Plant development and gas exchange of *Alnus maritima* in dry and flooded soil

by

Sarah Jane Gardner

A thesis submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Horticulture

Major Professor: William R. Graves

Iowa State University

Ames, Iowa

2001
Graduate College

Iowa State University

This is to certify that the Master's thesis of

Sarah Jane Gardner

has met the thesis requirements of Iowa State University

Signatures have been redacted for privacy
# TABLE OF CONTENTS

## CHAPTER 1. GENERAL INTRODUCTION
- Introduction 1
- Thesis Organization 2
- Literature Review 3

## CHAPTER 2. PLANT DEVELOPMENT AND PHOTOSYNTHESIS OF *ALNUS MARITIMA* IN DRY AND FLOODED SOIL
- Abstract 17
- Introduction 18
- Materials and Methods 20
- Results 23
- Discussion 24
- Literature Cited 27

## CHAPTER 3. DEVELOPMENT AND GAS EXCHANGE OF SEEDLINGS FROM THE THREE POPULATIONS OF *ALNUS MARITIMA*
- Abstract 33
- Introduction 34
- Materials and Methods 36
- Results 39
- Discussion 41
- Literature Cited 45

## CHAPTER 4. GENERAL CONCLUSIONS

## APPENDIX A. GAS EXCHANGE AND DESICCATION OF *ALNUS MARITIMA* SUBJECTED TO THREE RATES OF SOIL DRYING

## APPENDIX B. PRESENCE OF DEHYDRINS IN ROOT TISSUE OF *ALNUS MARITIMA*

## APPENDIX C. DEVELOPMENT AND GAS EXCHANGE OF THE THREE POPULATIONS OF *ALNUS MARITIMA* AND FOUR OTHER SPECIES OF *ALNUS*

## APPENDIX D. CHARACTERISTICS OF *ALNUS MARITIMA* MATERNAL SEED SOURCES

## ACKNOWLEDGMENTS
CHAPTER 1. GENERAL INTRODUCTION

Introduction

Plants improve urban areas. They cool and clean the air and provide a shelter for wildlife. The aesthetic contribution of trees and shrubs is obvious when one contrasts the appearance of a new suburban development with as yet no plantings and a neighborhood with extensive, mature plantings. However, much of the urban landscape is composed of only a few species known to survive the extreme stress of the environment. This homogeneity can be problematic, as evidenced by the catastrophic results of Dutch elm disease in the past century. Introduction of new taxa of trees and shrubs for managed landscapes is of paramount importance. One successful method used in identifying new plants for urban environments is to evaluate the drought-stress response of plants that normally grow in bottomland or riparian habitats. As a group, such plants are adapted to survive in the saturated soils known to occur in managed landscapes and there is evidence that genotypes that also possess drought resistance can be selected.

*Alnus maritima* [Marsh.] Muhl. ex Nutt. is a rare, riparian, woody plant species found in only three disjunct populations in the United States: the Delmarva Peninsula (southern Delaware and the Eastern Shore of Maryland), northwestern Georgia, and south-central Oklahoma. It is attractive, with glossy green, elegant leaves and yellow catkins in the fall. It also has the capacity to associate with filamentous actinomycetes that fix atmospheric nitrogen, which may be advantageous in soils of stressful environments.
The purpose of our research was twofold. One goal was to evaluate *A. maritima* for possible use at urban sites. The other goal was to further the knowledge of this rare and fairly unknown species. Only one study on the drought response of *A. maritima* had been published before my work, and no information was available concerning whether plants of *A. maritima* from the three disjunct populations differ in their responses to drought. To investigate these topics we first measured development and gas exchange in rooted stem cuttings from one plant from Oklahoma when subjected to a wide range of soil moisture. Later, to test for population differences in response to drought, we evaluated development and gas exchange of seedlings from multiple maternal parent plants in all three populations when subjected to drought. We also examined roots of *A. maritima* for the occurrence of dehydrin proteins, which may influence drought resistance in some plants. This research will broaden our knowledge about *A. maritima* and may help in the selection of genotypes of this species that possess traits that enhance the performance of plants in managed landscapes.

**Thesis Organization**

A literature review on *Alnus maritima* and effects of water stress on woody plants follows in this chapter. Chapter 2, a manuscript to be submitted to the *Journal of the American Society of Horticultural Science*, is a study of *A. maritima* subjected to a wide range of soil moisture. The third chapter is a manuscript to be submitted to the same journal. It reports on an experiment to test for population-based differences of drought response in *A. maritima*. General conclusions are presented in chapter 4. Four appendices follow. Each of
these is a summary of additional research done on *A. maritima* subjected to sub-optimal soil moisture.

**Literature Review**

*A. maritima*

*A. maritima* [Marsh.] Muhl. ex Nutt. (seaside alder) is a rare alder species that commonly grows to a height of 6-10 m in saturated soil. Propagation of *A. maritima* is easily accomplished by softwood cuttings or seed germination (Schrader, 1999; Schrader and Graves, 2000a). *A. maritima* has the capacity to benefit from fixed atmospheric nitrogen through a symbiotic relationship with the actinomycete *Frankia* (Furlow, 1979; Schrader, 1999). *A. maritima* is in the subgenus *Clethropsis* along with two other species (*Alnus nepalensis* Don, and *Alnus nitida* (Spach.) Endel) from southern Asia. All members of this subgenus flower in the fall (Furlow, 1979). The many other species of *Alnus* are in the subgenera *Alnus* or *Alnobetula* and flower in the spring (Furlow, 1979). *A. maritima* naturally occurs in three small areas of the United States: in northwestern Georgia, on the Delmarva Peninsula (southern Delaware and the Eastern Shore of Maryland), and south-central Oklahoma (Schrader and Graves, 2000a; 2000b). Schrader and Graves (2000b) have found winter seed dispersal of *A. maritima* may lower its germination percentage and limit population spread. Other limitations of population spread of *A. maritima* are the requirement of new seedlings for sufficient photosynthetically active radiation, fine soil texture, and nutrients (Schrader, 1999).
The cause of population isolation in *A. maritima* is controversial. Some theorize that indigenous people carried seeds from the Delmarva population to Oklahoma due to the use of *Alnus* in various medicinal remedies (Stibolt, 1981). However, the more recently discovered population in Georgia, and fossil evidence of other *Clethropsis* subgenera in the northwestern United States, tend to discount this theory (Furlow, 1979; Schrader, 1999; Schrader and Graves, 2000a; 2000b; Stibolt, 1981). The populations in Georgia and on the Delmarva Peninsula more closely resemble each other than the population in Oklahoma, both morphologically and developmentally (Schrader and Graves, 2000a). These findings, along with the fossil evidence, indicate the three populations are vestiges of a larger population, with the population in Oklahoma becoming isolated and diverging before the populations in Delmarva and Georgia (Furlow, 1979; Schrader and Graves, 2000a; Stibolt, 1981).

**Urban landscapes**

Plants are an integral part of our managed landscapes (Grey and Deneke, 1986). Trees in urban environments often suffer greater stress than trees in more natural settings. Urban trees are planted because of their aesthetic and physical benefits, while trees found in nature are adapted to their habitats (Kramer, 1987). In general, urban landscape plantings use only a few species known to tolerate the environmental stresses unique to urban areas (Hunt, 1989). In New York City, four species constituted 82% of 375 trees planted on land of the Consolidated Edison Company (Berrang et al., 1985). Two of the predominant abiotic stresses plants in managed landscapes must tolerate or avoid are extreme and varied soil moisture and compacted soil (Berrang et al., 1985; Hunt, 1989; Kramer, 1987). Often it is not clear whether the environmental stressor is saturated soil, dehydrated soil, compacted
soil, or a combination of these factors. Researchers found that trees in the New York City study, which were at a site that appeared prone to drought, were actually in very saturated soil (Berrang et al., 1985).

Root restriction may cause plants to exhibit symptoms associated with drought stress, even in flooded soil. In a study of *Alnus glutinosa* (L.) Gaertn, Tschaplinski and Blake (1985) found root restriction caused internal water deficits. Shoots of *A. glutinosa* continued to grow despite cessation of root growth, causing an imbalance in water-uptake to water-use. The first response of these plants was stomatal closure and then leaf abscission, which are also typical drought-avoidance responses.

Trees adapted to flooded soil frequently have the best survival rates in urban areas (Moll, 1989). Certain wetland tree species may sustain growth in dehydrated soil as well as in saturated soil. In a study on drought and flood stress of riparian species, Nash and Graves (1993) found *Magnolia virginiana* L. and *Taxodium distichum* (L.) Rich. maintained a positive relative growth rate and net assimilation rate in both a flood treatment and a drought treatment. The adaptations of the root system of trees normally found in saturated soil may be helpful in the low-oxygen environment of compacted soil (Moll, 1989).

**Drought**

Dry soil is considered to be a major problem in managed landscapes (Clark and Kjelgren, 1990; Whitlow and Bassuk, 1987). How a plant responds to soil desiccation can determine its long-term survival in urban areas. Ludlow (1989) characterized escape, avoidance, and tolerance as strategies plants may exhibit as they respond to drought. Most plants show an array of responses that may represent different categories. Plants that exhibit
an avoidance strategy will have tissues that are sensitive to drought. They will have deep roots, minimize water loss by stomatal control, have smaller leaves and shed those leaves when under desiccation stress. In general, plants using this strategy will demonstrate slow growth, reduced biomass, and good short-term but poor long-term survival. Plants that exhibit the tolerance strategy have tissues that can survive extreme dehydration. These plants do not avoid dehydration; they continue to fix carbon and sustain growth even during periods of drought, albeit at a lower growth rate. Plants that tolerate dehydration will be more likely to survive in a wide range of moisture environments (Ludlow, 1989). Hinckley et al. (1981) considered most trees to be capable of avoiding drought and considered delayed or repressed stomatal opening after drought a trait of drought-avoiding woody species. However, Kramer and Kozlowski (1979) categorize woody plants, like herbaceous plants, as drought tolerators as well as drought avoiders.

In general, drought reduces plant growth, primarily because cell expansion requires some minimum degree of cell turgor (Hsiao, 1973). Drought often changes the ratio of above and below ground biomass allocation, causing an increase in the root-to-shoot ratio. The proportion of total biomass involved with water uptake is favored at the expense of tissues that lose water (Hsiao, 2000; Monneveux and Belhassen, 1996). In a study by Joly et al. (1989) root-to-shoot ratio increased by 0.36 in *Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii* seedlings exposed to drought, and in a study of *Acer ×freemanii* E. Murray (Zwack et al., 1998), root-to-shoot ratio was increased by 0.12. Another mechanism of drought avoidance is to reduce leaf surface area and transpiration via leaf abscission (Dosmann et al., 2000; Kozlowski, 1976). Drought often increases the specific weight of leaves (Graves,
1994; Joly et al., 1989), perhaps as a result of increased leaf thickness. Cultivars of
*A. xfreemanii* increased their leaf thickness by 9.6 µm in response to drought (Zwack et al.,
1998). Leaf specific weight may influence gas exchange. Abrams et al. (1994) found that
maximum photosynthetic rate and leaf conductance were positively correlated to leaf mass
per unit area in 17 temperate, broad-leaved tree species.

Gas exchange is also affected by drought. A greater photosynthetic capacity is
correlated with increased drought tolerance (Kubiske and Abrams, 1993). Plants that
maintain a relatively high photosynthetic rate in low soil moisture and regain that rate faster
and more fully after rehydration are generally considered to be drought tolerant (Fan et al.,
1997). Boyer (1976) theorized that during high irradiance and drought, photosynthesis is
depressed due to stomatal closure and restricted influx of CO₂. A decrease in chloroplast
activity is the cause of depressed photosynthesis during low irradiance and drought (Boyer,
1976). In one study of drought stress among five *A. glutinosa* clones, Hennessey and Lorenzi
(1987) found different drought tolerances among the five clones and detected a positive
correlation between higher stomatal conductance and greater leaf area and height growth. In
another study Hennessey et al. (1985) found *A. maritima* had a lower stomatal diffusive
resistance than *A. glutinosa* and maintained low levels of stomatal resistance or high levels of
conductance. Drought effects on growth and morphology of plants of *A. maritima* were less
pronounced than the effects of drought on plants of *A. glutinosa*. Photosynthetic rate and
stomatal conductance may not be well correlated even in high irradiance. Photosynthetic rate
in seedlings of *Alnus rubra* Bong. subjected to drought was less than that of well-watered
controls, with no reduction in transpiration rate. When plants in the drought treatment were
rehydrated, their photosynthetic rate was greater than that of control plants with no concomitant increase in stomatal conductance or transpiration rate (Hawkins and McDonald, 1993). In a study done on *Alnus cordata* Loisel. seedlings Borghetti et al. (1989) also did not find strong stomatal control of photosynthesis.

The interaction of stomatal conductance and photosynthetic rate depends not only on irradiance levels, but also on the physiology and adaptations of the plant. Kubiske and Abrams (1993) found that a xeric tree species maintained relatively high rate of photosynthesis despite a decrease in stomatal conductance. Kubiske and Abrams (1992) also found differing responses to water stress among neighboring populations of *Quercus rubra* L., one in a wet site, one in a dryer site. Trees from the dryer site exhibited relatively few nonstomatal limits to photosynthesis in the beginning and middle of drought. These trees also had a greater leaf thickness, greater specific weight, and smaller leaf area compared to their neighbors. Stomata may take many days to recover after drought stress, depending on the rate, duration, and severity of the drought (Oliver and Bewley, 1997; Zwack et al., 1998). Photosynthesis may never attain its pre-stress rate after a period of drought (Davies and Kozlowski, 1977).

Transpiration or water-use efficiency is considered to be another indicator of drought tolerance. In a plant with high water-use efficiency, the amount of water used per CO₂ fixed is relatively small. However, in most environments with little water there is no advantage for high water-use efficiency if competitors could then use the water saved by the plant with the high water-use efficiency (Jones, 1993). Zhang et al. (1997) found no correlation between high water-use efficiency and drought tolerance in populations of ponderosa pine (*Pinus*
ponderosa Doug. ex Laws.). However, Wang et al. (1998) considered high water-use efficiency an indicator of drought tolerance in different populations of paper birch (*Betula papyrifera* Marsh.) treated with low soil moisture.

**Flooding**

Plants exposed to flooded root zones may exhibit some of the same responses plants show during drought. Flooding can decrease photosynthetic rate and stomatal conductance, reduce plant growth, cause leaf senescence, and decrease root-to-shoot ratio (Kawase, 1981; Kozlowski, 1984; 1985; Sharp, 1996). Gas exchange may be variable during flooding. In a study of *Fraxinus pennsylvanica* Marsh. and *T.* both flood-tolerant species, stomatal conductance was decreased 50%, with no concomitant decrease in photosynthetic rate when plants were subjected to flooding (Pezeshki and Chambers, 1986). In another study, *T.* *distichum* had higher stomatal conductance and photosynthetic rates in both flooded and aerated soil when compared to *Quercus falcata* var. *pagodaefolia* Ell. and *Quercus lyrata* Walt. (Pezeshki et al., 1996). Continuous flooding can cause plants to develop internal air spaces and adventitious roots that facilitate gas exchange (Kawase, 1981; Kozlowski, 1984). One adaptation of wetland trees is pressurized gas transport due to thermo-osmosis of gases (Grosse et al., 1998). A temperature gradient is established between the warmer outer stem when irradiated and the cooler ambient air. A pressure gradient is then created and the mean free path of the gas molecules is increased inside the stem, which prevents gas molecules from escaping into the outside air. Gas then accumulates inside the stem and is driven down to the roots by the increasing pressure. Pressurized gas transport has not been recorded for trees habituated to drier soils but has been found in unrelated genera, one of which is *Alnus.*
Gas flow to the roots of a leafless *A. glutinosa* can be four times greater in the light than in the dark. This mechanism is more significant in seedlings and leafless trees than in mature, fully foliated trees, and the mechanism may play an important role in seedling establishment. Thermo-osmosis may also cause oxygenated zones in the rhizosphere, which would encourage nitrogen fixation in the symbiotic *Frankia alni* (Grosse et al., 1998).

**Population differences involving species of *Alnus***

There are numerous studies on population differences in tree species. *Alnus* is a particularly large and variable genus with a large amount of intra-species variation (Hall and Maynard, 1979). In a study of *A. cordata* in Italy, Borghetti et al. (1989) found a particularly drought-sensitive population had the greatest leaf-area reduction and the lowest xylem potentials in a severe drought treatment. Dang et al. (1994) studied 40 *A. rubra* populations and found a weak geographic trend in gas exchange data, following a moisture and temperature gradient. They detected differences among the populations in photosynthetic rate, transpiration rate, and stomatal conductance but no difference in transpiration efficiency. In another study of populations of *A. rubra*, Ager et al. (1993) found a strong geographic pattern of variation. Developmental traits were associated with temperature and length of growing season.

In their study of *A. maritima*, Schrader and Graves (2000a) found seeds from Oklahoma had a higher germination percentage (55.0%) than seeds from Georgia and Delmarva (31.4% and 14.7%, respectively). Seedlings from Georgia had the greatest specific leaf weight. Leaves of seedlings from Oklahoma were longer, narrower, and less bullate than leaves from Georgia and Delmarva. Plants from Oklahoma also had a higher root-to-shoot
ratio and had more leaves and axillary shoots than plants from the other two populations. The leaf area ratio was the lowest for plants from Georgia (Schrader and Graves, 2000a).

The extensive variability in the Alnus genus and in the A. maritima species provides researchers with a number of traits for evaluation and selection. We are optimistic that the variability we found between the three populations of A. maritima can be exploited in future selection and research.

**Literature Cited**


CHAPTER 2. PLANT DEVELOPMENT AND PHOTOSYNTHESIS OF

ALNUS MARITIMA IN DRY AND FLOODED SOIL

A paper to be submitted to the Journal of the American Society for Horticultural Science

Sarah J. Gardner and William R. Graves

ADDITIONAL INDEX WORDS. seaside alder, water potential, water stress

ABSTRACT. Alnus maritima [Marsh.] Muhl. ex Nutt. (seaside alder) is a rare riparian species. Its natural occurrence in waterlogged soils suggests A. maritima could withstand flooding in managed landscapes, yet the responses of this species to a range of soil moisture conditions have not been characterized. The objective of this experiment was to measure photosynthesis, water potential, and growth responses of A. maritima treated with various irrigation regimens to evaluate the potential of A. maritima for use in managed landscapes. Rooted cuttings from one clone were arranged in a randomized complete block design and subjected to partial or total flooding of the root zone and to five watering frequencies: daily watering, slight drought, mild drought, moderate drought, and severe drought. Cycles of these four drought treatments ended when the mean moisture content of the upper 6 cm of the root zone had decreased to 0.4, 0.3, 0.2, and 0.1 m$^3$·m$^{-3}$, respectively. The treatments lasted from 30 to 45 days depending on the block. Dry weights of the root, shoot, and leaves were taken. Stem water potential and rate of photosynthesis of all plants were measured on the last day of treatment. The dry weight of plants in the partial flood and the slight drought
treatments was 41% greater than the dry weight of plants in other treatments. Total flooding resulted in plants with the lowest relative growth rate, net assimilation rate, and root and total dry weights, but root-to-shoot ratios of plants in total flooding were not different from those of plants in the partial flood and the mild drought treatments. Relative growth rates were greatest for plants in the partial flood and slight drought treatments. Photosynthetic rate was $> 3 \mu\text{mol CO}_2 \text{m}^{-2}\text{s}^{-1}$, except when soil moisture content was $\leq 0.1 \text{m}^3\text{m}^{-3}$ and stem water potential was $\leq -1.3 \text{MPa}$. *A. maritima* tolerates a wide range of soil moisture conditions, indicated by its responses to drought and flooding, and thus might be used successfully in managed landscapes.

**Introduction**

Plants in managed landscapes must tolerate a wide range of soil moisture conditions. Soil water content is especially variable and difficult to predict at urban planting sites, where trees and shrubs in close proximity may be subjected to both extremely dry soil and complete saturation of the root zone (Berrang et al., 1985; Whitlow and Bassuk, 1987). Drought and flooding can decrease photosynthetic rate (Davies and Kozlowski, 1977; Gravatt and Kirby, 1998), reduce plant growth (Kawase, 1981; Sharp, 1996), and cause leaf senescence (Kozlowski, 1985). The responses of a plant to drought and flooding can aid it in tolerating this stress. In plants exposed to drought, stomatal closure may decrease water loss due to transpiration and concomitantly lower photosynthetic rate (Davies and Kozlowski, 1977). Shoot growth may be retarded and root growth encouraged for a greater water absorption to
water loss ratio (Monneveux and Belhassen, 1996). Continuous flooding can cause plants to develop internal air spaces to facilitate gas exchange from the shoots to the roots (Kawase, 1981). Both flooding and drought can inhibit shoot growth for energy conservation.

Plants selected for managed landscapes should have the capacity to withstand the detrimental physiological, developmental, and aesthetic effects of drought and flooding. One strategy to choose taxa that can withstand a wide range of soil moisture content is to select for drought resistance among plants that occur naturally in wet soils (Nash and Graves, 1993). Bald cypress [*Taxodium distichum* (L.) Rich.] and sweetbay magnolia (*Magnolia virginiana* L.) are examples of wetland species that maintained positive relative growth rates and net assimilation rates during both drought and flooding (Nash and Graves, 1993), and bald cypress has survived in urban areas where many other species of woody plants fail. Additional taxa capable of flourishing regardless of soil water supply are needed.

*Alnus maritima* forms attractive large shrubs or small trees, with glossy leaves and yellow catkins in late summer or early fall. In its natural habitat, *A. maritima* is found exclusively on soils that are frequently or constantly saturated with fresh water (Schrader and Graves, 2000). Its capacity to thrive and sustain its ornamental appeal on wet soils has fostered our interest in the potential of *A. maritima* as a new nursery crop that could be marketed for use in managed landscapes. Although its responses to drought have not been characterized fully, Hennessey et al. (1985), suggested that *A. maritima* may have greater drought tolerance than other, more common members of its genus. As an initial step in evaluating the potential of *A. maritima* for use in managed landscapes, our objective was to measure photosynthesis, stem water potential, and development of plants of one clone that
were exposed to partial and complete root-zone inundation and to droughts of various intensities.

**Materials and Methods**

**PLANT MATERIAL AND EXPERIMENTAL DESIGN.** Terminal and subtending stem cuttings from one plant of *A. maritima* were taken on 11 June 1999. The greenhouse-grown stock plant was two years old and had been grown from a seed collected from the natural population of *A. maritima* in Oklahoma (latitude 34°19'40''N; longitude 96°35'30''W). All cuttings were from stems formed in 1999. Basipetal ends of the stems with two to three nodes were treated with Hormodin No. 2 (MSD AGVET, Merck & Co., Rahway, N.J.) and placed in flats of coarse perlite on the greenhouse mist bench as described by Schrader and Graves (2000). Nine weeks later (13 Aug.), rooted cuttings were potted singly in plastic containers (top diameter = 15.2 cm; height = 15 cm;) in a mix of 2 perlite : 2 *Sphagnum* peat : 1 soil (by volume). Pots were placed on a greenhouse bench under high-pressure sodium lamps (400 W) that provided 16-hour photoperiods. Plants were irrigated to container capacity that day and again two 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 11.0 mM N. Tap water was used to irrigate plants once daily on all days when fertilizer was not applied. Plants were sorted into a randomized complete block design with seven blocks. There were 24 plants of similar size in each of the seven blocks (168 total).
INITIAL HARVEST AND SUBSEQUENT TREATMENTS. Three plants per block were selected at random for an initial harvest on 10 Sept. The surface area of all leaf blades from these plants was determined with a LI-COR 3100 area meter (LI-COR, Lincoln, Nebr.), and the dry weight of root and shoot systems was determined. Three of the remaining 21 plants in each block were assigned randomly to each of seven irrigation treatments on the day of the initial harvest. There were two flooding treatments. Each pot assigned to the flooding treatments was placed within a 1.9 L (14 x 15 cm) specimen container (Fisher Scientific, Pittsburgh, Penn.). Entire root zones of plants in the total flood treatment were kept saturated with tap water by maintaining full volumes in the specimen containers. A 3.8-cm column of tap water was maintained in the specimen containers of plants in the partial flood treatment. Because of a gap between the bottom of the pot and the specimen container, only the lowest 1.5 cm of the root zone of each pot in the partial flood treatment was immersed in water. Plants in the other five treatments were subjected to moisture deficits of various severities by watering pots to container capacity at different frequencies. Pots in the daily watering treatment received 650 mL of water each day. Pots in the slight, mild, moderate, and severe drought treatments were watered with 1300, 1950, 2600, and 3250 mL water, respectively, upon completion of a drought cycle of appropriate severity. The various watering volumes applied were chosen so the amount of run-off would be similar for plants in these five drought treatments. We considered a drought cycle complete within blocks when the moisture content of the upper 6 cm of the root zone had decreased to 0.4, 0.3, 0.2, and 0.1 m$^3$·m$^{-3}$, respectively, for the slight, mild, moderate, and severe drought treatments. Moisture content was determined once each day with a model HH1 Theta Probe for organic
media with a model ML1 sensor (Delta-T Services, Cambridge, England). Fertilizer solution was applied instead of tap water to plants in all treatments at the first irrigation possible after plants in the severe drought treatment completed their third cycle. Plants in the flood treatments were fertilized at that time by flushing their root zones with fertilizer solution and then resuming root-zone inundation with tap water. The air temperature in the greenhouse was from 19.9 to 34.3 °C, relative humidity ranged from 12.1 to 71.4%, and photosynthetically active radiation was 0 to 1118 µmol·s⁻¹·m⁻².

**Final Harvest.** Harvesting was completed by block, beginning the day the severe drought treatment in each block completed its fifth cycle. Net photosynthetic rate and stem water potential were determined for two randomly chosen plants of the three per block in each treatment. These measurements were made before watering for the day was done. A LI-COR 6400 Portable Photosynthesis System (LI-COR, Lincoln, Nebr.) was used on the youngest fully expanded leaf of each plant at 1300 to 1500 HR central standard time. Immediately after each photosynthesis measurement, the stem adjacent to the petiole of the measured leaf was severed for determination of xylem water potential with a pressure bomb (PMS Instruments, Corvallis, Ore.). Surface area of leaf blades of these plants then was measured with the area meter. The length and maximum width of the longest leaf on each of the 14 plants were measured. Weights of leaf blades, stems plus petioles, and roots were determined after tissues dried 3 d at 67 °C. Relative growth rate and net assimilation rate were calculated as described by Harper (1977).

Data were analyzed by Statistical Analysis System Software (SAS Inst. Inc., 1988). The general linear models procedure was used, and means were separated by the Tukey-
Kramer multiple comparison procedure. A regression procedure (proc reg) was used for linear and quadratic effects of soil moisture content and for stem water potential on photosynthesis.

Results

The partial flood treatment and the slight drought treatment evoked the greatest plant dry weight; 41% more than the mean of plants in the other treatments combined and 137% more than plants in the total flood treatment (Table 1). Plants in the slight drought treatment had a greater root dry weight than plants in the mild drought, severe drought, and total flood treatments. Plants in the total flood treatment had the lowest plant and root dry weights and one of the lowest mean root-to-shoot ratios. The daily watering treatment led to plants with the greatest ratios of root to shoot dry weight and root dry weight to leaf surface area. The root-to-shoot ratio of those plants was 64% greater than that of plants in the other treatments combined, and their ratio of root dry weight to leaf surface area was 79% greater (Table 1).

Plants in the partial flood and slight drought treatments had the greatest relative growth rate (Table 1). Plants subjected to total flooding had the lowest relative growth rate, 41% lower on average than that of plants in the other treatments (Table 1). Totally flooded plants had the lowest net assimilation, 36% lower than the combined mean of all the other treatments. The other treatments were not different in net assimilation rate. The leaf area ratio was greatest for plants in the moderate drought, severe drought, partial flood, and mild
drought treatments and lowest in the total flood, daily irrigation, and slight drought treatments. No apparent leaf damage or abscission occurred, and all plants survived.

A quadratic analysis best represented data on photosynthetic rate. The quadratic function was 
\[ y = 2.53 + 16.11\text{[soil moisture content]} - 12.03\text{[soil moisture content]}^2; \]
\[ r^2 = 0.36; P \leq 0.0001 \] (Fig. 1). Soil moisture content of 0.7 m$^3$·m$^{-3}$ was associated with the greatest photosynthetic rate. A quadratic analysis of the stem water potential gave a regression equation of 
\[ y = 5.47 - 5.73\text{[stem water potential (MPa)]} - 5.52\text{[stem water potential (MPa)]}^2; \]
\[ r^2 = 0.54; P \leq 0.0001 \] (Fig. 2). This function predicted that a stem water potential near -0.5 MPa was associated with the greatest photosynthetic rate.

**Discussion**

The wide range of soil moisture conditions to which plants of this clone of *A. maritima* were exposed was sufficient to evoke several morphological and physiological responses. Our findings regarding foliar gas exchange are consistent with previous studies in which water stress was found to lower stomatal conductance of other woody species (Zwack et al., 1998). Stomata, and the rates of gas exchange they influence, may take many days to recover from drought, depending on the rate, duration, and severity of the water deficit (Oliver and Bewley, 1997; Zwack et al., 1998). Photosynthesis may not attain its pre-stress rate after a period of drought (Davies and Kozlowski, 1977). Both flooding and drought can reduce growth of woody plants (Kawase, 1981; Sharp, 1996), as occurred with this clone of *A. maritima* (Table 1). Growth reductions often are accompanied by leaf senescence.
(Kozlowski, 1985), but no chlorosis, necrosis, nor abscission were observed among the plants of *A. maritima* we treated. The lack of foliar damage during water stress may be a particularly important horticultural attribute of *A. maritima*.

Further research will be needed to discern the mechanisms underlying some of the treatment effects we have documented. The marked reduction in growth of plants treated with total flooding of the root zone was unexpected because this species typically is found in standing water in its native habitats. Perhaps the agitated water near roots of *A. maritima* in the wild would contain more dissolved oxygen than the unstirred water to which we exposed plants in the greenhouse. Flood-tolerant species may have a particularly efficient system for translocating oxygen from the shoot to the root (Kawase, 1981). One study found that lodgepole pine (*Pinus contorta* Dougl. ex Loud.) responded better to flooding when it had been acclimatized to that condition, because air spaces had developed in plants grown in flooded conditions but not in plants grown in well-drained soil (Kawase, 1981). One reason plants in the total flood treatment may not have done as well as we had expected is that our plants were not acclimated to saturated soil.

The daily watering treatment produced plants with lower than expected plant and root dry weight and relative growth rate. The cause for this is not obvious, but we speculate that it could be due to the nutrient balance of the root zone. Although we minimized leaching and applied similar amounts of fertilizer salts to plants in all treatments, plants in the daily watering treatment were, on average, watered more than twice as often as plants in the slight drought treatment. How this may have affected the pH, the localization of salts, or other characteristics of the root zone is not known.
The maintenance of foliar gas exchange over a wide range of stem water potentials and soil moisture contents supports our conclusion that this clone of *A. maritima* is tolerant of drought (Kubiske and Abrams, 1993). Plants exhibited only modest and gradual reductions in their photosynthetic rates with decrease of stem water potential or soil moisture over most of the ranges in these values we measured (Figs. 1 and 2). Photosynthetic rate did not decline precipitously until root-zone moisture content was < 0.1 m³·m⁻³ and stem water potential ≤ -1.3 MPa. Photosynthetic rate also declined slightly when soil moisture content and stem water potential were at their greatest (Figs. 1 and 2). The gradual decline of photosynthetic rate at the lowest and highest soil moisture contents we created indicate that the photosynthetic apparatus continues to function even after prolonged exposure to extreme root-zone moisture conditions.

Plants in the various drought treatments had similar relative growth rates. The relative growth rate of plants in the partial flood was similar to the growth rate of plants in the slight drought. Hennessey et al. (1985) found that well-watered controls and moderately drought stressed *A. maritima* produced similar leaf area and height, unlike *Alnus glutinosa* (L.) Gaertn., which exhibited reduced leaf area and height in response to drought. The net assimilation rate was not different for plants in any treatment except for plants in the total flood treatment. These data are similar to the findings of Nash and Graves (1993) for sweetbay magnolia, a taxon that sustained physiological functions during both drought and flooding. Although total flooding reduced the relative growth rate and net assimilation rate of the *A. maritima* clone we used, the rates remained positive (Table 1). This distinguishes *A. maritima* from some other woody plants treated with flooding, and the rates were greater than
those reported for flooded plants of sweetbay magnolia and bald cypress, another species native to sites where its roots remain in standing water (Nash and Graves, 1993). Plants of *A. maritima* in the drought treatments exhibited little reduction in dry weight compared with plants in the partial flood treatments, which had relatively high mean weights. This finding is also consistent with the Nash and Graves (1993) study, in which no reduction of root mass was found for sweetbay magnolia and bald cypress exposed to drought.

The number of taxa used in managed landscapes should be broadened. In 1970, 70% of urban trees were comprised of only seven species (Kielbaso, 1990). This homogeneity of plant taxa represents a serious vulnerability to disease and insect problems. Taxa that can withstand soil moisture extremes in urban environments with few detrimental effects need to be selected (Nash and Graves, 1993; Zwack et al., 1998). We conclude that its responses to drought and flooding indicate that *A. maritima* may be used as a new species for managed landscapes.

**Literature Cited**


Table 1. Plant dry weight (root and shoot), root dry weight, root-to-shoot ratio, root dry weight to leaf surface area ratio, relative growth rate, net assimilation, and leaf area ratio for each of seven treatments of *Alnus maritima*. Each value is a mean of 14 plants. We harvested the plants in each block at the end of the fifth cycle for plants in the severe drought treatment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dry weight (g)</th>
<th>Root-to-shoot ratio</th>
<th>Weight to leaf area (mg·cm⁻²)</th>
<th>Relative growth rate (mg·g⁻¹·day⁻¹)</th>
<th>Net assimilation (mg·cm⁻²·week⁻¹)</th>
<th>Leaf area ratio (cm²·g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total flood</td>
<td>4.32 d²</td>
<td>0.67 c</td>
<td>0.22 d</td>
<td>2.01 c</td>
<td>29.8 d</td>
<td>2.29 b</td>
</tr>
<tr>
<td>Partial flood</td>
<td>11.03 a</td>
<td>2.12 ab</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 watering</td>
<td>7.35 c</td>
<td>2.13 ab</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>9.41 ab</td>
<td>2.26 a</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>8.44 bc</td>
<td>1.66 b</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>8.42 bc</td>
<td>1.82 ab</td>
<td>0.31 bc</td>
<td>2.16 c</td>
<td>50.2 bc</td>
<td>3.33 a</td>
</tr>
<tr>
<td>Severe drought</td>
<td>7.68 bc</td>
<td>1.63 b</td>
<td>0.30 c</td>
<td>2.14 c</td>
<td>48.1 bc</td>
<td>3.26 a</td>
</tr>
</tbody>
</table>

²Means 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 $P \leq 0.0001$. 
Fig. 1. Photosynthesis as a function of volumetric soil moisture content. Photosynthetic rate and volumetric soil moisture content were measured for each block when plants in the severe drought treatment had finished their fifth cycle of drought. A quadratic function best fit the trend in these data: $y = 2.53 + 16.11[\text{soil moisture content}] - 12.03[\text{soil moisture content}]^2; r^2 = 0.36$. 
Fig. 2. Photosynthesis as a function of stem water potential. Photosynthetic rate and stem water potential were measured for each block when plants in the severe drought treatment had finished their fifth cycle of drought. A quadratic function best fit the trend in these data: $y = 5.47 - 5.73 \text{[stem water potential (MPa)]} - 5.52 \text{[stem water potential (MPa)]}^2; r^2 = 0.54$
CHAPTER 3. DEVELOPMENT AND GAS EXCHANGE OF
SEEDLINGS FROM THE THREE POPULATIONS OF *ALNUS*
*MARITIMA*

A paper to be submitted to the *Journal of the American Society for Horticultural Science*

Sarah J. Gardner and William R. Graves

**ADDITIONAL INDEX WORDS.** seaside alder, gas exchange, transpiration efficiency, water stress, stomatal conductance

**ABSTRACT.** Plants of *Alnus maritima* [Marsh.] Muhl. ex Nutt. (seaside alder) are attractive, associate with nitrogen-fixing bacteria, and grow in water-logged soils in three disjunct natural populations: the Delmarva Peninsula (southern Delaware and adjacent Maryland), northwestern Georgia, and south-central Oklahoma. Resistance to drought appears strong but has been tested only with genetically uniform plants from Oklahoma. Thus, our objective was to compare photosynthesis, stomatal conductance, transpiration, and development of plants from all populations during treatment with two root-zone moisture regimens. Six seedlings from each of 57 maternal sources, 19 maternal sources from each population (342 plants total) were arranged in randomized complete blocks, with two plants from each maternal source per block. One plant of each maternal source per block served as a control. We kept the lowest 1.5 cm of the root zones of these plants inundated with water. The other
plant from that maternal source in each block was randomly assigned to a treatment of five cycles of drought. Irrigation was withheld during these cycles until root-zone water content was as low as 0.05 m$^3$ m$^{-3}$. After treatment, control plants from Oklahoma had a 35% greater leaf area, 47% greater stem count, and a 44% greater leaf count than control plants from the other two populations. There was a greater reduction in stem and leaf count in plants from Oklahoma in the drought treatment. Drought caused an increase in specific leaf weight of plants from Oklahoma and Delaware, whereas specific leaf weight was high regardless of treatment among plants from Georgia. All populations increased their root-to-shoot ratio in response to drought. Plants in the drought treatment from Oklahoma and Delmarva had the greatest root-to-shoot ratio. Plants from Oklahoma had the highest transpiration rate and change in stem length across treatments. Within the two irrigation regimens, stomatal conductance, photosynthetic rate, transpiration efficiency, and shoot dry weight were similar regardless of population. We conclude that population-based differences in some developmental traits could be used to guide selection of genotypes for horticultural use.

**Introduction**

Although the benefits of species diversity are recognized, managed tree populations in many urban areas are dominated by a few taxa considered resistant to variable moisture supply and other environmental extremes (Berrang et al., 1985; Kielbaso, 1990). More species capable of withstanding the environmental stressors in urban areas are needed for use at harsh planting sites for which only a few taxa are now specified. Both flooding and
drought are considered common stressors that limit the life spans of trees in cities (Berrang et al., 1985; Kramer, 1987). Tree species adapted to flooding have some of the best survival rates in urban areas, presumably because the functions of their roots are sustained in the compacted, poorly aerated soils often found in managed landscapes (Moll, 1989). Some tree species that occur naturally only in wet to saturated soil are unusually resistant to drought as well as flooding (Nash and Graves, 1993).

*Alnus maritima* [Marsh.] Muhl. ex Nutt. (seaside alder) is a rare species that forms small trees in three disjunct populations in the United States: on the Delmarva Peninsula (southern Delaware and the Eastern Shore of Maryland), northwestern Georgia, and south-central Oklahoma (Schrader and Graves, 2000a; 2000b). *A. maritima* thrives in saturated soil. Its roots typically are found at least partially submerged in water in all three natural populations (Furlow, 1979; Schrader and Graves, 2000a; 2000b; Stibolt, 1981). Trees of *A. maritima* are attractive, particularly during autumn when their glossy leaves can become golden-brown and its yellow catkins are abundant. Compared with other members of its genus, *A. maritima* may be unusually drought resistant (Hennessey et al., 1985). Our previous research demonstrated drought resistance in one clone of *A. maritima* from Oklahoma (Chapter 2), indicating that a thorough evaluation of germplasm from all three populations is warranted.

Responses to different levels of water availability of other members of the genus *Alnus* are known to differ depending on the geographic origin of the individuals being evaluated. Dang et al. (1994) showed that photosynthetic rate, mesophyll conductance, transpiration rate, and stomatal conductance varied among *Alnus rubra* Bong. (red alder)
from different provenances in Canada. *Alnus cordata* Loisel. (Italian alder) from five geographic regions also differed in responses to drought (Borghetti et al., 1989). No such comparisons of germplasm from the disjunct populations of *A. maritima* have been made, but interspecific variation in other traits of this germplasm has been demonstrated (Schrader and Graves, 2000a; 2000b), including traits that might influence drought resistance, such as root-to-shoot ratio and specific leaf weight. These differences led us to hypothesize that there are population-based differences in responses to water stress among plants of *A. maritima*. Our objective was to determine whether origin of population is a significant source of variation in the development and gas exchange of seedlings of *A. maritima*. Population effects were examined among plants treated with drought along with plants in a control treatment intended to simulate the wet soils in which plants occur in the wild.

**Materials and Methods**

**PLANT MATERIAL AND EXPERIMENTAL DESIGN.** Seeds of *A. maritima* collected from the three natural populations were stratified for 21 d at 4°C. They were placed in petri plates in germination conditions on 1 Jan 2000, and germinated seeds were transferred to containers 8 d later as described by Schrader and Graves (2000a). On 14 Mar., seedlings were transplanted singly into plastic pots (top diameter = 15.2 cm; height =15cm) in a mix of 2 perlite : 2 *Sphagnum* peat : 1 soil (by volume). Six seedlings from each of 57 maternal sources, 19 maternal sources from each of the three populations, were potted (342 plants total) and randomized in three complete blocks on greenhouse benches, with two plants from
each maternal source represented in each block. Plants were subjected to the natural photoperiod in Ames, Iowa. Plants were irrigated once daily with tap water and fertilized once weekly with a solution of Peters® Excel® All-Purpose and Cal-Mag® (16.5N-2.2P-13.5K; Grace-Sierra Horticultural Products, Milpitas, Calif.) that contained 11.0 mM N. Beginning on 4 Apr., plants were provided water every other day and fertilized once weekly until treatments commenced.

TREATMENTS. Treatments began 16 May, and initial stem length of each plant was determined. Within each block, one plant from each maternal source was randomly assigned to a drought treatment, while the other plant from each source was designated to a control treatment. Containers with control plants were held in 1.5 cm of tap water that was maintained in sealed greenhouse benches with side walls. Each pot assigned to the drought treatment was placed within a 1.9-L specimen container (Fisher Scientific, Pittsburgh, Penn.) that prevented water in the bench from entering drainage holes in the pots. Five cycles of drought were imposed by withholding irrigation until mean moisture content of the root zones within seed source-block combinations decreased to prescribed levels as determined with a model HH1 Theta Probe for organic media with a model ML1 sensor (Delta-T Services, Cambridge, England). Mean moisture content of all plants from a seed source group in the drought treatment was allowed to decrease to 0.1 m$^3$·m$^{-3}$ for the first two cycles and to 0.05 m$^3$·m$^{-3}$ for the final three cycles of drought. Both the control and drought-treatment plants were fertilized by seed source groups at the end of each drought cycle 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 11.0 mM N for the first two cycles and
14 mM N for the later cycles. To rehydrate the plants, the specimen containers were filled with water and plants were maintained in water for 24 hours, after which any remaining water was discarded. Duration of drought treatment varied among plants from seed sources due to differences in evapotranspiration and averaged 89 d for plants from Oklahoma and Georgia and 95 d for plants from the Delmarva Peninsula. Gas exchange was measured with a LI-COR 6400 Portable Photosynthesis System (LI-COR, Lincoln, Nebr.). Three plants in the drought treatment from each population and their corresponding controls in each of the three blocks (54 plants total) were randomly chosen for measurement at least once weekly. The air temperature in the greenhouse was from 22.5 to 33.2 °C, relative humidity ranged from 40 to 82%, and photosynthetically active radiation was 2 to 1743 µmol·s⁻¹·m⁻².}

**Harvest.** Destructive harvest of plants in both irrigation treatments within seed source groups was completed as the fifth drought cycle ended for each seed source group. Surface area of leaves 1 cm or longer was measured with a model 3100 leaf area meter (LI-COR). 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 hr. The number of secondary stems and leaves longer than 1 cm were counted. Change in the primary stem length was calculated. Specific leaf weight was calculated by dividing the dry weight of the leaves by the leaf area.

**Data Analysis.** Data were analyzed by using the Statistical Analysis System software (SAS Institute Inc., 1988). The mixed procedure was used for development and gas exchange data. Populations were considered fixed effects, and seed sources were random effects nested within populations. Mean separations were performed with paired t-tests by
using the least square means statement with the pdiff option. A regression procedure was used to analyze the quadratic effects of soil moisture content on photosynthesis. The regression curves of plants from the different populations were analyzed. We used the error sums of squares to get a mean square of the regression curve of the pooled data from all three populations and the separate curves from each of the three populations. An F-statistic was then obtained. It indicated the probability of a difference in the three regression curves, one for each population, of photosynthetic rate on soil moisture content.

Results

Effects of population, treatment, and their interaction were significant for root-to-shoot ratio, leaf area, specific leaf weight, stem count, and leaf count (Table 1). Root-to-shoot ratio increased in response to drought for plants from all populations, and plants from the Delmarva Peninsula and Oklahoma populations in the drought treatment had the most root weight per unit shoot (Table 1). Plants from Oklahoma in the control treatment had the highest leaf area, 35% more leaf area on average than control plants from the other two populations (Table 1). Leaf area of plants subjected to drought was similar across populations; thus, relative to their respective controls, plants from Oklahoma exhibited the greatest percentage decrease in leaf area in response to reduced water supply (Table 1). Drought led to increased specific leaf weight for plants from the Delmarva Peninsula and Oklahoma, while specific leaf weight was high regardless of treatment among plants from Georgia (Table 1). Plants from Oklahoma in the control treatment had the lowest specific
leaf weight but had the highest stem and leaf count, 47% and 44% higher, respectively, than the combined means of control plants from Delmarva and Georgia (Table 1). Although plants from Oklahoma treated with drought had more stems and leaves than drought-stressed plants from the other populations, they exhibited the greatest reduction in stem and leaf count (Table 1).

Transpiration rate, change in primary stem length, and root dry weight varied due to the main effects of population and treatment, but there was not an interaction. Averaged over both irrigation treatments, plants from Oklahoma had the highest transpiration rate (Table 2). Plants from Oklahoma had shorter primary stems (15.4 cm) than plants from the Delmarva Peninsula and Georgia (20.0 and 19.2 respectively) when treatments began. After treatments, plants from the Oklahoma population were found to have the greatest gain in primary stem length, 17% more than the combined means of the plants from the other populations (Table 2).

Only the treatment main effect was a significant source of variation for stomatal conductance, photosynthetic rate, transpiration efficiency, and shoot dry weight. Stomatal conductance and photosynthetic rate were 63% and 21% greater, respectively, among plants in the control treatment than among plants subjected to drought (Table 3). Transpiration efficiency increased and shoot dry weight decreased in response to drought (Table 3).

A quadratic regression function best represented the relationship of photosynthesis rate and soil moisture content at the time photosynthesis was measured. The quadratic function was \( y = 2.74 + 35.03[\text{soil moisture content}] - 48.87[\text{soil moisture content}]^2; \ r^2 = 0.11; \ P \leq 0.0001 \) (Fig. 1). Photosynthetic data for plants of all populations were pooled due
to the similarity of regression functions that resulted from considering each population individually; the probability of no difference in the three curves was $P > 0.10$ (Fig. 1).

**Discussion**

Plants from all three populations exhibited some drought-tolerant characteristics. Drought treatments did not cause mortality, and photosynthesis was maintained at a relatively high rate (Fig. 1). However, our results show distinct differences between populations of *A. maritima* in their responses to low and high water supply. Population-by-treatment interactions for root-to-shoot ratio, leaf surface area, specific leaf weight, stem count, and leaf count indicate the influence of the irrigation regimens we used differed depending on the geographic origin of the seedlings. If such differences in response to water supply are found through subsequent research to be expressed in plants grown under field conditions, the information we present here could be used to guide efforts to select genotypes of *A. maritima* for use in managed landscapes.

This study strengthens the evidence that *A. maritima* seedlings from Oklahoma are capable of developing particularly high ratios of root to shoot dry mass. In a previous study using plants not subjected to drought, Schrader and Graves (2000a) found that seedlings from Oklahoma developed higher root-to-shoot ratios than seedlings from the Delmarva Peninsula and Georgia. While our experiment showed relatively small population differences in root-to-shoot ratios among well-irrigated seedlings (Table 1), all populations showed increased root-to-shoot ratios in response to drought. As indicated by the significant interaction of
population and treatment, populations differed in the degree to which the ratio increased due to drought; percentage increases ranged from 27% for plants from Georgia to 43% for plants from Oklahoma (Table 1).

Other measures of shoot development we made were consistent with the data for root-to-shoot ratio. Drought evoked large decreases in leaf surface area, stem count, and leaf count among plants from Oklahoma compared to plants of the other two populations (Table 1). An increase in the development of root tissue relative to shoot development can be advantageous during drought. Increases in root-to-shoot ratios lead to a comparatively high portion of the plant being involved in extraction of water from the root zone, while a relatively small portion of the plant loses water via transpiration (Hsiao, 2000; Monneveux and Belhassen, 1996). The relatively large curtailment of shoot development during drought among plants from Oklahoma might be considered detrimental among horticulturists because aesthetic traits of the shoot system are critical if a new landscape plant is to be accepted in the industry. Hence, while recognizing the potential significance of the large drought-induced increase in root-to-shoot ratio among plants from Oklahoma, plants from Oklahoma also had more stems and leaves after drought than did plants from the Delmarva Peninsula and Georgia (Table 1). These data suggest that the Oklahoma population could be the best source of genotypes that could be selected for the capacity to adjust root-to-shoot ratio based on prevailing water supply while also sustaining a comparatively well-developed shoot system.

Leaves with a high specific weight may have an unusually great photosynthetic capacity per unit area and a high water-use efficiency (Nobel, 1980). Taxa that form leaves with a high specific weight under all conditions, or taxa capable of increasing specific weight
when water supply becomes limiting, could be desirable at landscape planting sites prone to
drought. While our finding that plants from Georgia have comparatively high specific leaf
weights (Table 1) substantiates previous data pertaining to well-irrigated plants (Schrader and
Graves, 2000a), we also provide new evidence that only the population from Georgia does
not increase specific leaf weight in response to drought (Table 1). The capacity to adjust
specific leaf weight in response to soil moisture availability may be an important criterion on
which to base selections of *A. maritima* (Mooney, 1980). Highly variable moisture contents
are common in soils of managed planting sites. Leaves with low specific weights could
optimize carbon fixation during periods when moisture supply is ample and high rates of
transpiration do not risk depletion of soil moisture. This idea is consistent with our finding
that plants from Oklahoma had relatively low specific leaf weight (Table 1), the highest rates
of transpiration (Table 2), the greatest increase in stem length (Table 2), and relatively high
leaf surface area and root dry weight when provided a constant supply of soil moisture (Table
1). When water supplies decrease, however, the reduced foliar water loss per unit carbon
fixed that would result from increased specific leaf weight might be an important mechanism
of drought resistance. The relative value of a flexible or consistently high specific leaf
weight in *A. maritima* should be evaluated under field conditions. Our data show that both
population of origin and soil moisture conditions affect specific leaf weight in this species,
but further research is needed to discern the relative importance of specific leaf weight for
conferring drought resistance in *A. maritima*.

We did not detect differences in photosynthetic rate, stomatal conductance,
transpiration efficiency, and shoot dry weight between the three populations (Table 3, Fig. 1).
Photosynthetic data were taken over a very wide range of soil moisture and environmental conditions, and any subtle variability in photosynthetic rate could be masked by the extreme variability in soil moisture and ambient environment. Photosynthetic rate was lowered 18% by drought, while stomatal conductance was lowered by 39%. These data are consistent with a previous claim that *A. maritima* is a relatively drought-resistant member of its genus (Hennessey et al., 1985), because drought-tolerant trees tend to maintain a relatively high photosynthetic rate with a decreased stomatal conductance during periods of drought (Kubiske and Abrams, 1993). A study on ponderosa pine (*Pinus ponderosa*) did find stomatal conductance in the drought-tolerant population was very sensitive to water deficits. Stomatal conductance was relatively high when water was available to the root systems and decreased precipitously when water became limited (Zhang et al., 1997). In the study in chapter two, we found the optimal soil moisture for a high photosynthetic rate was 0.7 m$^3$•m$^{-3}$. In this study, photosynthesis appears to be reduced at high soil moistures because of measurements of plants in the drought treatment that had not regained their previous photosynthetic rate after rehydration (Fig. 1). Plants from all three populations increased their transpiration efficiency by 6% in the drought treatment (Table 3). Although this may be a mechanism to conserve water, some studies have found no correlation between transpiration efficiency and drought tolerance (Zhang et al., 1997).

Plants from the Oklahoma population thrived in the saturated soil of the control treatment as evidenced by much of the developmental data. In some urban areas, saturated soil may be more problematic than dry soil (Berang et al., 1985). Plants from the Oklahoma population may be the best choice in these areas, where an ornamental shrub with abundant
biomass is desired. However, plants from all three populations exhibited drought tolerant characteristics that may be valuable traits when selection criteria are evaluated. Field experiments need to be conducted to further investigate drought response in the three populations of *A. maritima*.

**Literature Cited**


Table 1. Root-to-shoot ratio, leaf area, specific leaf weight, stem count, and leaf count of the three *Alnus maritima* populations at harvest. All variables in this table shared significant effects of population, treatment, and population-by-treatment interaction. Each value is a mean of 57 plants. Plants in the drought treatment went through five cycles of drought. Plants from the same maternal source in both the drought and control treatments were harvested at the same time.

<table>
<thead>
<tr>
<th>Population</th>
<th>Root-to-shoot ratio</th>
<th>Leaf area (cm²)</th>
<th>Specific leaf weight (mg·cm⁻²)</th>
<th>Stem count</th>
<th>Leaf count</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Drought</td>
<td>Control</td>
<td>Drought</td>
<td>Control</td>
</tr>
<tr>
<td>Delmarva</td>
<td>0.74 c</td>
<td>1.01 a</td>
<td>684 b</td>
<td>265 c</td>
<td>4.93 b</td>
</tr>
<tr>
<td>Georgia</td>
<td>0.62 d</td>
<td>0.79 b</td>
<td>636 b</td>
<td>340 c</td>
<td>5.38 a</td>
</tr>
<tr>
<td>Oklahoma</td>
<td>0.70 cd</td>
<td>1.00 a</td>
<td>891 a</td>
<td>316 c</td>
<td>4.34 c</td>
</tr>
</tbody>
</table>

*Means within each pair of columns for each dependent variable followed by the same letter are not different at a P ≤ 0.05 according to paired t-tests in the mixed procedure of the Statistical Analysis System.*
Table 2. Transpiration rate, change in primary stem length, and root dry weight of the three *Alnus maritima* populations. All variables in this table are significant on a population and treatment level. Values for change in primary stem length and root dry weight represent means of 114 plants. Plants from the same maternal source in both the drought and control treatments were harvested at the same time.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Transpiration rate (mmol H₂O• m⁻²•s⁻¹)</th>
<th>Change in primary stem length (cm)</th>
<th>Root dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Population</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delmarva</td>
<td>2.82 c²</td>
<td>11.0 b</td>
<td>3.92 a</td>
</tr>
<tr>
<td>Georgia</td>
<td>2.99 bc</td>
<td>11.5 b</td>
<td>3.23 b</td>
</tr>
<tr>
<td>Oklahoma</td>
<td>3.21 a</td>
<td>13.2 a</td>
<td>3.93 a</td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.44 a</td>
<td>15.2 a</td>
<td>4.42 a</td>
</tr>
<tr>
<td>Drought</td>
<td>2.58 b</td>
<td>8.6 b</td>
<td>2.96 b</td>
</tr>
</tbody>
</table>

²Means within each column, by population and treatment, followed by the same letter are not different at a $P \leq 0.05$ according to paired t-tests in the mixed procedure of the Statistical Analysis System.
Table 3. Stomatal conductance, photosynthetic rate, transpiration efficiency, and shoot dry weight of plants from the three *Alnus maritima* populations. All variables in this table showed a significant treatment effect only. Values are means of 510 observations from all three populations. Plants from the same maternal source in both the drought and control treatments were harvested at the same time.

<table>
<thead>
<tr>
<th>Soil moisture</th>
<th>Stomatal conductance (mol H₂O·m⁻²·s⁻¹)</th>
<th>Photosynthetic rate (µmol CO₂·m⁻²·s⁻¹)</th>
<th>Transpiration efficiency (µmol CO₂/mmol H₂O)</th>
<th>Shoot dry weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.31a²</td>
<td>8.71 a</td>
<td>2.58 b</td>
<td>6.60 a</td>
</tr>
<tr>
<td>Drought</td>
<td>0.19 b</td>
<td>7.18 b</td>
<td>2.73 a</td>
<td>3.26 b</td>
</tr>
</tbody>
</table>

²Means within each column followed by the same letter are not different at a P ≤ 0.05 according to paired t-tests in the mixed procedure of the Statistical Analysis System.
Fig. 1. Photosynthesis as a function of volumetric soil moisture content. Data from the three populations were pooled. Photosynthetic rate and volumetric soil moisture were measured at least once weekly for an average of 89 d for plants from Oklahoma and Georgia and 95 d for plants from the Delmarva Peninsula. A quadratic function best fit the trend in these data: $y = 2.74 + 35.03[\text{soil moisture content}] - 48.87[\text{soil moisture content}]^2; r^2 = 0.11$. 
CHAPTER 4. GENERAL CONCLUSIONS

*A. maritima* is a unique and potentially valuable plant. The studies included in this thesis provide further evidence of the suitability of *A. maritima* for use in managed landscapes. Previous research in our lab has shown that *A. maritima* is easily propagated and exhibits variation between the populations. *A. maritima* is particularly promising due to both its ability to withstand saturated soil and its capacity to associate with bacteria that fix atmospheric nitrogen. Previous research suggests *A. maritima* will not exhibit invasive characteristics if introduced into managed landscapes.

In our first study (Chapter 2), we found *A. maritima* (propagated from one plant from Oklahoma) withstood a wide range of soil moisture levels. All plants of *A. maritima* exposed to the four drought treatments had relatively high plant dry weights. Plants in every treatment were similar in their net assimilation rate, except for plants in the total flood treatment, which had a lower rate. In general, we did not find extreme differences in plant development subjected to a wide range of soil moisture treatments. This confirms our hypothesis that *A. maritima*, like some other woody plants normally found in wetlands, will not have extreme detrimental responses to drought.

In our second study (Chapter 3), we investigated the variability of drought response in the three populations. The three populations responded differently to drought as evidenced by the interaction between the main effects of population and treatment for many of the developmental characteristics. Because of the statistical analysis, large number of seed sources, and the small sizes of the wild populations, we believe our data represent the
populations as a whole. The variations in response to drought present us with a number of characteristics to evaluate and use for selection. If a plant with a large leaf area and greater number of stems and leaves is desired, then a seed source or clone from Oklahoma should be selected. However, because plants from Oklahoma had the greatest reduction in above ground biomass in response to drought, their ornamental characteristics could be compromised when soil becomes dry. If a consistently high specific leaf weight is confirmed as important during subsequent field studies, then plants from Georgia will be most promising.

There are many more areas of research to be conducted on *A. maritima*. A study should be done on *A. maritima* and soil moisture under field conditions. Particularly promising seed sources should be investigated further. Because of the developmental variation seen in this study and previous studies, possible genotypic variation of the three populations should be examined on the molecular level.
APPENDIX A. GAS EXCHANGE AND DESICCATION OF ALNUS MARITIMA SUBJECTED TO THREE RATES OF SOIL DRYING

Introduction

We often use greenhouse studies to predict what will happen in the field. However, the imposed treatments and environment in greenhouses may not duplicate field conditions. A drought treatment can be much more severe and more rapid than what usually takes place in nature. Our objective was to investigate what influence the rate of soil desiccation had on the drought response of Alnus maritima [Marsh.] Muhl. ex Nutt. To accomplish this we examined photosynthetic rate, transpiration efficiency, and the ratio of desiccated leaves to non-desiccated leaves in A. maritima exposed to three rates of soil drying.

Material and Methods

Thirty plants propagated from the same Oklahoma plant were transplanted into three container volumes (1430, 3160, and 5510 cm$^3$). There were 10 plants per container size. On Dec. 10, 1999 pots were completely randomized in the greenhouse. The initial primary stem length was measured and number of leaves counted. Five plants per each container size were randomly assigned a drought treatment and the other five a control treatment. Six cycles of drought were imposed on plants assigned to the drought treatment by withholding irrigation until moisture content of the root zones was $< 0.10$ m$^3$·m$^{-3}$, as determined by individual plant,
with a model HH1 Theta Probe for organic media with a model ML1 sensor (Delta-T Services, Cambridge, England). Water was maintained in saucers placed under pots assigned to the control. Moisture content of pots in the control was 0.35 m$^3$·m$^{-3}$ - 0.50 m$^3$·m$^{-3}$. Gas exchange measurements were taken daily on the first fully expanded leaf through a full drought cycle. The desiccated leaves of each plant were collected throughout the experiment. At the end of the sixth cycle, a destructive harvest was performed on the plant in the drought treatment and a corresponding plant in the flood treatment. A final primary stem length was taken and the weight of desiccated and non-desiccated leaves were determined after at least 3 d in 67 °C. The study lasted 45 d total.

Data were analyzed by using the Statistical Analysis System software (SAS Institute Inc., 1988). The glm procedure was used for leaf and gas exchange data. Means separation of the leaf data was accomplished with the Tukey-Kramer option. Mean separations for gas exchange data were performed with paired t-tests by using the lsmeans statement with the pdiff option. A regression procedure was used to analyze the quadratic effects of soil moisture content on photosynthesis and the difference or similarities of the three curves were analyzed as in Chapter 3.

Results

The rate of drying was different for plants in each container size, with the large size displaying the slowest rate and the small size, the fastest rate (Table 1). Plants in soil that dried at a rapid and moderate rate had the largest ratio of desiccated leaves to non-desiccated
leaves and the lowest photosynthetic rate. Transpiration efficiency was highest in plants in soil with the rapid rate of drying. Plants in the three drought treatments had greater transpiration efficiencies than plants in the control treatments (Table 1).

A quadratic regression function best represented the relationship of photosynthesis rate and soil moisture content at the time photosynthesis was measured (Fig. 1). The quadratic function of the rapid rate of drying was $y = 1.41 + 45.80[\text{soil moisture content}] - 73.83[\text{soil moisture content}]^2; r^2 = 0.24; P \leq 0.0001$ (Fig. 1). The quadratic function for the moderate rate was $y = 0.59 + 53.59[\text{soil moisture content}] - 89.57[\text{soil moisture content}]^2; r^2 = 0.26; P \leq 0.0001$ (Fig. 1). The quadratic function for the slow rate of drying was $y = -2.79 + 99.31[\text{soil moisture content}] - 184.76[\text{soil moisture content}]^2; r^2 = 0.46; P \leq 0.0001$ (Fig. 1). An analysis of the three curves determined a difference in the curves.

**Discussion**

The rate the soil dried did affect the variables reported. Leaf desiccation was greater with a rapid and moderate rate of soil dehydration. Photosynthetic rate also was depressed in plants with roots in soil that dried quickly and moderately (Table 1). The rate of soil drying affected the regression of photosynthesis on soil moisture (Fig. 1). This information underscores the importance of carefully evaluating all aspects of a study. Many researchers have contrasted the severity of imposed droughts and the accelerated rate of drying in greenhouse studies as opposed to field studies. Often times, how plants respond to the artificial environment of greenhouses and the treatments imposed by researchers, will not
exactly correlate with how the plant will respond in the field. However, greenhouse studies provide us with preliminary data that can be utilized in subsequent field studies.
Table 1. Mean number of days in the cycle, ratio of weight of desiccated leaves to non-desiccated, photosynthetic rate, and transpiration efficiency of *Alnus maritima* subjected to three rates of soil drying. The numbers in each cell for cycle and leaf data represent a mean of 5 plants.

<table>
<thead>
<tr>
<th>Container size (cm³)</th>
<th>Soil moisture</th>
<th>Mean number of days in cycle</th>
<th>Ratio of weight of desiccated leaves/non-desiccated leaves</th>
<th>Photosynthetic rate (µmol CO₂•m⁻²•s⁻¹)</th>
<th>Transpiration efficiency (µmol CO₂/mmol H₂O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1430</td>
<td>Drought</td>
<td>4.0 c²</td>
<td>0.71 a</td>
<td>7.05 c</td>
<td>2.83 a</td>
</tr>
<tr>
<td>3160</td>
<td>Drought</td>
<td>5.8 b</td>
<td>0.78 a</td>
<td>7.30 c</td>
<td>2.63 b</td>
</tr>
<tr>
<td>5510</td>
<td>Drought</td>
<td>7.2 a</td>
<td>0.42 b</td>
<td>8.53 b</td>
<td>2.65 b</td>
</tr>
<tr>
<td>1430</td>
<td>Control</td>
<td>-</td>
<td>0.03 c</td>
<td>8.82 b</td>
<td>2.45 c</td>
</tr>
<tr>
<td>3160</td>
<td>Control</td>
<td>-</td>
<td>0.08 c</td>
<td>8.33 b</td>
<td>2.37 c</td>
</tr>
<tr>
<td>5510</td>
<td>Control</td>
<td>-</td>
<td>0.17 bc</td>
<td>9.61 a</td>
<td>2.45 c</td>
</tr>
</tbody>
</table>

²Means within each column, by population and treatment, followed by the same letter are not different at a P ≤ 0.05 according to paired t-tests in the glm procedure for gas exchange data and Tukey-Kramer for leaf and cycle data.
Rapid rate (1430 cm³)

Moderate rate (3160 cm³)

Slow rate (5510 cm³)

Rate of photosynthesis (μmol CO₂·m⁻²·s⁻¹)

Volumetric soil moisture content (m³·m⁻³)
APPENDIX B. PRESENCE OF DEHYDRINS IN ROOT TISSUE OF

**ALNUS MARITIMA**

Introduction

One way in which plants may survive moisture stress is the transcription and accumulation of drought-induced proteins. Dehydrins are a class of late-embryogenesis-abundant (LEA) proteins found in plants. They are hydrophilic proteins that may play a role in binding water in cells of drought-stressed plants so that the cellular structure is not compromised beyond repair (Ingrams and Bartels, 1996; Celleir et al., 1998). In one study a correlation between drought survival and LEA protein accumulation but not transcript accumulation was found (Reid and Walker-Simmons, 1993). Dehydrin induction may be tissue specific in some plants. In wheat (*Triticum durum* Desf.) seedlings an accumulation of LEA proteins was found in the shoot, but not in the root, when the plants were subjected to severe drought (Bray, 1993). Artlip and Wisniewski (1997) found a high accumulation of a drought-induced dehydrin protein in bark tissue of *Prunus persica* (L.) batsch trees, but a low level of accumulation in leaves. In that study protein levels remained high one week after drought stress was relieved, while transcript levels were negligible. Research in our lab has found the presence of dehydrin proteins in leaves of *Acer rubrum* L. cv. Franksred (Red Sunset®) in both a drought and well-watered control treatment. However, the presence of dehydrin protein was not detected in leaves of drought or control plants of *Alnus maritima* [Marsh.] Muhl. ex Nutt., even though the presence of dehydrin genes were ascertained in the
genome by Southern analysis (Foster and Graves, unpublished research). The objective of this study was to analyze root tissue of *A. maritima* for the presence of dehydrin proteins.

**Materials and Methods**

At the end of the sixth drought cycle, roots of plants in the study in Appendix A were immediately frozen in liquid N₂. Proteins were extracted from this tissue with a modified version of a protocol used by Wisniewski et al. (1996). Root tissue, 4-5 g, was homogenized with 10 ml Borate Buffer. The homogenate was centrifuged twice to clarify the proteins. The resultant supernatant was heated to 95°C to precipitate any heat-sensitive proteins. After cooling on ice the sample was centrifuged again to pellet the precipitated proteins. Proteins were precipitated from the resultant supernatant by the addition of 0.1 ml of trichloroacetic acid (about 10% v/v). The precipitate was collected by centrifugation then washed with ice-cold acetone and dried. The sample was then resuspended with Borate Buffer. Protein concentration was determined with the Bradford assay (Bradford, 1976) and a discontinuous SDS-PAGE was conducted (Laemmli, 1970). Seventy micrograms of protein was loaded per well. Gels were either stained with 0.01% Coomassie Brilliant Blue R-250 to visualize all the proteins, or proteins were transferred from the gels onto 0.45-µm nitrocellulose membranes (Towbin et al., 1979). A Bio-Rad Immun-Star Chemiluminescent Detection Kit (Bio-Rad) was used to detect dehydrin proteins after the membranes were incubated with a dehydrin antibody or a blocked dehydrin antibody provided by T. Close (Close et al., 1993). The membrane was then exposed to x-ray film for 2 min.
Results and Discussion

Protein bands were evident on the x-ray film at 40 and 49 kD and were not apparent on the x-ray film exposed to the membrane with blocked antibody, indicating there was specific binding of the dehydrin antibody. However, as in the research on *Acer rubrum*, the protein was detected in both the plants in the drought treatment and plants in the control treatment. Furthermore, protein accumulation exhibited no apparent pattern related to the rate of drying. It is possible that protein was not loaded equally in all the wells; any small deviation in the amount of sample loaded could have a large effect on the amount of protein in each band. Protein tended to smear in the gels stained with Coomassie, so that equal loading of proteins was difficult to visualize. Further work needs to be conducted on leaf, root, and possibly bark tissues of *A. maritima*, to understand the role or lack of a role dehydrins play in the drought response of *A. maritima*.

Literature Cited


APPENDIX C. DEVELOPMENT AND GAS EXCHANGE OF THE
THREE POPULATIONS OF *ALNUS MARITIMA* AND FOUR OTHER
SPECIES OF *ALNUS*

In the study described in Chapter 3, we had included four seed sources (six plants per seed source) from other *Alnus* species (*Alnus glutinosa* (L.) Gaertn, *Alnus serrulata* Willd., *Alnus nepalensis* Don, and *Alnus nitida* (Spach.) Endel. *A. glutinosa* plants were propagated from seeds from Eastern Europe. *A. serrulata* seeds were collected from trees in Georgia. *A. nepalensis* and *A. nitida* seeds were from India. Due to the small representation of each of these taxa relative to the representation of *Alnus maritima* [Marsh.] Muhl. ex Nutt., they were not included in the statistical analysis. However, we think it is interesting to compare the four other species with the three populations of *A. maritima*, especially the two southern Asian *Alnus* species (*A. nepalensis* and *A. nitida*), the other members of the *Clethropsis* subgenus. We compare developmental data in Table 1. The very large amount of aboveground biomass of *A. nitida* is quite evident. *A. nitida* had a large shoot dry weight, change in primary stem length, and leaf area, this is especially apparent for plants in the control treatment. The dry weight of both the root and the shoot, as well as the root-to-shoot ratio of *A. serrulata* plants in the control treatment fell within the range of values of the *A. maritima* populations. *A. serrulata* and *A. maritima* share similar habitats on the Delmarva peninsula and in Georgia.

Due to the large number of comparisons, gas exchange data were pooled for both the control and drought plants and analyzed (Table 2). Gas exchange data did exhibit some
differences (Table 2). \textit{A. nitida} had the greatest stomatal conductance. Photosynthetic rate was similar for all species and populations, except \textit{A. serrulata}, which had a lower rate than all taxa except \textit{A. glutinosa}. All three populations of \textit{A. maritima} had greater transpiration efficiencies than the other four species of \textit{Alnus}. Table 3 is a comparison of that same data with the means of the drought and control plants separated.
Table 1. Shoot and root dry weight, root-to-shoot ratio, change in primary stem length, leaf area, and specific leaf weight of *A. glutinosa*, *A. serrulata*, *A. nitida*, *A. nepalensis*, and the three populations of *A. maritima*. Each value of *A. maritima* is a mean of 57 plants. Each value of *A. glutinosa*, *A. serrulata*, *A. nitida*, and *A. nepalensis* is a mean of 3 plants.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Dry weight (g)</th>
<th>Change in primary stem length (cm)</th>
<th>Leaf area (cm²)</th>
<th>Specific leaf weight (mg·cm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shoot</td>
<td>Root</td>
<td>Root-to-shoot ratio</td>
<td></td>
</tr>
<tr>
<td><em>A. maritima</em></td>
<td>Control Drought</td>
<td>Control Drought</td>
<td>Control Drought</td>
<td>Control Drought</td>
</tr>
<tr>
<td>population</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delmarva</td>
<td>6.57</td>
<td>3.30</td>
<td>4.63</td>
<td>3.21</td>
</tr>
<tr>
<td>Georgia</td>
<td>6.11</td>
<td>3.50</td>
<td>3.76</td>
<td>2.70</td>
</tr>
<tr>
<td>Oklahoma</td>
<td>7.11</td>
<td>3.00</td>
<td>4.88</td>
<td>2.98</td>
</tr>
<tr>
<td>Species</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. glutinosa</em></td>
<td>14.20</td>
<td>5.54</td>
<td>7.41</td>
<td>4.37</td>
</tr>
<tr>
<td><em>A. serrulata</em></td>
<td>6.53</td>
<td>2.94</td>
<td>4.48</td>
<td>2.32</td>
</tr>
<tr>
<td><em>A. nepalensis</em></td>
<td>4.73</td>
<td>4.79</td>
<td>1.68</td>
<td>1.80</td>
</tr>
<tr>
<td><em>A. nitida</em></td>
<td>23.23</td>
<td>8.66</td>
<td>4.69</td>
<td>2.73</td>
</tr>
</tbody>
</table>
Table 2. Stomatal conductance, photosynthetic rate, transpiration rate, and transpiration efficiency of *A. glutinosa*, *A. serrulata*, *A. nitida*, *A. nepalensis*, and the three populations of *A. maritima*. Gas exchange data were gathered at least once weekly.

The study continued for an average of 89 d for plants from Oklahoma and Georgia and 95 days for plants from the Delmarva peninsula. Treatment lasted 42, 105, 30, and 83 days for *A. glutinosa*, *A. serrulata*, *A. nitida*, and *A. nepalensis* plants, respectively and ended when each seed source group or species finished its fifth cycle of drought.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Stomatal conductance (mol H₂O•m⁻²•s⁻¹)</th>
<th>Photosynthetic rate (µmol CO₂•m⁻²•s⁻¹)</th>
<th>Transpiration rate (mmol H₂O•m⁻²•s⁻¹)</th>
<th>Transpiration efficiency (µmol CO₂/mmol H₂O)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. maritima</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Population</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delmarva</td>
<td>0.24 b</td>
<td>7.59 a</td>
<td>2.82 c</td>
<td>2.67 ab</td>
</tr>
<tr>
<td>Georgia</td>
<td>0.25 b</td>
<td>8.05 a</td>
<td>2.99 bc</td>
<td>2.73 a</td>
</tr>
<tr>
<td>Oklahoma</td>
<td>0.27 b</td>
<td>8.18 a</td>
<td>3.21 b</td>
<td>2.57 bc</td>
</tr>
<tr>
<td>Species</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. glutinosa</em></td>
<td>0.24 b</td>
<td>6.30 ab</td>
<td>2.87 c</td>
<td>2.00 d</td>
</tr>
<tr>
<td><em>A. serrulata</em></td>
<td>0.23 b</td>
<td>5.79 b</td>
<td>2.68 c</td>
<td>2.01 d</td>
</tr>
<tr>
<td><em>A. nepalensis</em></td>
<td>0.39 b</td>
<td>8.85 a</td>
<td>3.79 a</td>
<td>2.38 c</td>
</tr>
<tr>
<td><em>A. nitida</em></td>
<td>0.48 a</td>
<td>8.54 a</td>
<td>3.95 a</td>
<td>2.06 d</td>
</tr>
</tbody>
</table>

*Means within each column followed by the same letter are not different at a P ≤ 0.05 according to paired t-tests in the mixed procedure.*
Table 3. Stomatal conductance, photosynthetic rate, transpiration rate, and transpiration efficiency of *A. glutinosa*, *A serrulata*, *A. nitida*, *A. nepalensis*, and the three populations of *A. maritima*. Gas exchange data were gathered at least once weekly.

The study continued for an average of 89 d for plants from Oklahoma and Georgia and 95 days for plants from the Delmarva peninsula. Treatment lasted 42, 105, 30, and 83 days for *A. glutinosa*, *A. serrulata*, *A. nitida*, and *A. nepalensis* plants, respectively and ended when each seed source group or species finished its fifth cycle of drought.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Stomatal conductance (mol H₂O•m⁻²•s⁻¹)</th>
<th>Photosynthetic rate (µmol CO₂•m⁻²•s⁻¹)</th>
<th>Transpiration rate (mmol H₂O•m⁻²•s⁻¹)</th>
<th>Transpiration efficiency (µmol CO₂/mmol H₂O)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Drought</td>
<td>Control</td>
<td>Drought</td>
</tr>
<tr>
<td><em>A. maritima</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>population</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delmarva</td>
<td>0.30</td>
<td>0.17</td>
<td>8.41</td>
<td>6.77</td>
</tr>
<tr>
<td>Georgia</td>
<td>0.29</td>
<td>0.21</td>
<td>8.77</td>
<td>7.33</td>
</tr>
<tr>
<td>Oklahoma</td>
<td>0.34</td>
<td>0.21</td>
<td>8.94</td>
<td>7.43</td>
</tr>
<tr>
<td>Species</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. glutinosa</em></td>
<td>0.29</td>
<td>0.19</td>
<td>6.87</td>
<td>5.73</td>
</tr>
<tr>
<td><em>A. serrulata</em></td>
<td>0.28</td>
<td>0.18</td>
<td>6.39</td>
<td>5.20</td>
</tr>
<tr>
<td><em>A. nepalensis</em></td>
<td>0.52</td>
<td>0.27</td>
<td>9.49</td>
<td>8.21</td>
</tr>
<tr>
<td><em>A. nitida</em></td>
<td>0.69</td>
<td>0.27</td>
<td>10.05</td>
<td>7.03</td>
</tr>
</tbody>
</table>
APPENDIX D. CHARACTERISTICS OF *ALNUS MARITIMA* 

MATERNAL SEED SOURCES

This appendix includes the longitude and latitude as well as several characteristics of *Alnus maritima* maternal seed sources. The following tables and figures may help in selecting seed sources for desired traits. Tables 1, 2, and 3 illustrate means of maternal seed source variables measured at harvest of plants from Oklahoma, Georgia, and the Delmarva peninsula respectively. Figures 1, 2, 3, 4, 5, and 6 illustrate means of the maternal seed source groups for change in primary stem length, stem count, leaf count, leaf area, root-to-shoot ratio, and specific leaf weight. The x axis represents the means of the three plants per maternal seed source in the control treatment and the y axis the means of the three plants per maternal seed source in the drought treatment, so that each point on the figures represent the maternal seed source group as a whole.
<table>
<thead>
<tr>
<th>Leaf area (cm²)</th>
<th>Root dry weight (g)</th>
<th>Shoot dry weight (g)</th>
<th>Root-to-shoot ratio</th>
<th>Specific leaf weight (mg•cm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Drought</td>
<td>Control</td>
<td>Drought</td>
<td>Control</td>
</tr>
<tr>
<td>619</td>
<td>349</td>
<td>3.98</td>
<td>3.27</td>
<td>5.64</td>
</tr>
<tr>
<td>807</td>
<td>179</td>
<td>4.64</td>
<td>1.83</td>
<td>5.65</td>
</tr>
<tr>
<td>687</td>
<td>499</td>
<td>4.21</td>
<td>3.39</td>
<td>5.61</td>
</tr>
<tr>
<td>988</td>
<td>274</td>
<td>4.72</td>
<td>4.65</td>
<td>7.45</td>
</tr>
<tr>
<td>866</td>
<td>312</td>
<td>5.03</td>
<td>2.96</td>
<td>7.73</td>
</tr>
<tr>
<td>1046</td>
<td>422</td>
<td>4.16</td>
<td>3.36</td>
<td>7.96</td>
</tr>
<tr>
<td>761</td>
<td>300</td>
<td>4.91</td>
<td>2.43</td>
<td>6.30</td>
</tr>
<tr>
<td>1036</td>
<td>575</td>
<td>3.52</td>
<td>3.55</td>
<td>6.48</td>
</tr>
<tr>
<td>1382</td>
<td>289</td>
<td>7.46</td>
<td>2.77</td>
<td>11.70</td>
</tr>
<tr>
<td>744</td>
<td>303</td>
<td>3.72</td>
<td>2.90</td>
<td>5.62</td>
</tr>
<tr>
<td>705</td>
<td>305</td>
<td>2.72</td>
<td>3.35</td>
<td>4.24</td>
</tr>
<tr>
<td>1182</td>
<td>394</td>
<td>7.46</td>
<td>3.34</td>
<td>10.32</td>
</tr>
<tr>
<td>678</td>
<td>271</td>
<td>2.97</td>
<td>2.52</td>
<td>4.81</td>
</tr>
<tr>
<td>1028</td>
<td>252</td>
<td>5.33</td>
<td>1.95</td>
<td>7.69</td>
</tr>
<tr>
<td>759</td>
<td>203</td>
<td>5.06</td>
<td>2.52</td>
<td>7.98</td>
</tr>
<tr>
<td>1032</td>
<td>299</td>
<td>6.50</td>
<td>2.96</td>
<td>8.55</td>
</tr>
<tr>
<td>787</td>
<td>249</td>
<td>5.91</td>
<td>2.53</td>
<td>6.75</td>
</tr>
<tr>
<td>934</td>
<td>242</td>
<td>5.25</td>
<td>3.12</td>
<td>7.68</td>
</tr>
<tr>
<td>881</td>
<td>292</td>
<td>5.08</td>
<td>3.20</td>
<td>6.94</td>
</tr>
<tr>
<td>Leaf area (cm²)</td>
<td>Root dry weight (g)</td>
<td>Shoot dry weight (g)</td>
<td>Root-to-shoot ratio</td>
<td>Specific leaf weight (mg·cm⁻²)</td>
</tr>
<tr>
<td>----------------</td>
<td>---------------------</td>
<td>----------------------</td>
<td>---------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>Control</td>
<td>Drought</td>
<td>Control</td>
<td>Drought</td>
<td>Control</td>
</tr>
<tr>
<td>754</td>
<td>196</td>
<td>4.99</td>
<td>2.17</td>
<td>7.32</td>
</tr>
<tr>
<td>509</td>
<td>242</td>
<td>3.14</td>
<td>3.08</td>
<td>4.49</td>
</tr>
<tr>
<td>530</td>
<td>352</td>
<td>3.58</td>
<td>2.77</td>
<td>5.13</td>
</tr>
<tr>
<td>453</td>
<td>372</td>
<td>3.03</td>
<td>2.52</td>
<td>4.35</td>
</tr>
<tr>
<td>1045</td>
<td>241</td>
<td>4.94</td>
<td>2.54</td>
<td>9.19</td>
</tr>
<tr>
<td>615</td>
<td>273</td>
<td>3.81</td>
<td>3.04</td>
<td>7.40</td>
</tr>
<tr>
<td>788</td>
<td>293</td>
<td>4.45</td>
<td>2.51</td>
<td>8.03</td>
</tr>
<tr>
<td>429</td>
<td>212</td>
<td>2.84</td>
<td>2.39</td>
<td>4.70</td>
</tr>
<tr>
<td>646</td>
<td>232</td>
<td>4.11</td>
<td>2.41</td>
<td>6.14</td>
</tr>
<tr>
<td>454</td>
<td>358</td>
<td>2.87</td>
<td>2.03</td>
<td>3.99</td>
</tr>
<tr>
<td>532</td>
<td>412</td>
<td>3.18</td>
<td>2.60</td>
<td>5.09</td>
</tr>
<tr>
<td>628</td>
<td>310</td>
<td>3.88</td>
<td>2.61</td>
<td>5.81</td>
</tr>
<tr>
<td>682</td>
<td>324</td>
<td>3.24</td>
<td>2.75</td>
<td>5.98</td>
</tr>
<tr>
<td>644</td>
<td>478</td>
<td>4.02</td>
<td>2.93</td>
<td>6.34</td>
</tr>
<tr>
<td>578</td>
<td>444</td>
<td>3.98</td>
<td>2.76</td>
<td>6.08</td>
</tr>
<tr>
<td>789</td>
<td>402</td>
<td>4.62</td>
<td>3.30</td>
<td>7.11</td>
</tr>
<tr>
<td>920</td>
<td>584</td>
<td>4.25</td>
<td>3.80</td>
<td>8.25</td>
</tr>
<tr>
<td>530</td>
<td>398</td>
<td>3.22</td>
<td>2.67</td>
<td>5.95</td>
</tr>
<tr>
<td>552</td>
<td>328</td>
<td>3.22</td>
<td>2.40</td>
<td>4.68</td>
</tr>
<tr>
<td>Leaf area (cm²)</td>
<td>Root dry weight (g)</td>
<td>Shoot dry weight (g)</td>
<td>Root-to-shoot ratio</td>
<td>Specific leaf weight (mg•cm⁻²)</td>
</tr>
<tr>
<td>---------------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Control</td>
<td>Drought</td>
<td>Control</td>
<td>Drought</td>
<td>Control</td>
</tr>
<tr>
<td>719</td>
<td>158</td>
<td>5.80</td>
<td>3.51</td>
<td>6.85</td>
</tr>
<tr>
<td>712</td>
<td>427</td>
<td>4.05</td>
<td>3.14</td>
<td>6.60</td>
</tr>
<tr>
<td>676</td>
<td>231</td>
<td>4.71</td>
<td>2.77</td>
<td>6.95</td>
</tr>
<tr>
<td>1830</td>
<td>315</td>
<td>7.50</td>
<td>3.29</td>
<td>16.07</td>
</tr>
<tr>
<td>693</td>
<td>397</td>
<td>4.69</td>
<td>4.26</td>
<td>7.46</td>
</tr>
<tr>
<td>812</td>
<td>316</td>
<td>5.97</td>
<td>3.74</td>
<td>8.44</td>
</tr>
<tr>
<td>511</td>
<td>245</td>
<td>4.77</td>
<td>3.23</td>
<td>4.57</td>
</tr>
<tr>
<td>483</td>
<td>235</td>
<td>3.26</td>
<td>3.09</td>
<td>4.05</td>
</tr>
<tr>
<td>401</td>
<td>317</td>
<td>3.05</td>
<td>2.86</td>
<td>3.63</td>
</tr>
<tr>
<td>408</td>
<td>246</td>
<td>3.33</td>
<td>2.94</td>
<td>3.86</td>
</tr>
<tr>
<td>619</td>
<td>226</td>
<td>4.12</td>
<td>2.75</td>
<td>5.79</td>
</tr>
<tr>
<td>523</td>
<td>352</td>
<td>3.75</td>
<td>3.59</td>
<td>4.32</td>
</tr>
<tr>
<td>442</td>
<td>267</td>
<td>3.25</td>
<td>2.99</td>
<td>4.39</td>
</tr>
<tr>
<td>892</td>
<td>193</td>
<td>6.15</td>
<td>2.88</td>
<td>8.52</td>
</tr>
<tr>
<td>765</td>
<td>273</td>
<td>5.66</td>
<td>3.43</td>
<td>7.85</td>
</tr>
<tr>
<td>621</td>
<td>229</td>
<td>4.36</td>
<td>3.42</td>
<td>6.07</td>
</tr>
<tr>
<td>834</td>
<td>199</td>
<td>6.66</td>
<td>3.82</td>
<td>9.63</td>
</tr>
<tr>
<td>614</td>
<td>197</td>
<td>4.41</td>
<td>3.14</td>
<td>5.87</td>
</tr>
<tr>
<td>441</td>
<td>207</td>
<td>2.56</td>
<td>2.21</td>
<td>3.90</td>
</tr>
</tbody>
</table>
Fig. 1. Change in primary stem length by maternal seed source groups. The y axis represents the average of three plants per seed source group in the drought treatment. The x axis represents the average of three plants per seed source group in the control treatment. The symbol O represents plants from Oklahoma, G represents plants from Georgia, and E represents plants from the Delmarva peninsula.
Fig. 2. Stem count by maternal seed source groups. The y axis represents the average of three plants per Seed source group in the drought treatment. The x axis represents the average of three plants per seed source group in the control treatment. The symbol O represents plants from Oklahoma, G represents plants from Georgia, and E represents plants from the Delmarva peninsula.
Fig. 3. Change in leaf count by maternal seed source groups. The y axis represents the average of three plants per seed source group in the drought treatment. The x axis represents the average of three plants per seed source group in the control treatment. The symbol O represents plants from Oklahoma, G represents plants from Georgia, and E represents plants from the Delmarva peninsula.
Fig. 4. Leaf area by maternal seed source groups. The y axis represents the average of three plants per seed source group in the drought treatment. The x axis represents the average of three plants per seed source group in the control treatment. The symbol O represents plants from Oklahoma, G represents plants from Georgia, and E represents plants from the Delmarva peninsula.
Fig. 5. Root-to-shoot ratio by maternal seed source groups. The y axis represents the average of three plants per seed source group in the drought treatment. The x axis represents the average of three plants per seed source group in the control treatment. The symbol O represents plants from Oklahoma, G represents plants from Georgia, and E represents plants from the Delmarva peninsula.
Fig. 6. Specific leaf weight by maternal seed source groups. The y axis represents the average of three plants per seed source group in the drought treatment. The x axis represents the average of three plants per seed source group in the control treatment. The symbol O represents plants from Oklahoma, G represents plants from Georgia, and E represents plants from the Delmarva peninsula.
ACKNOWLEDGMENTS

There are many people I need to thank. First and foremost, thank you to Dr. William Graves, I would not have been able to get this far without you. I would like to thank my committee members, Dr. Jeffery Iles and Dr. Richard Hall for their assistance, and patience. I also need to thank Dr. Carol Foster, who gave up so much of her time and energy to help me and teach me new techniques. Another person instrumental to this thesis is soon to be Dr. James Schrader, who germinated all the seeds and helped me in numerous ways, Alnus maritima could not have a more enthusiastic or erudite spokesperson. I need to thank Mark Kroggel for all his help and Dr. Phillip Dixon for all his statistical help. I would like to thank the Horticulture Department that supported me in my graduate studies and all the faculty and staff who have assisted me.

I also need to thank my family, especially my sister Anna, for her editing and support, my daughter Rivkah, for allowing me to use every available flat surface in her room for journal articles and books, and finally, my husband Tom, for his grace and fortitude for putting up with me all these years. Thanks.