickness, specific leaf mass, stomatal density, and guard cell length were measured on fully expanded, mature leaves from five plants for each of the three redbud sources. Leaf area was measured using an area meter (Li-COR Model LI-3100, Lincoln, Nebraska). Stomatal density and guard cell length were measured with cellulose acetate impressions of the lower leaf surface (Payne 1970). Free-hand transverse sections of leaves taken from the approximate midpoint between the midrib and leaf margin were measured under a light microscope for leaf thickness. Specific leaf mass was determined by weighing oven-dried (80°C, 48 h) sections of fresh material of known area.

Statistical analysis of the data was accomplished using a one-way ANOVA and Scheffe’s multiple-range test at P < 0.05 (Steel and Torrie 1960, Hicks 1982). Leaf conductance data were averaged per plant prior to analysis, resulting in five replicates per treatment.

Results
Plant Water Relations
Predawn ψleaf during the four sample periods (Figure 1) and soil moisture content at Day 13 (range = 10.5 – 11.5%) were not significantly different among the three redbud sources. Predawn ψleaf remained relatively high (> −0.70 MPa) through five days without water, but it significantly declined to an average value of −1.75 MPa (range = −1.63 to −1.82 MPa) for the three sources by Day 13.

Leaf conductance (gₑₒ) significantly decreased for all seedlings during the drought (Figure 1). A significant decrease in gₑₒ occurred between Days 1–5 for all sources, despite only a slight decrease in predawn ψleaf. Kansas prairie redbud maintained significantly greater gₑₒ than did the Kansas understory and Indiana seedlings throughout the drought, except on Day 1 when no statistical difference in gₑₒ was detected between the Kansas prairie and understory seedlings. During days 1–5, Kansas understory redbud had greater gₑₒ than did leaves of the Indiana source, but no significant difference in gₑₒ was seen between these sources on Days 6–13 (Figure 1). At the end of the experiment, gₑₒ for Kansas understory redbud had decreased by 81%, compared to decreases of 70% and 67% in Indiana and Kansas prairie redbud, respectively.

Tissue Water Relations
Kansas prairie redbud had significantly lower values for ϕ₀₀ and ϕ₀ than did Indiana redbud at both high ψleaf (Day 1) and low ψleaf (Day 13, Table 1). Kansas understory redbud had significantly lower ϕ₀ at low ψleaf than that of Indiana redbud. No statistical difference was seen in the osmotic parameters between the two Kansas sources, nor was there a decrease in these parameters from high to low ψleaf for any of the sources.

Relative water content at zero turgor (RWC₀) and the bulk modulus of elasticity (e) did not differ among the three sources at either high or low ψleaf. However, RWC₀
FIGURE 1. Changes in leaf conductance to water vapor diffusion and predawn leaf water potential (averaged for all sources) during a 13-day drought for *Cercis canadensis* (redbud) seedlings from 3 different habitats. Vertical bars represent the standard error of the mean.

and $e$ decreased significantly in Kansas understory redbud from high to low $\Psi_{\text{leaf}}$; these changes were not detected in the other sources.

**Leaf Morphology**

Substantial differences in leaf morphology were evident among the redbud sources (Table 2). Leaves of Kansas prairie redbud were significantly smaller and thicker and had higher specific leaf mass than were leaves of Kansas and Indiana understory redbud. Both Kansas sources had greater stomatal density, and Kansas understory redbud had smaller guard cells compared to those of the Indiana source.
TABLE 1. Summary of tissue water relation variables ($\bar{\bar{\psi}} \pm s.e.$) derived from pressure-volume analysis of the three redbud sources. Means in a column followed by the same letter are not significantly different. $\psi_{w}^{0}$ = osmotic potential at full turgor; $\psi_{l}$ = osmotic potential at zero turgor; RWC$_{a}$ = relative water content at zero turgor; $\epsilon$ = bulk modulus of elasticity.

<table>
<thead>
<tr>
<th>Drought status and seed source</th>
<th>$\psi_{w}^{0}$ (MPa)</th>
<th>$\psi_{l}$ (MPa)</th>
<th>RWC$_{a}$ (%)</th>
<th>$\epsilon$ (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High $\psi_{l}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kansas prairie</td>
<td>$-2.19 \pm 0.08^{a}$</td>
<td>$-2.43 \pm 0.12^{a}$</td>
<td>90.1 $\pm 0.71^{b}$</td>
<td>16.76 $\pm 0.70^{b}$</td>
</tr>
<tr>
<td>Kansas understory</td>
<td>$-2.02 \pm 0.07^{a,b}$</td>
<td>$-2.20 \pm 0.10^{a}$</td>
<td>91.5 $\pm 0.63^{b}$</td>
<td>18.57 $\pm 1.12^{b}$</td>
</tr>
<tr>
<td>Indiana understory</td>
<td>$-1.88 \pm 0.06^{a,c}$</td>
<td>$-2.12 \pm 0.10^{a}$</td>
<td>89.8 $\pm 0.40^{b}$</td>
<td>14.16 $\pm 1.13^{b}$</td>
</tr>
<tr>
<td>Low $\psi_{l}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kansas prairie</td>
<td>$-2.15 \pm 0.07^{a}$</td>
<td>$-2.39 \pm 0.07^{a}$</td>
<td>88.1 $\pm 0.72^{a}$</td>
<td>13.16 $\pm 1.39^{a}$</td>
</tr>
<tr>
<td>Kansas understory</td>
<td>$-2.03 \pm 0.09^{a,b}$</td>
<td>$-2.25 \pm 0.06^{a}$</td>
<td>86.7 $\pm 0.68^{b}$</td>
<td>11.38 $\pm 0.18^{c}$</td>
</tr>
<tr>
<td>Indiana understory</td>
<td>$-1.77 \pm 0.04^{a,c}$</td>
<td>$-2.00 \pm 0.01^{c}$</td>
<td>88.6 $\pm 0.42^{a}$</td>
<td>11.15 $\pm 0.29^{a}$</td>
</tr>
</tbody>
</table>

DISCUSSION

Based on microenvironmental conditions and regional differences in climate, environments for the redbud sources used in this study might be ranked from mesic to xeric as: (1) Indiana understory, (2) Kansas understory, and (3) Kansas prairie. Many of the differences in plant and tissue water relations and leaf morphology reported in this study were correlated with this ranking.

In terms of plant water relations, Kansas prairie seedlings maintained higher $g_{ww}$ compared to Indiana and Kansas understory seedlings throughout the imposed drought, despite similar decreases in predawn $\psi_{l,\text{leaf}}$ in all three sources. These findings are similar to those reported in a field study in Kansas in which prairie redbud had significantly greater $g_{ww}$ than understory redbud at both early and midseason, when soil and leaf water potentials were high and low, respectively (Abrams 1986b). In that study, understory redbud had a decrease in midday $g_{ww}$ from early to midseason, whereas no corresponding decrease in $g_{ww}$ was observed in prairie redbud. Even at a $\psi_{l,\text{leaf}}$ of $-2.7$ MPa, redbud saplings growing in the prairie had $g_{ww}$ values over 6.0 mm/s (Abrams 1986b). In contrast, Kansas prairie redbud in this study had a significant decrease in $g_{ww}$ with increasing drought. This difference in the relationship of $g_{ww}$ with decreasing $\psi_{l,\text{leaf}}$ between greenhouse seedlings in this study and field plants may suggest a potential for physiological plasticity in addition to genetic adaptation for open-grown redbud in Kansas prairie.

Genetic variation in physiological characteristics has been related to environ-

TABLE 2. Leaf structural characteristics of the three redbud sources. Means ($\pm s.e.$) in a row followed by the same letter are not significantly different. Values in parentheses are the number of replications per leaf.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No. leaves</th>
<th>Kansas Prairie</th>
<th>Kansas Understory</th>
<th>Indiana Understory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf area (cm$^2$)</td>
<td>10</td>
<td>53.6 $\pm$ 3.1$^{a}$</td>
<td>74.6 $\pm$ 4.4$^{b}$</td>
<td>84.9 $\pm$ 2.2$^{b}$</td>
</tr>
<tr>
<td>Leaf thickness (mm)</td>
<td>10</td>
<td>0.134 $\pm$ 0.004$^{c}$</td>
<td>0.122 $\pm$ 0.004$^{d}$</td>
<td>0.111 $\pm$ 0.003$^{d}$</td>
</tr>
<tr>
<td>Specific leaf mass (mg/cm$^2$)</td>
<td>10</td>
<td>3.63 $\pm$ 0.22$^{a}$</td>
<td>2.73 $\pm$ 0.17$^{f}$</td>
<td>2.66 $\pm$ 0.08$^{f}$</td>
</tr>
<tr>
<td>Stomatal density (no./mm$^2$)</td>
<td>10 (3)</td>
<td>95.6 $\pm$ 8.1$^{a}$</td>
<td>102.9 $\pm$ 6.8$^{a}$</td>
<td>61.7 $\pm$ 6.9$^{b}$</td>
</tr>
<tr>
<td>Guard cell length (µm)</td>
<td>10 (3)</td>
<td>17.4 $\pm$ 0.3$^{a}$</td>
<td>16.4 $\pm$ 0.4$^{a}$</td>
<td>18.2 $\pm$ 0.9$^{a}$</td>
</tr>
</tbody>
</table>

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mental gradients for a wide variety of species (Mooney and Billings 1961, Berry and Bjorkman 1980, Gurevitch et al. 1986). Several studies have compared plant populations from coastal versus desert habitats or from points along altitudinal gradients and have found optimum photosynthesis at temperature or light levels that correspond to the population's habitat (Peary and Harrison 1974, Berry and Bjorkman 1980, Ledig and Koroboro 1983, Slatyer and Ferrar 1977). In terms of drought responses, eastern cottonwood (Populus deltoides) from sand-dune areas had higher net photosynthesis at low ψleaf than plants from strip mine or floodplain habitats (McGee et al. 1981). Genetic variation in photosynthesis and gsw was also reported for drought-stressed Populus clones in Washington (Scrascia-Mugnozza et al. 1986).

Values calculated for the tissue water parameters in this study were similar to those reported for several other Eastern Deciduous Forest tree species (Roberts et al. 1980, Parker et al. 1982, Abrams and Knapp 1986). The most distinct drought adaptation that emerged from the pressure-volume data were the lower osmotic potentials of Kansas prairie source redbud seedlings. Despite relatively low gsw values during the later stages of drought, Kansas understory redbud had lower Ψe at low ψleaf than did Indiana source seedlings and it was the only source to undergo a significant decrease RWCg and ε (i.e., more elastic leaf tissue) from high to low ψleaf. One somewhat unexpected result of this study was a general lack of change in the tissue water parameters from the early to later stages of drought. In general, drought-tolerant plants have the ability to osmotically adjust, to maintain cellular turgor at lower water potentials, and to increase tissue elasticity during dry periods (Parker et al. 1982, Abrams and Knapp 1986). These changes may not occur, however, if drought is imposed too rapidly (cf. Turner and Jones 1980). An average decrease in Ψleaf of 0.1 MPa/day for redbud in this study compares to that in a study of sorghum leaves in which only partial turgor was maintained (Turner and Jones 1980). In contrast, full turgor was maintained in sorghum, through increased osmotic adjustment, when ψleaf decreased at a rate of 0.008 MPa/day. Nonetheless, the relatively high gsw values recorded in Kansas prairie redbud in this study was probably related to its ability to maintain lower Ψc values during drought than the other redbud sources (cf. Turner and Jones 1980).

Genetic variation in tissue water relations has been investigated for several herbaceous species (Turner and Kramer 1980), but few examples exist for trees. In one such study, three populations of Eucalyptus viminalis displayed differences in cell water content and the shape of their moisture release curves (ψleaf versus relative water content) that were consistent with their drought resistances (Ladiges 1975). Genetic variation in osmotic adjustment and tissue elasticity has been reported for black walnut seedlings along a longitudinal transect from New York to Iowa (Parker and Pallardy 1983). However, the capacity for altering these parameters was not always consistent with the habitat conditions of the black walnut seed sources.

Differences in leaf morphology among the sources used in this study were generally consistent with previous comparisons of redbud individuals from xeric and mesic portions of its range and between redbud saplings growing in a Kansas prairie and nearby forest understory (Donselman and Flint 1982, Abrams 1986b). Redbud grown from seed from the western, more xeric portions of its range had thicker and smaller leaves with smaller and more dense stomata compared with plants from more mesic areas (Donselman and Flint 1982). These differences are consistent with those reported for a wide variety of xeric versus mesic species (Jackson 1967, Carpenter and Smith 1975, 1981, Abrams 1987). In this study, leaf area was reduced and leaf thickness, specific leaf mass, and stomatal density were higher for Kansas prairie redbud compared with plants of Indiana and/or Kansas understory sources. Leaf morphology of greenhouse-grown Kansas understory redbud in this study was similar to that observed in field-grown saplings, except for the latter having greater stomatal density (cf. Abrams 1986b). In contrast, many differences in leaf morphology were evident between prairie redbud in this study and saplings in the field, with field-grown plants having greater stomatal density, leaf thickness, and specific leaf mass and smaller leaf area. These differences may be attributed to the more xeric nature of the Kansas prairie and the greater light intensities developed in the

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field compared to the greenhouse environment used in this study. The observed differences between greenhouse and field-grown Kansas prairie sources suggest a capacity for morphological plasticity in redbud in addition to the genetic differences described above.

In summary, redbud is widely distributed in mature hardwood forest understories throughout the eastern United States, and in eastern Kansas it also grows in relatively xeric open prairies. Kansas prairie redbud maintained higher $g_{an}$ with decreasing $\psi_{leaf}$, exhibited lower values for osmotic parameters at high and low $\psi_{leaf}$, and produced more xerophytic leaves compared to understory redbud. Moreover, Kansas understory redbud had intermediate characteristics in terms of $g_{an}$ at the early to middle stages of drought, osmotic potentials at high and low $\psi_{leaf}$ and leaf area and thickness compared to Kansas prairie and Indiana understory redbud. Physiological and morphological adaptation through genetic or plastic changes, as suggested by this and an earlier study (Abrams 1986b), should facilitate the survival of redbud in these greatly contrasting habitats.

LITERATURE CITED


