Resistance to water stress of *Alnus maritima*: intraspecific variation and comparisons to other alders

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Abstract

*Alnus maritima* (seaside alder) is a rare and threatened woody species that occurs naturally as three disjunct subspecies. While its limited numbers and distribution have prompted investigations into the cause of its rarity, the aesthetic appeal and apparent flood resistance of *A. maritima* have led to a strong interest in its horticultural potential. The occurrence of all three subspecies of *A. maritima* exclusively in waterlogged soils in nature suggests that water availability may limit both the success of plants in the wild and the potential for their use in landscapes. We conducted three experiments to assess how *A. maritima* would respond to a wide range of root-zone moisture contents. First, we examined foliar gas exchange and growth of one clone during drought and flooding. Plants survived, grew, and avoided damage to and abscission of leaves during seven treatments that ranged from complete root-zone inundation to severe drought. Partial flooding and slight drought maximized plant dry weight, while total flooding and mild, moderate, and severe drought limited growth. High rates of photosynthesis were sustained except during severe drought. Next, we examined genotypic variation in drought resistance among the subspecies of *A. maritima* and compared the resistance of *A. maritima* to that of four other species of *Alnus*. Only subtle differences were detected among subspecies in how deficit irrigation influenced the physiology and development of plants. Of the five species evaluated, *A. maritima* and *Alnus nepalensis* most strongly expressed indicators of drought resistance. Lastly, a field trial with 270 seedlings of subspecies *oklahomensis* confirmed that *A. maritima* can be grown on soils with moisture contents ranging from dry (≈10% water by volume) to saturated. We conclude that, even though *A. maritima* thrives when a portion of its root zone is constantly inundated, it is more drought tolerant than some other species indigenous to well drained soils. The drought resistance of *A. maritima* can be attributed in part to its continued high rate of photosynthesis, fine control of stomatal conductance, increased specific leaf weight, and increased root:shoot ratio under conditions of drought. Considering the natural habitat to which *A. maritima* is restricted, our data illustrate that the niche a species occupies in nature does not necessarily represent how adapted the species may be to dissimilar environmental conditions. We also conclude that *A. maritima* and *A. nepalensis* show promise for increased use as ornamentals resistant to disparate soil water contents.

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Keywords: Seaside alder; *Alnus glutinosa*; *Alnus serrulata*; *Alnus nitida*; *Alnus nepalensis*; Drought and flood resistance; Stress tolerance; Photosynthetic rate; Water potential; Foliar gas exchange

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1. Introduction

Alnus maritima is the rarest of North American Alnus species and occurs as three disjunct subspecies in the United States: in Delaware and Maryland on the Delmarva Peninsula (subspecies maritima), in northwestern Georgia (subspecies georgiensis), and in south-central Oklahoma (subspecies oklahomensis) (Schrader and Graves, 2002). While its rarity and peculiar distribution have heightened interest in its conservation and ecology, its aesthetic appeal has sparked interest in its development as a horticultural crop. Efforts to identify factors contributing to the rarity of A. maritima have revealed physiological and ecological traits that seemingly represent competitive advantages in nature. Seed production and germinability of A. maritima is more than sufficient for plant replacement and range increase in the wild (Schrader and Graves, 2000a), and plants grow well under a wide range of soil-chemical conditions (Schrader and Graves, 2002). Although A. maritima is found only in relatively warm temperate climates, all three subspecies are extremely cold hardy (Schrader and Graves, 2003).

One determinant of ecological fitness that has not been examined sufficiently is the resistance of A. maritima to water stress. In nature, all three subspecies of A. maritima are isolated to habitats where their root systems are partially to completely submerged in fresh water (Furlow, 1979; Stibolt, 1981; Schrader and Graves, 2002). The restriction of plants to water-logged soils suggests that poor drought-stress resistance may be a factor limiting the distribution of A. maritima. We questioned whether A. maritima, an apparent obligate-wetland species, could be resistant to both drought and flood stress. Some wetland species have proven tolerant of both wet and dry soils. For example, bald cypress (Taxodium distichum) sustains positive relative growth and net assimilation rates during episodes of drought and flooding that evoke negative rates among other species (Naish and Graves, 1993). There are indications that A. maritima may be similarly resistant to water stress. Graves et al. (2002) showed that A. maritima sustained photosynthesis during prolonged flooding, and a study by Hennessey et al. (1985) suggested that A. maritima may have greater drought tolerance than do more common Alnus species. Observations of plants in the wild suggest that A. maritima subspecies georgiensis may be particularly adapted to dry soil conditions. Plants of this subspecies are found further above the water table than are plants of the other two subspecies within their habitats, but the consistently greater root:shoot ratio of subspecies oklahomensis suggests a possible morphological advantage for drier conditions (Schrader and Graves, 2002). A comprehensive analysis of all subspecies is needed to characterize their water-stress resistance thoroughly.

Our goals were to define the basic physiological responses of A. maritima to flooding and water-deficit stress, to determine the effects of flooding and water-deficit conditions on the growth and health of A. maritima, to compare the water-stress resistance of A. maritima to that of four other species of Alnus, to compare the water-stress resistance of the three subspecies of A. maritima, and then to apply these experimental findings to ecological and horticultural issues surrounding A. maritima and the other species of Alnus. As an initial step in evaluating the response of A. maritima to water stress, we measured leaf gas exchange, stem water potential, and growth of ramets of subspecies oklahomensis. Plants were exposed to partial and complete root-zone inundation and to four severities of deficit irrigation. In a second experiment, we evaluated the effects of water stress on the gas exchange and growth of seedlings of all three subspecies of A. maritima and of four other species of Alnus. Along with these greenhouse experiments, we measured survival and growth of seedlings of subspecies oklahomensis exposed to a wide range of soil-moisture conditions in the landscape for over 2 years. Our results characterize the water-stress resistance of A. maritima and four other species of Alnus, provide new insights regarding the cause of the rarity of A. maritima in the wild, and contribute new information to support the advancement of A. maritima as a nursery crop.

2. Materials and methods

2.1. Experiment 1: responses of a clone of subspecies oklahomensis to drought and flooding

2.1.1. Plant material and experimental design

Softwood cuttings with two or three nodes from one A. maritima (Marsh.) Muhl. ex Nutt. were taken...
on 11 June 1999. The greenhouse-grown stock plant was 2 years old and had been grown from seed collected from the natural population of *A. maritima* subspecies *oklahomensis* Schrader & Graves (latitude 34° 19′ 40′′N; longitude 96° 35′ 30′′W). Hormodin No. 2 (MSD AGVET, Merck & Co., Rahway, NJ) was used, and cuttings were placed in flats of coarse perlite on a greenhouse mist bench (Schrader and Graves, 2000b). Nine weeks later (13 August), rooted cuttings were potted singly in plastic pots (top diameter = 15.2 cm, height = 15 cm) in a mix of 2 perlite:2 *Sphagnum* peat:1 soil (by volume). Pots were placed in a completely randomized design in a glass-glazed greenhouse under high-pressure sodium lamps (400 W) that provided 16 h photoperiods. Plants were watered to container capacity that day and 2 weeks later with a solution of Peters® Excel® All-Purpose and Cal-Mag® (16.5N-2.2P-13.5K; Grace-Sierra Horticultural Products, Milpitas, CA) that contained N at 11.0 mM. Tap water was applied to plants once daily on all other days. The experimental design was a 7 × 2 factorial (LI-COR, Lincoln, NE), and dry weight of roots and shoots was determined. Fertilizer solution was applied instead of tap water to plants in all treatments at the first irrigation after severe-drought plants completed three drought cycles. Fertilizer solution was applied instead of tap water to plants in all treatments at the first irrigation after severe-drought plants completed three drought cycles. Flooded plants were fertilized at that time by flushing their root zones with fertilizer solution and then resuming inundation with tap water. Air in the greenhouse during treatments was 20–34 °C, relative humidity (RH) ranged from 12 to 71%, and maximum photosynthetically active radiation (PAR) on cloudless days was $1120 \mu$mol m$^{-2}$s$^{-1}$. Three randomly chosen plants of each plant size were harvested on 10 September. Surface area of leaf blades was determined with a LI-COR 3100 area meter (LI-COR, Lincoln, NE), and dry weight of roots and shoots was determined. The initial mean plant dry mass for the seven plant-size groups was 0.84, 0.91, 1.13, 1.30, 1.62, 1.99, and 3.12 g for size groups one through seven, respectively. Three of the remaining 21 plants per plant-size group were assigned randomly to each of seven root-zone moisture treatments. The seven levels of initial plant size were selected in order to examine how plant size affects species responses to water-deficit stress and to quantify any plant size-by-treatment interaction.

### 2.1.2. Initial harvest and subsequent treatments

Three randomly chosen plants of each plant size were harvested on 10 September. Surface area of leaf blades was determined with a LI-COR 3100 area meter (LI-COR, Lincoln, NE), and dry weight of roots and shoots was determined. The initial mean plant dry mass for the seven plant-size groups was 0.84, 0.91, 1.13, 1.30, 1.62, 1.99, and 3.12 g for size groups one through seven, respectively. Three of the remaining 21 plants per plant-size group were assigned randomly to each of seven root-zone moisture treatments. The seven levels of initial plant size were selected in order to examine how plant size affects species responses to water-deficit stress and to quantify any plant size-by-treatment interaction.

All plants within a plant-size group were harvested the day plants in the severe-drought treatment within the group completed their fifth cycle. Averaged across plant-size groups, the number of cycles completed for the other three drought treatments were 16, 10, and 7 cycles, respectively, for the slight-, mild-, and moderate-drought treatments. Average cycle lengths for the four drought treatments across plant-size groups were 2, 3, 5, and 7 days, respectively, for the slight-, mild-, moderate-, and severe-drought treatments. Moisture was measured once daily with a model HH1 Theta Probe for organic media with a ML1 sensor (Delta-T Services, Cambridge, England). Because the concept of permanent wilting percentage (PWP, soil water content at which plants remain wilted overnight) involves the integration of plant type, condition of rhizosphere, and substrate hydraulic properties (Fonteno, 1993), we chose to establish this parameter empirically rather than by extrapolation based on simple physical properties of the medium. The PWP was at a root-zone moisture content of ≤0.1 m$^{-3}$ and we used that moisture content for our severe-drought treatment. Fertilizer solution was applied instead of tap water to plants in all treatments at the first irrigation after severe-drought plants completed three drought cycles. Flooded plants were fertilized at that time by flushing their root zones with fertilizer solution and then resuming inundation with tap water. Air in the greenhouse during treatments was 20–34 °C, relative humidity (RH) ranged from 12 to 71%, and maximum photosynthetically active radiation (PAR) on cloudless days was $1120 \mu$mol m$^{-2}$s$^{-1}$.
The youngest fully expanded leaf on these plants was dried for 3 days. Surface area of leaf blades of these plants then was measured. Weights of all leaf blades, stems plus petioles, and root-zone moisture content were determined for each plant. Relative growth and net assimilation rates were calculated for each plant.

### Data analysis

We used the general linear models (GLMs) procedure of SAS/STAT®, Version 6.12 (1989–1996) to examine main effects, and the Tukey–Kramer method was used to separate means. Analysis of variance showed no interaction between the main effects of initial plant size and root-zone moisture treatment, so these were independent (Cochran and Cox, 1992) and could be analyzed and reported separately. Regression procedures of SAS/STAT® and JMP®, Version 3.2.6 (1989–1999) were used to assess effects of root-zone moisture content on photosynthesis and xylem water potential to evaluate effects of xylem water potential on foliar gas exchange. To meet requirements of continuous variability and to prevent skewing of the data set by the large increase in root-zone moisture, regressions over root-zone moisture excluded data from the total-flood treatment.

### Experiment 2: water stress in Alnus maritima and four other species of Alnus

#### Plant material and experimental design

We collected seeds of A. glutinosa (L.) Gaertn. in Ames, Iowa, and seeds of Alnus serrulata (Ait.) Willd. and A. maritima from plants in their native habitats. Seeds of Alnus nitida (Spach) Endl. and A. nepalensis D. Don indigenous to India were purchased. All seeds were stratified for 21 days at 4 °C, were placed in germination conditions on 1 January 2000, and germinated seeds were transferred to pots 8 days later as described by Schrader and Graves (2000a). On 14 March, seedlings were transplanted by using the same type of pots and medium, and the same methods, used in experiment one. The experimental design was a 5 × 2 factorial (Cochran and Cox, 1992) at the species level with five treatments of species (A. maritima, A. serrulata, A. glutinosa, A. nitida, and A. nepalensis) crossed with two treatments of root-zone moisture (control and drought). There was a 3 × 2 factorial at the infraspecific level with three treatments for subspecies of A. maritima (subspecies oklahomensis, georgiaensis Schrader & Graves, and maritima) crossed with two treatments of root-zone moisture (control and drought). Six seedlings from each of 57 maternal sources of A. maritima (19 sources from each of the subspecies) and from 1 maternal source each of the other 4 species were potted and randomized in 3 complete blocks in a glass-glazed greenhouse, with 2 plants from each maternal source in each block. No supplemental irradiance was provided. Plants were irrigated once every 1 or 2 days with tap water and fertilized once weekly with the same source and concentration of fertilizer used in experiment one.

#### Treatments

Treatments began 16 May. Length of the primary stem of each plant was measured, and, within blocks, one plant of each maternal source was randomly assigned to a drought treatment, the other to a control treatment. Pots with controls were maintained with the bottom 1.5 cm of their root zone in tap water. Five cycles of drought were imposed on the remaining plants by withholding irrigation until mean moisture of the root zones (measured as during experiment one) within combinations of seed sources and blocks decreased to 0.1 m³ m⁻³ for the first two cycles and to 0.05 m³ m⁻³ for the final three cycles. The PWP for this experiment occurred at a root-zone moisture content of ≈0.05 m³ m⁻³, a value that thus was used as the final root-zone moisture content for the drought treatment. All plants by seed-source groups were provided the same source and concentration of fertilizer as used previously at the end of each drought cycle for the first two cycles; N at 14 mM was used for
the remaining cycles. To rehydrate root medium after drought, pots were held in water for 24 h and drained. Air during treatments ranged from 23 to 33 °C, RH was 40–82%, and PAR was ≤ 1743 μmol m⁻² s⁻¹.

2.2.3. Measurements
Gas exchange was measured at least once weekly in the same way as in experiment one. Six plants of each subspecies of A. maritima, three from both treatments in each block (54 plants total), were randomly chosen for measurement, along with all plants of the other four species. Instantaneous transpiration efficiency was calculated by dividing net photosynthetic rate by transpiration rate. Destructive harvest of plants in both treatments within seed-source groups was completed as the fifth drought cycle ended. Surface area of leaves ≥1 cm long was measured. Length of the longest stem was measured, and weights of leaves, stems, and roots were determined after they dried at 67 °C for at least 48 h. The number of secondary stems and leaves >1 cm long were counted. The root:shoot dry weight ratio, stem elongation (change in the primary stem length during treatments), and the mean individual leaf area per plant was calculated by dividing total leaf area by leaf number. Leaf area ratio was calculated as described by Harper (1977).

2.2.4. Data analysis
Main effects and mean separations were determined as during experiment one. Data sets with unequal replication among groups were tested for homogeneity of variance by using Levene’s test, and non-homogeneous data were transformed by a log or square-root function. Means were calculated from raw data, while the mean-separation statistics were calculated from raw or transformed data as necessary. The Tukey-Kramer method was used for mean-separation statistics because it is considered more powerful than other methods for pair-wise comparisons of unequal groups (SAS Inst. Inc., 1989; Stevens, 1990), and it provides strong control of comparison- and experiment-wise error rates (Milliken and Johnson, 1984; SAS Inst. Inc., 1989). Individual seed sources of each subspecies of A. maritima were cross-classified with root-zone moisture treatments and therefore were averaged to obtain a representative mean for each subspecies. Regression procedures, conducted as during experiment one, tested effects of root-zone moisture content on leaf gas exchange. Regression curves were examined statistically at the species and subspecies levels by using the GLM procedure of SAS/STAT® to compare two reduced models (one quadratic for all taxa, parallel quadratics for all taxa) with the full model (separate quadratics for all taxa). When separate quadratics were warranted, regression curves were separated by pair-wise contrasts by using the contrast function of the GLM procedure of SAS/STAT® (SAS Inst. Inc., 1989; Statistician Philip Dixon, personal communication).

2.3. Experiment 3: survival and growth in the landscape in response to water supply

2.3.1. Site and experimental design
Seven-month-old seedlings of three half-sibling groups of subspecies oklahomensis were installed on a 40% slope of sandy-clay-loam soil (55% sand, 23% silt, and 22% clay) in Ames, IA, on 18 July 1998. Seed had been germinated and seedlings grown in a greenhouse as described by Schrader and Graves (2000a). Seedlings were installed in a randomized complete block design in nine parallel rows, each parallel to and at a different distance from a continuous waterway at the base of the slope. Rows were 2 m apart. Root zones of plants in the lowest row (row nine) were flooded continuously. Rows eight through one were at progressively higher elevations in an attempt to achieve progressively drier root zones. Each row contained 30 plants set 1 m apart and arranged as 10 randomized blocks; each block contained one seedling from each half-sibling group. All plants were irrigated as they established in 1998 and then were maintained without irrigation or fertilization through two more growth seasons. Soil moisture was measured once in 1999 and once in 2000 with a model HH1 Theta Probe for organic media with a ML1 sensor (Delta-T Services, Cambridge, England). Theta probe readings were taken at six evenly spaced locations within each row. Canopy volume (Schrader and Graves, 2002) and plant health were measured 21 September 1999 and 2000, and seasonal growth rate was calculated as the increase in canopy volume from 1999 to 2000. Health was rated subjectively...
from 1 to 10 (1 = least healthy, 10 = most healthy). Precipitation, potential evapotranspiration, and other climatic data were recorded daily by an automated weather station. The PWP for sandy-clay-loam soils is $\approx 0.13 \text{ m}^3 \text{ m}^{-3}$ soil moisture content (Kramer and Boyer, 1995).

2.3.2. Data analysis

The same software used previously revealed treatment effects via the GLM procedure, and the least significant difference test (LSD) was used to separate means. Our plot design allowed us to assess non-treatment effects across the plot in two ways: by using blocks within each row and by using second-level blocks (vertical columns of nine blocks, one from each of the parallel rows).

3. Results

3.1. Experiment 1: responses of a clone of subspecies oklahomensis to drought and flooding

All plants in the seven root-zone moisture treatments survived. Leaves on plants treated with moderate and severe drought wilted between irrigations, but no permanent damage resulted to leaves in any treatment, nor did any leaves abscise. Net photosynthesis was 63% lower during severe drought compared with the average across all other treatments (Table 1). Partial flooding led to a mean plant dry weight that was at least 15% greater than that of plants in any other treatment (Table 1). Total flooding evoked the lowest mean plant dry weight and led to a lower mean root-shoot ratio than that of most other treatments (Table 1). In contrast, daily irrigation led to a mean root-shoot ratio that was at least 31% greater than that associated with other treatments and to the highest ratio of root dry weight to leaf surface area (Table 1). Partially flooded plants had a comparatively high mean relative growth rate, though it was not different from the mean after slight drought (Table 1). Total flooding led to the lowest mean relative growth and net assimilation rates (Table 1).

Initial plant size affected the time required for the completion of each drought cycle. Initial plant size had either no effect on measured parameters (photosynthetic rate, root-shoot ratio, ratio of root dry weight to leaf area, relative growth rate, net assimilation rate) or plants remained in the same size ranking after treatments as they were before treatments (data not shown).

Quadratic functions best represented the relationships between root-zone moisture content and photosynthetic rate and between root-zone moisture and xylem water potential of this clone (Fig. 1). Photosynthesis increased with increasing root-zone moisture content to an optimum rate of 7.5 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$ at 0.360 m$^3$ m$^{-3}$ and then decreased with increasing root-zone moisture content (Fig. 1A). Xylem water potential increased with increasing root-zone moisture content to $-0.38 \text{ MPa}$ at 0.40 m$^3$ m$^{-3}$ and then decreased with

Table 1 Photosynthetic rate, plant dry weight, root-to-shoot ratio, root dry weight to leaf surface area ratio, relative growth rate, and net assimilation for each of seven treatments applied to one clone of Alnus maritima subspecies oklahomensis

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Photosynthetic rate$^a$ ($\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$)</th>
<th>Plant dry weight (g)</th>
<th>Root-shoot ratio</th>
<th>Ratio of root dry weight to leaf area (mg cm$^{-2}$)</th>
<th>Relative growth rate (mg g$^{-1}$ day$^{-1}$)</th>
<th>Net assimilation rate (mg cm$^{-2}$ week$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total flood</td>
<td>6.80 ab</td>
<td>4.32 d</td>
<td>0.22 d</td>
<td>2.01 a</td>
<td>29.8 d</td>
<td>2.29 b</td>
</tr>
<tr>
<td>Partial flood</td>
<td>6.89 ab</td>
<td>11.03 a</td>
<td>0.26 cd</td>
<td>1.94 c</td>
<td>58.2 a</td>
<td>3.94 a</td>
</tr>
<tr>
<td>Daily irrigation</td>
<td>5.78 b</td>
<td>7.35 c</td>
<td>0.47 a</td>
<td>3.90 a</td>
<td>45.6 c</td>
<td>3.66 a</td>
</tr>
<tr>
<td>Slight drought</td>
<td>6.66 ab</td>
<td>9.41 ab</td>
<td>0.36 b</td>
<td>2.80 b</td>
<td>52.7 ab</td>
<td>3.89 a</td>
</tr>
<tr>
<td>Mild drought</td>
<td>7.63 a</td>
<td>8.44 bc</td>
<td>0.27 cd</td>
<td>2.05 c</td>
<td>50.4 bc</td>
<td>3.47 a</td>
</tr>
<tr>
<td>Moderate drought</td>
<td>7.49 a</td>
<td>8.42 bc</td>
<td>0.33 bc</td>
<td>2.16 c</td>
<td>50.2 bc</td>
<td>3.33 a</td>
</tr>
<tr>
<td>Severe drought</td>
<td>2.52 c</td>
<td>7.68 bc</td>
<td>0.30 c</td>
<td>2.14 c</td>
<td>48.1 bc</td>
<td>3.26 a</td>
</tr>
</tbody>
</table>

Plants were grouped by size into seven groups with three plants per treatment in each group. There were 21 plants per treatment. We harvested the plants in each group at the end of the fifth cycle for plants in the severe drought treatment.

$^a$ Means for photosynthetic rate of plants just before final harvest ($N = 14$).

$^b$ Means within each column followed by the same letter are not different at $P \leq 0.05$ according to the Tukey-Kramer multiple comparison procedure. There was a treatment difference for all dependent variables at $P \leq 0.0001$. 

from 1 to 10 (1 = least healthy, 10 = most healthy). Precipitation, potential evapotranspiration, and other climatic data were recorded daily by an automated weather station. The PWP for sandy-clay-loam soils is $\approx 0.13 \text{ m}^3 \text{ m}^{-3}$ soil moisture content (Kramer and Boyer, 1995).

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Fig. 1. Photosynthesis (A) and stem water potential (B) as a function of volumetric root-zone moisture content for clones of one genotype of *A. maritima* subspecies *oklahomensis*. Photosynthetic rate, stem water potential, and volumetric root-zone moisture content were measured for each block when plants in the severe drought treatment had finished their fifth cycle of drought. Quadratic functions best fit the trends in these data ($P < 0.0001$). Equations are included in the figure along with the $r^2$ value for each.

Increasing root-zone moisture (Fig. 1B). A quadratic function also best described the relationship between xylem water potential and photosynthesis (Fig. 2A), which was highest, 6.96 μmol CO$_2$ m$^{-2}$ s$^{-1}$, at a water potential of −0.52 MPa. Stomatal conductance (Fig. 2B) and transpiration (Fig. 2C) increased linearly with increasing xylem water potential.

3.2. Experiment 2: water stress in *Alnus maritima* and four other species of *Alnus*

3.2.1. Foliar gas exchange

*A. maritima*, *A. nitida*, and *A. nepalensis* had the greatest net photosynthetic rates within both treatments (Table 2). Reductions in photosynthesis due to drought ranged from 13% (*A. nepalensis*) to 30% (*A. nitida*). Among plants in the control treatment,
A. nitida and A. nepalensis had the greatest stomatal conductance, had the greatest decrease in stomatal conductance due to drought (60 and 48%, respectively), yet also had the greatest stomatal conductance during drought (Table 2). The instantaneous transpiration efficiency of A. maritima was greater than that of all other species in the control treatment and greater than that of all species except A. nepalensis during drought (Table 2).

Net photosynthetic rate, stomatal conductance, and instantaneous transpiration efficiency as functions of root-zone moisture content were best described by quadratic functions. A single quadratic function was not appropriate for all five species, nor did parallel quadratics characterize the species adequately. Regression curves for photosynthesis by root-zone moisture showed peaks of optimum photosynthesis for all five species within the range of moisture that we measured (0.024–0.560 m $^3$ m $^{-2}$), but revealed differences among the species in the magnitude of their optimum photosynthetic rate and in their response to varying moisture conditions (Fig. 3A). A. nitida and A. nepalensis or much of it (A. nitida) (Table 2, Fig. 3A). The optimum root-zone moisture content for photosynthesis was lowest for A. glutinosa (0.316 m $^3$ m $^{-2}$) and highest for A. nitida (0.363 m $^3$ m $^{-2}$) (Table 2). Varying root-zone moisture conditions had the greatest effect on the photosynthesis of A. glutinosa, which showed the most pronounced decrease of the five species at sub- and supra-optimal moistures (Fig. 3A). A. maritima showed the least change in net photosynthesis at non-optimal root-zone moisture contents (Fig. 3A).

The net photosynthetic rate of A. maritima was greater than that of A. serrulata over the entire range of moisture and greater than that of A. glutinosa over most of the range. A. nepalensis was the only species that had a greater rate of net photosynthesis than A. maritima over the entire range of root-zone moisture contents (Fig. 3A).

Trends for stomatal conductance by root-zone moisture were similar for A. maritima, A. glutinosa, and A. serrulata (Fig. 3B). Regressions for all three of these species showed similar curves that reached their peak between 0.4 and 0.5 m $^3$ m $^{-3}$ and showed peak values within 23 mmol m $^{-2}$ s $^{-1}$ of each other (Table 2, Fig. 3B). Trends were different for A. nitida and A. nepalensis. Stomatal conductance of both species increased in a mildly quadratic fashion with increasing root-zone moisture over the entire range (Fig. 3B).

\textit{Alnus glutinosa} showed the highest optimal transpiration efficiency of the five species, but it, along with

### Table 2

Mean photosynthetic rate, stomatal conductance, and transpiration efficiency of plants from five species of \textit{Alnus} after control (partial flood) or drought treatments

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<thead>
<tr>
<th>Species</th>
<th>Photosynthetic rate (μmol CO$_2$ m$^{-2}$ s$^{-1}$)</th>
<th>Stomatal conductance (mmol m$^{-2}$ s$^{-1}$)</th>
<th>Instantaneous transpiration efficiency (μmol CO$_2$/mmol H$_2$O)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control Drought Regression optimum</td>
<td>Control Drought Regression optimum</td>
<td>Control Drought Regression optimum</td>
</tr>
<tr>
<td>A. maritima</td>
<td>$8.70 \pm b$ $7.17 \pm e$ $9.02 \pm (0.356)$</td>
<td>$310 b$ $194 c$ $287 (0.463)$</td>
<td>$2.58 a$ $2.73 a$ $2.95 (0.282)$</td>
</tr>
<tr>
<td>A. glutinosa</td>
<td>$8.77 c$ $5.74 d$ $9.08 (0.316)$</td>
<td>$286 b$ $185 c$ $291 (0.404)$</td>
<td>$2.08 cd$ $2.03 cd$ $2.97 (0.310)$</td>
</tr>
<tr>
<td>A. serrulata</td>
<td>$5.39 a$ $5.79 c$ $7.15 (0.347)$</td>
<td>$284 b$ $181 c$ $266 (0.469)$</td>
<td>$2.05 cd$ $1.98 cd$ $2.47 (0.328)$</td>
</tr>
<tr>
<td>A. nitida</td>
<td>$10.05 a$ $7.03 c$ $11.27 (0.363)$</td>
<td>$695 a$ $274 b$ $9.08 (0.358)$</td>
<td>$2.08 cd$ $2.03 cd$ $2.97 (0.310)$</td>
</tr>
<tr>
<td>A. nepalensis</td>
<td>$9.49 a$ $8.21 bc$ $10.12 (0.356)$</td>
<td>$520 a$ $268 b$ $268 (0.469)$</td>
<td>$2.17 bc$ $2.58 ab$ $2.86 (0.265)$</td>
</tr>
</tbody>
</table>

* Means within each pair of columns followed by the same letter are not different at \( P \leq 0.05 \) according to the Tukey–Kramer multiple comparison procedure. Values are the means of raw data. Mean separation statistics are for either raw data (if the raw data met the assumption of homogeneity of variance according to Levene’s test) or for data transformed to meet the requirement of homogeneity of variance. At the species level, \( N = 50 \) for \( A. \) maritima, \( N = 45 \) for \( A. \) glutinosa, \( N = 55 \) for \( A. \) serrulata, \( N = 42 \) for \( A. \) nitida, \( N = 51 \) for \( A. \) nepalensis.

* No optimum could be calculated for these species because stomatal conductance increased through the entire range of soil moisture.
A. nitida, showed the greatest decrease at moistures above and below their optimum (Table 2, Fig. 3C). Instantaneous transpiration efficiency of A. maritima was minimally affected by varying root-zone moisture conditions, and A. maritima showed greater efficiency than other species over the entire range of moisture, except for A. glutinosa and A. nitida near their optimum (Fig. 3C). A. nepalensis and A. maritima showed peak efficiency at lower root-zone moisture than the other three species, while efficiency of A. serrulata was optimal at comparatively high moisture (Table 2).

At the subspecies level, there were no differences among subspecies within each treatment for net photosynthetic rate and stomatal conductance, but there were treatment effects on these parameters for each of the subspecies. Relative to controls, photosynthesis and stomatal conductance were an average of 17 and 37% lower, respectively, among plants in the drought treatment. There was a treatment effect on transpiration efficiency only for subspecies oklahomensis, which had lower transpiration efficiency under the control treatment (2.45 μmol CO₂/mmol H₂O) than it did under drought (2.69 μmol CO₂/mmol H₂O) and had lower transpiration efficiency than subspecies georgiensis (2.68 μmol CO₂/mmol H₂O) under the control treatment. Regression analyses of measures of gas exchange as a function of root-zone moisture showed little difference in the responses of the three subspecies (data not shown).

Alnus species names and their corresponding line types appear in the legend. Regression curves within each graph followed by the same lower case letter are not different at P < 0.05 according to post-hoc contrast statistics of SAS. Variables were measured at least once weekly for up to 95 days. Quadratic functions best fit the trends in these data. Equations, along with the r² value and significance for each, appear below.

(A) Photosynthesis (A) = -48.9x² + 35.0x + 2.7, r² = 0.11, P = 0.0001. Alnus glutinosa: y = -98.8x² + 62.5x - 0.29, r² = 0.43, P < 0.0001. Alnus serrulata: y = -58.2x² + 40.4x + 0.13, r² = 0.15, P < 0.0001. Alnus nitida: y = -90.4x² + 65.6x - 0.15, r² = 0.57, P = 0.0001. Alnus nepalensis: y = -54.1x² + 38.5x + 3.3, r² = 0.26, P = 0.0001. (B) Stomatal conductance (B) = y, root-zone moisture = x. Alnus maritima: y = -988x² + 974x + 47, r² = 0.11, P < 0.0001. Alnus glutinosa: y = -174x² + 1413x + 4.9, r² = 0.31, P < 0.0001. Alnus serrulata: y = -114x² + 107x + 17.2, r² = 0.09, P = 0.004. Alnus nitida: y = -97.4x² + 1270x + 13.8, r² = 0.37, P < 0.0001. Alnus nepalensis: y = 782x² + 386x + 142, r² = 0.29, P < 0.0001. (C) Transpiration efficiency (C) = y, root-zone moisture = x. Alnus maritima: y = -9.5x² + 5.5x + 2.2, r² = 0.03, P = 0.0001. Alnus glutinosa: y = -42.8x² + 25.7x - 0.6, r² = 0.35, P < 0.0001. Alnus serrulata: y = -18.3x² + 12.0x + 0.5, r² = 0.15, P < 0.0001. Alnus nitida: y = -28.1x² + 17.4x + 0.3, r² = 0.20, P < 0.0001. Alnus nepalensis: y = -13.6x² + 8.3x + 1.8, r² = 0.11, P = 0.0032.
3.2.2. Morphological effects
Drought increased the root:shoot ratio of *A. maritima* (Table 3). *A. maritima* had a greater root:shoot ratio than the other two members of its subgenus (*A. nitida* and *A. nepalensis*), and among drought-treated plants, *A. nitida* and *A. nepalensis* had lower root:shoot ratios than the other three species (Table 3). Among plants subjected to drought, *A. nepalensis* had the greatest leaf area ratio, and, among species, drought led to the largest reduction of leaf area ratio for *A. glutinosa* (Table 3). Unlike other species, *A. maritima* showed increased specific leaf weight after drought, and its specific leaf weight after drought exceeded that of all other species except *A. nepalensis* (Table 3). *A. glutinosa* showed the greatest drought-induced reduction in mean individual leaf area (56%), while no effect was found for *A. nitida* and *A. nepalensis*, which had individual leaves with the largest area after drought (Table 3). *A. serrulata* and *A. nitida* tended to branch the most and least, respectively, regardless of treatment (Table 3).

Drought increased the root:shoot ratio of all three subspecies, and plants of subspecies *maritima* and *oklahomensis* had the highest ratios after drought (Table 3). Drought reduced leaf area ratio by an average of 26% across subspecies (Table 3). Among controls, subspecies *oklahomensis* had the greatest leaf area ratio, while subspecies *maritima* had the greatest reduction (33%) in response to drought and had the lowest ratio after drought. Drought led to increased specific leaf weight for subspecies *maritima* and *oklahomensis*, while specific leaf weight was high regardless of treatment for subspecies *georgiensis* (Table 3). Plants of subspecies *oklahomensis* had the lowest specific leaf weight within both treatments, and had the greatest reduction in mean leaf size due to drought (38%), the most stems per unit length of primary shoot (Table 3) and the most leaves (data not shown) regardless of treatment.

3.2.3. Growth effects
*Alnus nitida* had the greatest stem elongation within both treatments, while stems of *A. serrulata* elongated the least during drought and showed the greatest drought-induced percentage reduction in elongation (Table 3). Shoot dry weight was reduced by over 50% for all species except *A. nepalensis*. Drought caused the largest percentage reductions in shoot and plant dry weight for *A. nitida* and *A. glutinosa*. Stem elongation, shoot dry weight, and plant dry weight of all three subspecies of *A. maritima* decreased in response to drought, and these measures of growth were similar among all subspecies treated with drought (Table 3). Among controls, subspecies *oklahomensis* had the greatest stem elongation and acquired greater shoot and plant dry weight than subspecies *georgiensis*. Subspecies *oklahomensis* showed the greatest percentage decrease for these three growth parameters in response to drought, while subspecies *georgiensis* showed the lowest percentage decrease.

3.3. Experiment 3: survival and growth in the landscape in response to water supply
Precipitation during the first full season (1999) was greater than the 50-year mean for each month except September and greater than the 50-year mean for the season (Fig. 4). The above-average precipitation kept the potential soil-moisture deficit (precipitation minus potential evapotranspiration) minimal throughout the 1999 growth season (Fig. 4). In 2000, precipitation was below average for each month except May and was 17 cm below the 50-year seasonal mean (Fig. 4). Thus, the potential soil moisture deficit increased (became more negative) throughout the 2000 growth season, reaching –40 cm H2O by early September (Fig. 4). Only 1.22 cm of precipitation fell at the plot during the 30 days before data were taken on 21 September.

There was a moisture gradient measurable in the upper 6 cm of soil for rows seven through nine at both times data were collected, but there was little difference in the upper 6 cm of soil moisture in the remaining rows (Table 4). The root zones of plants in row nine were completely inundated throughout 1998 and 1999, but soil moisture in row nine decreased to 0.468 m3 m–3 during the drought in 2000 (Table 4). The root zones of plants in row eight were flooded only occasionally (after heavy rain and spring snow melt), and root zones of plants in row seven were never completely flooded. Although rows seven and eight were not generally in standing water, inspection after the trial revealed that the roots of plants from row seven did reach the water table and up to half of the root system of each plant in row eight was below the water table during much of the trial. Soil moisture...
Table 3  Morphology and growth of five species of Alnus and the three subspecies of Alnus maritima grown under control and drought conditions

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Treatment</th>
<th>Alnus</th>
<th>Alnus maritima subspecies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>maritima</td>
</tr>
<tr>
<td>Morphology</td>
<td>Control</td>
<td>0.69 b</td>
<td>0.53 bc</td>
</tr>
<tr>
<td>Root:shoot ratio</td>
<td>Drought</td>
<td>0.93 a</td>
<td>0.80 ab</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf area ratio</td>
<td>Control</td>
<td>68 b</td>
<td>62 bc</td>
</tr>
<tr>
<td>(cm² g⁻¹)</td>
<td>Drought</td>
<td>50 c</td>
<td>37 d</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Specific leaf</td>
<td>Control</td>
<td>4.9 b</td>
<td>4.8 b</td>
</tr>
<tr>
<td>weight (mg cm⁻²)</td>
<td>Drought</td>
<td>5.2 a</td>
<td>4.3 bc</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean individual</td>
<td>Control</td>
<td>7.4 b</td>
<td>13.5 a</td>
</tr>
<tr>
<td>leaf area (cm²)</td>
<td>Drought</td>
<td>4.8 c</td>
<td>6.0 c</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stems per cm of</td>
<td>Control</td>
<td>0.66 b</td>
<td>0.47 b</td>
</tr>
<tr>
<td>primary shoot</td>
<td>Drought</td>
<td>0.70 ab</td>
<td>0.40 bc</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stem elongation</td>
<td>Control</td>
<td>15.2 c</td>
<td>5.0 de</td>
</tr>
<tr>
<td>(cm)</td>
<td>Drought</td>
<td>8.5 d</td>
<td>8.9 ed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot dry</td>
<td>Control</td>
<td>6.6 c</td>
<td>14.2 ab</td>
</tr>
<tr>
<td>weight (g)</td>
<td>Drought</td>
<td>3.3 d</td>
<td>5.5 cd</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant dry</td>
<td>Control</td>
<td>11.0 b</td>
<td>21.6 a</td>
</tr>
<tr>
<td>weight (g)</td>
<td>Drought</td>
<td>6.2 c</td>
<td>9.9 bc</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* At each taxonomic level, means within each pair of rows followed by the same letter are not different at \( P = 0.05 \) according to the Tukey–Kramer multiple comparison procedure. Values are the means of raw data. Mean separation statistics are for either raw data (if the raw data met the assumption of homogeneity of variance according to Levene’s test) or for data transformed to meet the requirement of homogeneity of variance. At the species level, \( N = 171 \) for \( A. maritima \) and \( N = 3 \) for the other four species of Alnus. \( N = 57 \) for each of the three subspecies of \( A. maritima \).

b Percentage change in the mean from control to drought treatment (+: increase; —: decrease).

in the upper rows became very low during the drought in 2000 (≤0.11 m³ m⁻³ in rows one through six) (Table 4). It is clear that plants in these rows experienced severe drought conditions during this time because soil moisture in sandy–clay–loam soils becomes unavailable to mesophytes at soil moisture contents <0.13 m³ m⁻³ (Kramer and Boyer, 1995).

Even under the severe drought conditions of 2000, none of the plants died as a result of water-deficit. By 21 September 2000, all plants in rows one through four had between 20 and 90% of their leaves permanently wilted, all plants in rows five through seven showed permanent wilting of some leaves, while plants in rows eight and nine had no wilted leaves. The percentage of wilted leaves on each plant was quantified as a factor that determined relative health rating (Table 4). Plants in row eight had greater relative health, final canopy volume, and growth per year than plants in all other
rows. Plants in row eight also showed a greater percentage of their total growth during 2000 than plants in all other rows except rows seven and nine (Table 4). Plants in row seven were healthy, were the second largest overall, and showed a large percentage of their growth during 2000, but were smaller than plants in rows seven and eight (Table 4). Plants in rows five and six were moderately healthy but had lower final canopy volumes than those of plants in rows seven and eight. Growth was minimal in rows one through three, and the severe drought of 2000 led to poor health (Table 4).

Our assessment of non-treatment effects showed no differences across the plot for any of the measured parameters when plants were grouped into second-level blocks (data not shown). By analyzing within each row, we discovered a slight edge effect that was evident only in the two upper corners of the plot and for the "growth per season" and "percentage of total growth" parameters only (data not shown). We have attributed this non-treatment effect to the fact that plants in the corners had less competition for available soil water during the year of severe drought (2000). This edge effect helps to explain why the results for these two parameters were greater for plants in row one than for plants in row two (Table 4).

4. Discussion

4.1. Physiology, growth, and health

Drought and flooding decrease photosynthesis (Davies and Kozlowski, 1977; Gravatt and Kirby, 1998), reduce growth (Kawase, 1981; Sharp, 1996), and cause leaf senescence (Kozlowski, 1985) in plant species intolerant of soil-water extremes. Stomatal closure conserves water during unfavorable conditions
and can be an important mechanism for avoiding or tolerating stress, but it also compromises carbon fixation (Davies and Kozlowski, 1977). Drought may reduce growth of shoots more than roots, increasing the capacity of the root system to sustain hydration of the plant (Monneveux and Belhassen, 1996). During experiments one and two, \textit{A. maritima} maintained foliar gas exchange in both dry and flooded soil (Tables 1 and 2). This is consistent with the findings of Graves et al. (2002), who reported that prolonged flooding of \textit{A. maritima} does not reduce photosynthesis. Although growth diminished during total flooding (Table 1), rates of relative growth and net assimilation remained positive. This not only distinguishes \textit{A. maritima} from many woody species that show negative rates when flooded but also is noteworthy because the growth and assimilation rates for totally flooded \textit{A. maritima} were greater than those for flooded bald cypress (Nash and Graves, 1993), which, like \textit{A. maritima}, is restricted in nature to saturated or flooded soils. Even though photosynthesis was sustained when plants in greenhouses were flooded (Table 1), flooded plants in the field appeared healthy (Table 4), and \textit{A. maritima} withstands flooding better than other members of the Betulaceae (Graves et al., 2002), \textit{A. maritima} subjected to long-term, complete root-zone inundation does not realize its potential to grow rapidly (Table 1).

\textit{A. maritima} thrives when a portion of its root zone is constantly inundated with water. Such conditions led to high net photosynthetic, relative growth, and net assimilation rates (Table 1), and to comparatively high dry mass (Table 1), large canopy volume, the most seasonal growth, and excellent health (Table 4, row eight). The low root:shoot and root dry weight: leaf area ratios of partially flooded plants indicate \textit{A. maritima} preferentially partitions growth to shoots instead of roots when root-zone conditions are favorable, and that roots that develop in partially flooded soils transport water efficiently to relatively large, rapidly growing shoots. Partial inundation appears to minimize physiological stresses related to water and represents the best root-zone moisture status for \textit{A. maritima}.

Plants of subspecies \textit{oklahomensis} exhibited only modest and gradual reductions in net photosynthesis with decreased stem water potential or root-zone moisture over most of the ranges we measured (Figs. 1A and 2A). Net photosynthetic rate did not decline precipitously until root-zone moisture was <0.17 m$^3$ m$^{-3}$ and xylem water potential was $<−1.2$ MPa. This was

### Table 4

<table>
<thead>
<tr>
<th>Row</th>
<th>Soil moisture in 1999 ($m^3$)</th>
<th>Soil moisture in drought ($m^3$)</th>
<th>Final canopy volume ($dm^3$)</th>
<th>Growth per season ($dm^3$)</th>
<th>Percentage of total growth (%)</th>
<th>Relative health rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>One</td>
<td>0.239 cd</td>
<td>0.097 cd</td>
<td>173 d</td>
<td>76 ed</td>
<td>40.1 cd</td>
<td>4.4 e</td>
</tr>
<tr>
<td>Two</td>
<td>0.228 d</td>
<td>0.104 c</td>
<td>181 d</td>
<td>47 d</td>
<td>25.4 d</td>
<td>3.9 e</td>
</tr>
<tr>
<td>Three</td>
<td>0.243 cd</td>
<td>0.102 c</td>
<td>263 d</td>
<td>87 ed</td>
<td>26.6 d</td>
<td>4.5 e</td>
</tr>
<tr>
<td>Four</td>
<td>0.255 cd</td>
<td>0.091 c</td>
<td>375 cd</td>
<td>155 cd</td>
<td>34.3 cd</td>
<td>5.4 d</td>
</tr>
<tr>
<td>Five</td>
<td>0.296 cd</td>
<td>0.106 c</td>
<td>388 cd</td>
<td>215 cd</td>
<td>52.6 bc</td>
<td>7.2 c</td>
</tr>
<tr>
<td>Six</td>
<td>0.248 cd</td>
<td>0.112 c</td>
<td>397 cd</td>
<td>241 cd</td>
<td>59.7 bc</td>
<td>7.5 c</td>
</tr>
<tr>
<td>Seven</td>
<td>0.285 c</td>
<td>0.122 c</td>
<td>402 b</td>
<td>709 b</td>
<td>68.8 ab</td>
<td>8.6 b</td>
</tr>
<tr>
<td>Eight</td>
<td>0.336 b</td>
<td>0.285 b</td>
<td>1861 a</td>
<td>1547 a</td>
<td>83.5 a</td>
<td>9.4 a</td>
</tr>
<tr>
<td>Nine</td>
<td>0.913 a</td>
<td>0.468 a</td>
<td>647 c</td>
<td>529 b</td>
<td>72.0 ab</td>
<td>8.6 b</td>
</tr>
</tbody>
</table>

Values are means of 30 plants.

- **a** Sucessive row treatments from one to nine. Row one was the furthest up the slope and the furthest from standing water. Row nine was the lowest row and had plant root zones completely flooded.
- **b** Soil moisture content for the upper 6 cm of root zone, measured on 21 August 1999 (4 days after precipitation, N = 6).
- **c** Soil moisture content for the upper 6 cm of root zone, measured on 13 September 2000 (last of 10 days without precipitation, N = 6).
- **d** Canopy size calculated from three dimensional shoot measurements taken on 21 September 2000.
- **e** Change in canopy volume from 21 September 1999 to 21 September 2000.
- **f** Percentage of total growth that took place during 2000 ([growth per season 2000]/[final canopy volume] $\times 100$).
- **g** Visual shoot health rating from 1 to 10, with 1 representing the poorest health and 10 representing the best health.
- **h** Means within each column followed by the same letter are not different at $P < 0.05$ according to Fisher’s least significant difference test.
consistent with treatment-mean data that showed high rates during mild and moderate drought but very low rates during severe drought (Table 1). Although partially flooded plants grew best and were deemed the healthiest, they did not have the highest rate of photosynthesis (Table 1). Plants in the mild and moderate drought treatments showed some of the highest individual rates of photosynthesis. While their averaged rates were not greater than those of plants in some other treatments according to mean separation statistics, their high rates at low root-zone moisture and water potential shifted the plotted regression peaks for photosynthesis to the left (Figs. 1A and 2A), thereby reducing the optimum root-zone moisture and xylem water potential for photosynthesis to 0.360 m$^3$ m$^{-3}$ and −0.519 MPa, respectively. Although rates of photosynthesis were high during mild and moderate drought, plants in those treatments showed lower plant dry weight and lower relative growth rate than partially flooded plants (Table 1). This indicates that, although photosynthesis can remain high under mild and moderate drought, some photosynthetic energy is used to resist drought stress. This physiological effect on A. maritima was confirmed during experiment two, when photosynthetic rate was slightly reduced during moderate drought (Table 2), but plants again had a disproportionate reduction in total dry weight due to drought (Table 3). One way that photosynthate is used to resist drought is through its role in osmotic adjustment. When growth of agricultural crops slows due to root-zone water limitation, photosynthate continues to be imported into cells at a relatively high rate (Meyer and Boyer, 1981; Sharp et al., 1988, 1990). Import of photosynthate then exerts its use in biosynthesis and the osmotic potential becomes more negative, giving cells an increased capacity to extract water from the xylem and ultimately the soil (Kramer and Boyer, 1995).

The strategy of drought-stress resistance in A. maritima appears to change when drought is severe. Plants in experiment one showed a steep decline in net photosynthesis when root zones were very dry, yet plant weights were similar for plants in all four drought treatments (Table 1). Rather than continuing to expend considerable energy to resist stress, A. maritima apparently attempts to avoid drought stress through marked reductions in gas exchange. The relatively high dry weight of plants subjected to severe drought suggests that plants quickly recovered the capacity for photosynthesis and growth after water was supplied at the end of each drought cycle. This was confirmed by the repeated measures of photosynthesis in experiment two. Mean net photosynthesis was 80% greater during the wetter portions of the drought cycles (7.77 μmol CO$_2$ m$^{-2}$ s$^{-1}$ for root-zone moisture >0.1 m$^3$ m$^{-3}$) than during the driest portions (4.32 μmol CO$_2$ m$^{-2}$ s$^{-1}$ for root-zone moisture ≤0.100 m$^3$ m$^{-3}$).

Alnus glutinosa reportedly does not control stomatal conductance at low xylem water potential (Eschenbach and Kappen, 1999). Conductance and transpiration thereby govern water potential, a strategy that allows maximal productivity when moisture is abundant while limiting plants to sites where roots can access water (Eschenbach and Kappen, 1999). Although we did not re-evaluate the relationship of conductance and xylem water potential for A. glutinosa, we saw that the stomatal conductance of this species varied with root-zone moisture (Fig. 3B), a response often attributed to root-shoot signaling via abscisic acid (Davies and Zhang, 1991; Jones, 1998). Unlike A. glutinosa, the stomatal conductance of A. maritima appears dependent on both root-zone moisture and xylem water potential. Xylem water potential of ramets in experiment one was closely related to both root-zone moisture (Fig. 1B) and stomatal conductance (Fig. 2B). There was a stronger correlation of xylem water potential and stomatal conductance ($r = 0.64$) than there was for root-zone moisture and stomatal conductance ($r = 0.44$), suggesting that control of stomatal aperture in A. maritima may rely more heavily on signals specific to xylem water potential than on root-shoot signaling, or that A. maritima adjusts stomatal aperture based directly on xylem water potential and only indirectly on root-zone moisture. Regardless of the underlying mechanisms, under moderate to severe water deficits, A. maritima became very responsive to changing root-zone moisture and xylem water potential and strictly controlled stomatal aperture. This control is most evident at xylem water potentials ≤−0.7 MPa (Fig. 2B). When data at ≤−0.7 MPa xylem water potential, which coincided with root-zone moistures <0.156 m$^3$ m$^{-3}$, were analyzed separately, water potential increased linearly with root-zone moisture ($y = 7.3(x) −1.83$, $r^2 = 0.80$), stomatal conductance increased linearly with
xylem water potential \((y = 0.09x + 0.16, r^2 = 0.87)\), and stomatal conductance increased linearly with root-zone moisture \((y = -0.01x + 0.70, r^2 = 0.86)\), demonstrating that stomatal control in \(A. \text{maritima}\) is especially acute under severe-drought conditions.

Taxa with leaves of consistently high specific weight or taxa that increase specific leaf weight during periods of low water supply may have unusually great photosynthetic capacity and water-use efficiency (Nobel, 1980). \(A. \text{maritima}\) had a higher specific leaf weight after drought than all other species except \(A. \text{nepalensis}\) (Table 3), mean instantaneous transpiration efficiencies during drought were greatest for these two species (Table 2), and their optimum efficiency occurred with less root-zone moisture than for the other species (Table 2). Transpiration efficiency of the subspecies appears to have been linked especially closely to specific leaf weight. Subspecies \(oklahomensis\) had both the lowest specific leaf weight (Table 3) and transpiration efficiency among controls (data not shown). But drought increased specific leaf weight most for subspecies \(oklahomensis\) (Table 3), and transpiration efficiency was not different among subspecies after drought (data not shown). For our clone of subspecies \(oklahomensis\), specific leaf weight appeared increasingly important as drought intensified. Specific leaf weight and transpiration efficiency were more strongly correlated for plants during moderate and severe drought \((r = 0.55\) and \(r = 0.52\), respectively) than for plants during slight and mild drought \((r = 0.03\) and \(r = 0.07\), respectively).

The two North American species we studied, \(A. \text{serrulata}\) and \(A. \text{maritima}\), differed in their responses to the treatments we imposed. Drought evoked little change in morphology but reduced growth of \(A. \text{serrulata}\) (Table 3). Plants of \(A. \text{serrulata}\) were small and grew slowly regardless of treatment, and \(A. \text{serrulata}\) had the lowest net photosynthetic rates and instantaneous transpiration efficiencies at most root-zone moisture. Although it is more widely distributed and common in the wild than \(A. \text{maritima}\), our results indicate that \(A. \text{serrulata}\) is comparatively prone to water stress. \(A. \text{maritima}\) contrasted \(A. \text{serrulata}\) in several ways. Its resistance to total root-zone inundation appears linked to the consumption of photosynthetic energy that otherwise could promote rapid growth (Table 1), yet \(A. \text{maritima}\) can grow and remain healthy with its roots completely inundated indefinitely (Table 4). \(A. \text{maritima}\) is also quite drought resistant. With the exception of \(A. \text{nepalensis}\), \(A. \text{maritima}\) showed the least reduction in growth due to water-deficit, and it maintained photosynthesis and transpiration efficiency comparatively well over the entire root-zone moisture range (Table 2, Fig. 3). This illustrates that \(A. \text{maritima}\), like many drought-tolerant species, maintains relatively high photosynthetic rates with decreased stomatal conductance during water deficits (Kubitske and Abrams, 1993).

Of the five species we evaluated, \(A. \text{glutinosa}\) was the least tolerant of root-zone moisture extremes. Drought had the most severe effect on \(A. \text{glutinosa}\) for almost all parameters of morphology, growth, and physiology, results consistent with those of Hennessey et al. (1985), who compared \(A. \text{glutinosa}\), \(A. \text{maritima}\), and \(A. \text{serrulata}\). Although \(A. \text{glutinosa}\) attained high net photosynthetic rates and instantaneous transpiration efficiencies at certain root-zone moisture, it sustained adequate values over a narrow range of root-zone moisture (Fig. 3). One possible explanation is its limited control of stomatal conductance. If stomatal apertures are controlled solely by root-zone moisture content (Fig. 3B) with little or no regulation based on xylem water potential (Eschenbach and Kappen, 1999), effects of other environmental factors like wind speed, temperature, and relative humidity on plant water potential go unnoticed (Kramer and Boyer, 1995). In dry soil, stomates might limit CO\(_2\) availability when xylem water potential is acceptable for photosynthesis, and in wetter soils, stomates may remain open when xylem water potentials become detrimental.

Our results offer new insights regarding \(A. \text{nepalensis}\) and \(A. \text{nitida}\). These Asian species are taxonomically allied with \(A. \text{maritima}\), and together these three late-season-blooming species comprise the \(Alnus\) subgenus Clethropsis (Spach) Regel. Drought minimally affected the growth and morphology of \(A. \text{nepalensis}\) (Table 3). Even though \(A. \text{nepalensis}\) had comparatively low root:shoot ratios, root-zone water deficit caused no manifest symptoms of stress. \(A. \text{nepalensis}\) also had the highest net photosynthetic rates over the range of root-zone moisture in our regression analysis but never attained the high instantaneous transpiration efficiencies shown by \(A. \text{maritima}\) (Fig. 3). \(A. \text{nitida}\) showed exceptional growth when provided ample water (Table 3) but showed substantial reductions
in photosynthesis and growth due to drought (Tables 2 and 3) and had a limited range of root-zone moistures at which photosynthesis and transpiration efficiency were favorable (Fig. 3).

4.2. Ecological implications

A. maritima is indigenous exclusively on saturated or flooded soils (Schrader and Graves, 2002), which might imply that poor drought resistance is the major cause of its limited distribution. Our results, however, demonstrate that A. maritima is more flexible regarding root-zone moisture than might be expected based on its restricted occurrence in wetlands (Table 1, Fig. 1), and A. maritima is more drought tolerant than some of the more widespread species of its genus (Tables 2 and 3, Fig. 3). Water availability alone is not responsible for the limited distribution of A. maritima, but water availability likely is a major contributing factor. Schrader and Graves (2002) proposed that, as a water-loving pioneer species, the realized niche of A. maritima is an environment with very wet soil and abundant direct sunlight. A. maritima grows best with about half of its root system submerged in fresh water, and its growth decreases in dryer soils (Tables 1 and 4). Its abundant growth under very wet conditions gives A. maritima a competitive advantage over other tree species in saturated soils, but in dryer soils, reduced growth and an apparent lack of shade tolerance make it unable to compete with species better-suited to dry soils. Nonetheless, the capacity of A. maritima to grow in dryer soils and to use fixed nitrogen gas via symbioses with Frankia bacteria (Benson and Sylvester, 1993), may enable the species to invade dryer areas denuded by natural or human-produced devastation.

The next logical step in determining the primary causes for the rarity of A. maritima should be to determine its shade tolerance. If A. maritima is relatively intolerant of shade, we should be able to conclude that slow growth in dry soils together with shade intolerance are the main factors responsible for the limited numbers and distribution of A. maritima.

4.3. Horticultural implications

A few species dominate the managed tree populations in many regions. The prevalent taxa survive and appear healthy despite environmental extremes, such as excessive and deficient soil moisture, poor soil nutrient levels, and high and low temperatures (Berrang et al., 1985; Kramer, 1987; Kielbaio, 1990). Many species of the genus Alnus naturally occur on wet to flooded soils and also are adapted to soil low in nitrogen because of their nitrogen-fixing symbioses (Benson and Sylvester, 1993). Along with these qualities, A. maritima responds more favorably to drought than all of the species we studied except A. nepalensis. A. maritima is best suited to consistently wet soils where a portion of the root zone is saturated, but plants can perform acceptably on drained soils except where they may be prone to episodes of severe drought. A. maritima also is extremely cold hardy and can be used in areas as harsh as USDA zone 3a (Schrader and Graves, 2003). Variation among and within the three subspecies during experiment two shows the potential for selecting genotypes especially resilient to extreme conditions. Subspecies georgienensis showed the least degree of change in response to drought for all measures of morphology and growth except individual leaf size and stems per centimeter of primary shoot (Table 3). Although this might imply that subspecies georgienensis is better adapted to drought than the other subspecies, our findings that subspecies oklahomensis adjusts well to root-zone moisture conditions, combined with its particularly attractive growth habit (Schrader and Graves, 2000c), lead us to recommend that subspecies oklahomensis be favored for managed landscapes.

It is ironic that the Alnus species used most often in the landscape, A. glutinosa, is comparatively susceptible to soil-moisture stress. Our results and those of others (Hennessey et al., 1985; Eschenbach and Kappen, 1999) should prompt reconsideration of the use of A. glutinosa in landscapes where soil moisture is not consistently high. A. serrulata has been planted more often in landscapes than all species we evaluated except A. glutinosa, but its slow growth and relatively poor response to drought might constitute disadvantages for commercial producers and consumers. While A. nepalensis shows exceptional promise as a landscape tree resistant to soil-moisture stress, its minimal cold hardiness prevents its use in temperate climates (Schrader and Graves, 2003). In landscapes with uniform moisture, A. nitida might grow larger than the other four species we studied and develop a symmet-
rical habit with a dominant central leader (Schrader, 2002). Although A. nitida was not hardy in USDA hardiness zone 5a (Schrader and Graves, 2003), it occurs at higher elevations than A. nepalensis in the Himalayan Mountains (Manandhar, 2002) and thus may have more potential than A. nepalensis for use in cold climates.

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References


