Phenology and Depth of Cold Acclimation in the Three Subspecies of *Alnus maritima*

James A. Schrader¹ and William R. Graves

Department of Horticulture, Iowa State University, Ames, IA 50011–1100

ADDITIONAL INDEX WORDS. seaside alder, subspp. *oklahomensis* and *georgiensis*, cold hardiness, tissue discoloration, woody landscape plants, stress tolerance, plant biogeography

ABSTRACT. *Alnus maritima* (Marsh.) Muhl. ex Nutt. is a large shrub or small tree with potential for use in managed landscapes. Because the three subspecies of *A. maritima* are indigenous only to areas with mild winter temperatures (USDA hardiness zones 7a and 7b), knowledge of their cold acclimation and cold hardiness is vital if they are to be used where winters are more harsh. Phenology and depth of cold hardiness were assessed by collecting stem samples seven times from 25 Sept. 2000 to 23 Apr. 2001, subjecting the samples to cold temperature ramping, and determining the lowest survival temperature (LST) via the tissue discoloration method. Samples were collected from indigenous plants of the three subspecies and from plants growing in a common garden near Ames, Iowa (USDA zone 5a). Results indicated that some plants from all three subspecies can survive midwinter extremes as low as -80°C ; that plants grown in Ames achieved a greater depth of cold hardiness for most of the winter and were more uniform in cold hardiness than plants growing in warmer native sites; and that the three subspecies did not differ in phenology or depth of cold acclimation. Results of field trials with plots of 150 plants each installed in three northern hardiness zones (USDA zones 5a, 4a, and 3a) supported these conclusions by showing survival of all 450 plants. We resolved differences among subspecies by rating the percentage of stem tissue survival for each plant in the field plots. Subspecies *maritima*, from the northernmost provenance (the Delmarva Peninsula), showed the least stem death across all three plots (3.9% tissue death), followed by subsp. *georgiensis* from northwestern Georgia (10% tissue death), and subsp. *oklahomensis* from southern Oklahoma (12.8% tissue death). Our results suggest that low temperatures should not limit the use of *A. maritima* in areas as harsh as USDA zone 3a. Selections based on cold hardiness may allow the use of *A. maritima* in areas with even colder winters.

Alnus maritima (seaside alder) is a rare woody species that occurs naturally as three subspecies from widely disjunct provenances in the United States. *Alnus maritima* subsp. *oklahomensis* Schrader & Graves is found naturally in only two counties in south-central Oklahoma, *A. maritima* subsp. *georgiensis* Schrader & Graves is isolated to one county in northwestern Georgia, and *A. maritima* subsp. *maritima* is found only on the Delmarva Peninsula in four counties in Maryland and two counties in Delaware (Schrader and Graves, 2002). There is a growing interest in the use of *A. maritima* in managed landscapes, but the proper geographical range for the use of this species has not been established. The natural distribution of *A. maritima* is limited to areas with relatively mild winter temperatures. Subspecies *oklahomensis* and *maritima* occur in USDA hardiness zone 7a, where the mean minimum temperature is -15 to -18°C , and subsp. *georgiensis* occurs in zone 7b with a mean minimum of -12 to -15°C (Agriculture Research Service, 1990). Although it appears that a lack of cold hardiness has limited *A. maritima* from areas with harsher winters, the present distribution of the species may be only a remnant of a much larger range that included areas as far north as Washington and Idaho (Furrow, 1979; Schrader and Graves, 2000). While *Alnus* is among the most cold hardy genera in the world (Vezina et al., 1997), little information is available on *A. maritima*. Therefore, our overall goal was to model the phenology and cold acclimation of this species.

The peculiar natural distribution of *A. maritima* provided a unique situation for the study of plant cold hardiness. Their isolation and extreme geographic disjunction suggest that the three subspecies of *A. maritima* may differ in phenology and depth of cold acclimation, therefore it is possible that genotypes from one of the subspecies may be more suitable for use in colder areas. We examined the cold hardiness of plants from all three subspecies growing both in natural

environments and in field plots we established in Iowa and Minnesota. Our objectives were to characterize the phenology of winter dormancy, cold acclimation, and return of active shoot growth; to determine the effects of colder climates on the cold hardiness of *A. maritima*; to compare the cold hardiness of *A. maritima* with that of four other species of *Alnus*; and to establish the safe geographical range for *A. maritima* in managed landscapes as defined by plant cold hardiness. Our results will be used to develop guidelines for proper use of *A. maritima* in managed landscapes and will help guide plant breeding and selection efforts. They also provide new clues concerning the origin of the disjunct distribution of *A. maritima*.

Materials and Methods

Cold acclimation and cold hardiness

LABORATORY ASSESSMENTS. Two laboratory assessments of stem tissue hardiness were conducted. The first assessment was performed during Fall, Winter, and Spring 2000–01 by using the tissue discoloration method (Howell and Weiser, 1970), which can be more accurate than use of specific conductivity, tetrazolium staining, and multiple freezing point exotherms (Stergios and Howell, 1973). Tissue discoloration also has proven consistently accurate with numerous woody-plant taxa (Lindstrom and Del Hierro, 1992; Lindstrom and Dirr, 1991; McNamara and Pellet, 1996; Pellet et al., 1981). We examined the cold acclimation and cold hardiness of plants of the three subspecies of *A. maritima* and compared the acclimation and hardiness of plants growing in natural habitats (USDA hardiness zone 7) with those growing in a common garden near Ames, Iowa (USDA hardiness zone 5a).

Samples of stems formed in 2000 were collected on 25 Sept., 30 Oct., and 4 Dec. 2000, and 8 Jan., 12 Feb., 18 Mar., and 22 Apr. 2001 from plants of all three subspecies in situ (growing in Oklahoma, Georgia, and on the Delmarva Peninsula) and ex situ (growing together in a randomized plot in Ames). Five mature trees of each subspecies were sampled in situ, along with five 3-year-old, half-sibling groups of each subspecies ex situ. Each half-sibling group

Received for publication 5 Sept. 2002. Accepted for publication 16 Jan. 2003. We thank Stanley Rice for assistance with tissue collection. We are grateful to Cynthia Haynes and Jeffery Iles for their helpful critiques of the manuscript.

¹Corresponding author; e-mail jschrade@iastate.edu.

consisted of six plants propagated from the seed of one parent tree in its native provenance. Therefore, the experimental design was a 3×2 factorial (Cochran and Cox, 1992) executed on seven different dates and consisting of three treatments for subspecies (subsp. *oklahomensis*, *georgiensis*, and *maritima*) crossed with two treatments of habitat (native/in situ and Iowa/ex situ).

Stem samples from all collection sites were transported on ice to Iowa State Univ. within 24 h. Each stem section was trimmed to 5 cm in length after removing the terminal 5 cm of the shoot apex, was wrapped in moist paper toweling, and was placed in a 16-mm-diameter culture tube that was held at 4 °C in a programmable freezer (ScienTemp, Adrian, Mich.) until the start of temperature ramping (≤ 12 h). After all samples were prepared, the temperature was lowered and held at -2 °C for 1 h. Each sample then was nucleated with ice crystals, controls were removed and placed in a refrigerator at 4 °C, and the temperature was lowered at 2 °C per hour. Temperatures were monitored in culture tubes by using a datalogger and four thermocouple probes (CR23X; Campbell Scientific, Logan, Utah) positioned at disparate locations in the freezer. Four replicates from each plant source were removed at each 4 °C interval. Upon removal, samples were allowed to thaw slowly by placing them on ice for 1 h and then holding them at 4 °C for 12 h. After thawing, culture tubes were covered with parafilm and incubated in a dark growth chamber at 22 °C for 14 d. After incubation, each stem sample was sectioned longitudinally and examined for tissue discoloration under a dissecting microscope (Fig. 1). Each stem section was rated as alive (cambium and phloem light green or clear) or dead ($\geq 60\%$ of cambium and phloem brown). The lowest survival temperature (LST) (Fuchigami et al., 1971) was determined for each source plant as the minimum treatment temperature at which $\geq 75\%$ (three out of four) of the replications survived.

We assessed response to seven temperatures (range of 24 °C) on each date. Ranges were -2 to -22 °C on 25 Sept., -6 to -30 °C on 30 Oct., -34 to -58 °C on 4 Dec., -56 to -80 °C on 8 Jan., -38 to

-62 °C on 12 Feb., -34 to -58 °C on 18 Mar., and -30 to -54 °C on 22 Apr. There were samples on each date that were not killed by the lowest temperature applied. For analysis purposes, these samples were assigned an LST that coincided with the most severe treatment for that assessment date, even though the LST may have actually been lower. Analyses focused on cold acclimation (increase in cold hardiness) and deacclimation (loss of cold hardiness) of plants of the three subspecies through the fall, winter, and spring and on defining their peak cold hardiness in midwinter. The effects of collection date, in situ vs. ex situ habitat, and subspecies were determined by analysis of variance and Fisher's least significant difference test. Analysis of variance showed no interaction between the main effects of date, habitat, or subspecies, establishing that the main effects of these parameters were independent (Cochran and Cox, 1992). Because the main effects were independent we were able to analyze and report these effects separately. Replication was $n = 10$ for subspecies, $n = 15$ for habitat, and $n = 30$ for collection date. Cold acclimation and hardiness results were also modeled with the current season's daily minimum temperatures and record daily minimum temperatures for locations in zones 5a, 4a, and 3a to predict the safe geographical range for the use of *A. maritima* in managed landscapes.

The second laboratory assessment examined the midwinter cold hardiness of *A. maritima* and its three subspecies in another way. Whole shoots were collected on 30 Jan. 2002 from 4-year-old trees of all subspecies at a research station of Iowa State Univ. near Ames. After wrapping the basal 5 to 10 cm of each shoot with moist paper towel surrounded by parafilm, the frozen, whole shoots were placed in the programmable freezer and held at -6 °C for 10 h. The temperature then was lowered 3 °C per hour. One shoot from each subspecies was removed at each of five treatments (-30, -40, -50, -60, and -70 °C) and allowed to thaw slowly by placing it outdoors for ≥ 12 h at -2 to -6 °C. For the sixth treatment at -80 °C, samples were allowed to remain in the freezer as the programmed temperature slowly returned from -80 to -6 °C over the 12-h period. We

Fig. 1. Diagnostic characteristics for the tissue-discoloration method of cold-hardiness analysis. After receiving chilling treatments followed by 14 d of incubation at 22 °C, stem samples of *Alnus maritima* were rated as dead when cambium and phloem tissues were dark brown and necrotic (left) or alive when cambium and phloem tissues appeared light green and healthy (right). This figure may be viewed in color at <http://www.hort.iastate.edu/graves/>.

Fig. 2. Field trials to evaluate the winter hardiness of *Alnus maritima* and four other species of *Alnus* were established at three sites in the upper-midwestern United States: at a research station of Iowa State Univ. near Ames, Iowa (USDA hardiness zone 5a, mean annual minimum -26 to -29 °C); at the Minnesota Landscape Arboretum in Chanhassen, Minn. (USDA hardiness zone 4a, mean annual minimum -32 to -34 °C); and on a site near Lake George, Minn. (USDA zone 3a, mean annual minimum -37 to -40 °C).

believe this provided a stringent test of cold-hardiness for these samples by maintaining them at extremely cold temperatures for a long period ($\leq -60^{\circ}\text{C}$ for ≈ 10 h) and by providing a realistic rate of temperature change during warming. After thawing, the basal end of each shoot was held in forcing solution of gibberellic acid ($25\text{ mg}\cdot\text{L}^{-1}$), 8-hydroxyquinoline ($200\text{ mg}\cdot\text{L}^{-1}$), and sucrose ($20\text{ g}\cdot\text{L}^{-1}$) in distilled-deionized H_2O . The shoots were held on a greenhouse bench at 20 to 22°C under a 16-h photoperiod by using two 400-W high-pressure sodium lamps to supplement natural irradiance. Shoots were monitored each day for evidence of budbreak and new growth, as an estimate of the coldest temperature at which the trees could survive in midwinter.

FIELD TRIALS. In Spring 2000, 1-year-old seedlings of *A. maritima*, *Alnus serrulata* (Ait.) Willd., *Alnus glutinosa* (L.) Gaertn., *Alnus nitida* (Spach) Endl., and *Alnus nepalensis* D. Don were installed at three sites that differed in winter climate severity. These field plots were established at a research station of Iowa State Univ. near Ames (USDA hardiness zone 5a, mean annual minimum of -26 to -29°C); at the Minnesota Landscape Arboretum in Chanhassen (USDA hardiness zone 4a, mean annual minimum of -32 to -34°C); and on a site near Lake George (USDA zone 3a, mean annual minimum of -37 to -40°C) (Fig. 2). At all three locations, ten half-sibling groups of seedlings of each subspecies of *A. maritima*, three half-sibling groups of seedlings of *A. serrulata* (one group each from Delaware, Georgia, and Arkansas), and one half-sibling group each of *A. glutinosa*, *A. nitida*, and *A. nepalensis* were placed in a completely randomized design, with five replications per half-sib group. Therefore, the experimental design was a 5×3 factorial (Cochran and Cox, 1992) at the species level with five treatments of species (*A. maritima*, *A. serrulata*, *A. glutinosa*, *A. nitida*, and *A. nepalensis*) crossed with three treatments of plot location (Ames, Chanhassen, and Lake George) and a 6×3 factorial at the infraspecific level with six treatments for subspecies/population (*A. maritima* subsp. *oklahomensis*, *georgiensis*, and *maritima* and *A. serrulata* populations in Delaware, Georgia, and Arkansas) crossed with three treatments of plot location (Ames, Chanhassen, Lake George). *Alnus serrulata* is a species with a continuous distribution that covers nearly the same range as the disjunct distribution of *A. maritima*. The population of *A. serrulata* from Georgia is sympatric with *A. maritima* subsp. *georgiensis*, the population of *A. serrulata* from Delaware is sympatric with subsp. *maritima*, and the population of *A. serrulata* in Arkansas is close to subsp. *oklahomensis*, but 150 km farther north.

Plots were irrigated twice each month from 1 May to 1 Oct. during the two growth seasons to ensure that drought did not affect growth and survival. Plants were measured at the end of the first season (early October 2000) and again the following spring (mid-June 2001) to assess winter survival and growth in the three different climates. Measurements were plant survival (percentage of trees from each taxon that exhibited any new growth from any position on the existing shoot by mid-June 2001), tissue survival (percentage of the existing shoot system that broke bud and grew), and canopy volume in June 2001. The canopy volume was calculated by multiplying the shoot height by the horizontal canopy area (area of an ellipse calculated from the north-south and east-west canopy diameter measurements) (Schrader and Graves, 2002). The effects of genotype (species, subspecies, or population) and plot location were determined by analysis of variance and Fisher's least significant difference test. Analysis of variance showed no interaction between the main effects of genotype and plot location, establishing that the main effects of these parameters were independent (Cochran and Cox, 1992). Because the main effects were independent, we were able to analyze and report these effects separately, taking advantage

of hidden replication. At the species level, replication was $n = 450$ for *A. maritima*; $n = 45$ for *A. serrulata*; and $n = 15$ for *A. glutinosa*, *A. nitida*, and *A. nepalensis* across all three plots. At the infraspecific level, replication was $n = 150$ for each subspecies of *A. maritima* and $n = 15$ for each population of *A. serrulata* across the three plots. Replication was $n = 50$ for the subspecies of *A. maritima* within each plot and $n = 150$ for the species *A. maritima* at each plot.

Phenology of dormancy and active growth

The phenology of budbreak, shoot growth, and leaf abscission was characterized for 2-year-old *A. maritima* growing at a research station of Iowa State Univ. near Ames during 2001. Data were collected from 125 plants each from subsp. *maritima* and *georgiensis* (25 half-sibling groups each) and 105 plants from subsp. *oklahomensis* (21 half-sibling groups) that were installed in June 2000 in a completely randomized plot. Plants were measured each day during the time of budbreak, once a month (24 May, 24 June, 25 July, 24 Aug., 24 Sept., and 5 Oct.) during the time of active growth, and once every 3 d during the time of leaf abscission. Percentage budbreak was the percentage of plants that had broken bud on or before each assessment date. A plant was considered to have broken bud when three or more of its buds had opened sufficiently that the adaxial side of a leaf could be seen. Shoot growth was a rate calculated as the increase in canopy volume divided by the number of days of growth and measured in cubic centimeters per day. Leaf abscission was measured as the percentage (estimated to the nearest 10%) of total seasonal leaves remaining on a plant on each assessment date.

Daily minimum temperatures for the Ames plot were recorded by using a CR23X datalogger (Campbell Scientific, Logan, Utah). Daily minimum temperatures for the sites in Minnesota were obtained from the Historical Data Retrieval and Climate Summaries web page (<http://climate.umn.edu/doc/historical.htm>). All data for 100-year record daily minima and 100-year mean daily minima were obtained from the Climatology Index Page (The Weather Channel, 1995–2002). Data for the 50-year record daily minima for Ames were obtained from the Climatological Data Selection Page (<http://mesonet.agron.iastate.edu/climodat/table.html>). To help determine in which part of the winter season seedlings may have been damaged and to establish where *A. maritima* can safely be used in the landscape, we plotted the phenology of cold hardiness of *A. maritima* (LSTs for the plants in Ames) recorded in the first lab assessment with the daily minimum temperatures for winter 2000–01 and the 50- or 100-year record minima for the three cold hardiness sites. Data were analyzed by using the general linear models (GLM) procedure and the least significant difference (LSD) option of SAS/STAT software, Version 6.12 (1989–96).

Results

Cold acclimation and cold hardiness

LABORATORY ASSESSMENTS. Results from the first laboratory assessment showed ample cold hardiness for all three subspecies on all seven dates throughout the winter of 2000–01 when compared with the 100-year record daily minima from regions where they are found naturally (Table 1). Although the winter climates where the three subspecies are indigenous vary, as shown by the mean and record minimum temperatures for these regions (Table 1), the LSTs were not different among the three subspecies on any of the seven dates assessed. On four of the assessment dates, cold hardiness was greater (lower LST) for plants in Ames than for plants in their native habitats. Cold acclimation (shown by the decreasing LSTs for 25 Sept., 30 Oct., 4 Dec., and 8 Jan.) and midwinter peak cold

Table 1. Lowest survival temperature (LST) in °C for *Alnus maritima* subsp. *oklahomensis*, *georgiensis*, and *maritima* assessed on seven dates during winter 2000–01. Samples were collected on the same dates both from trees growing in native habitats and from trees of the three subspecies growing in a common garden near Ames, Iowa. Mean LST for *A. maritima* in each habitat and the mean LST for *A. maritima* across habitats are included along with the number of samples out of 30 that exceeded the test range on each assessment date. Also included are the 100-year record daily minimum temperature (°C) and the 100-year mean daily minimum temperature (°C) for the seven dates, recorded for Ames, Iowa, and the areas where the three subspecies are native^z.

Category and source	Date 2000–01						
	25 Sept.	30 Oct.	4 Dec.	8 Jan.	12 Feb.	19 Mar.	23 Apr.
Subspecies LST ^y							
<i>oklahomensis</i>	–13 a	–26 a	–50 a	–61 a	–55 a	–48 a	–36 a
<i>georgiensis</i>	–16 a	–25 a	–52 a	–66 a	–57 a	–45 a	–40 a
<i>maritima</i>	–12 a	–25 a	–53 a	–62 a	–56 a	–50 a	–41 a
Record daily minimum temperature ^x							
Tishomingo, Okla.	7 (14)	–10 (7)	–7 (1)	–18 (–3)	–17 (–1)	–11 (6)	3 (12)
Cartersville, Ga.	4 (13)	–4 (6)	–9 (2)	–13 (–1)	–14 (0)	–6 (5)	–2 (9)
Seaford, Del.	1 (11)	–6 (4)	–12 (–1)	–12 (–4)	–19 (–4)	–11 (1)	–1 (6)
Ames, Iowa	2 (9)	–7 (1)	–20 (–7)	–27 (–13)	–28 (–11)	–16 (–2)	–3 (5)
Habitat means ^w							
Native habitat	–12.7 a	–23.9 a	–49.2 a	–64.1 a	–50.3 a	–40.9 a	–30.0 a
Ames, Iowa	–14.8 a	–27.1 b	–53.5 a	–61.9 a	–61.7 b	–54.5 b	–48.1 b
<i>A. maritima</i> ^v	–13.7 a	–25.5 b	–51.3 d	–63.0 f	–56.0 e	–47.7 d	–39.1 c
Number of samples exceeding test range ^u	2 @ –22	6 @ –30	16 @ –58	7 @ –80	18 @ –62	8 @ –58	5 @ –54

^zSubsp. *oklahomensis* is found near Tishomingo, Okla.; subsp. *georgiensis* is found near Cartersville, Ga.; and subsp. *maritima* is found near Seaford, Del.

^ySubspecies means across habitats (main effects of subspecies, $n = 10$). Subspecies means within each column followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's least significant difference test.

^xNumbers outside parentheses are the 100-year record daily minimum temperatures; numbers inside parentheses are the 100-year mean daily minimum temperatures.

^wMean LST for the species *A. maritima* in situ and ex situ (main effects of habitat, $n = 15$). Habitat means within each column followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's least significant difference test.

^vMean LST for the species *A. maritima* across subspecies and habitats (main effects of collection date, $n = 30$). Species means followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's least significant difference test.

^uNumber of samples out of 30 that exceeded the temperature range for the test date, followed by the minimum temperature (°C) tested on that date.

hardiness (LSTs for 8 Jan.) were similar for plants growing in the two habitats, but plants in native habitats deacclimated (lost cold hardiness) more quickly than did plants growing in Ames (Table 1). Even though plants deacclimated sooner in native sites, their LSTs were more than sufficient for plants to withstand the record minima in their natural environments. The mean LST for all 30 samples on 8 Jan. indicates that, as a species, *A. maritima* can withstand midwinter extremes as low as –63 °C. Because seven samples exceeded the lower limit of the test range on 8 Jan. (–80 °C), *A. maritima* may be cold hardy at even lower temperatures, and at least some of the genotypes were cold hardy at –80 °C or lower.

The strong midwinter cold hardiness of *A. maritima* was confirmed by our second laboratory assessment. Budbreak was observed on all samples from all three subspecies and all six treatments (–30, –40, –50, –60, –70, and –80 °C) by 20 Feb. 2002, within three weeks of cold-temperature ramping. New foliage was produced by all samples until the termination of the experiment on 13 Mar. 2002. New growth took place from both apical and axillary buds, with no visible signs of damage from cold stress on any of the shoots (Fig. 3).

FIELD TRIALS. Two-year-old trees of *A. maritima*, *A. serrulata*, and *A. glutinosa* showed 100% plant survival at all three of the cold-hardiness plots during their first winter (2000–01), while the other two species, *A. nitida* and *A. nepalensis*, showed 0% survival (Table 2). Of the three species that survived, *A. maritima* showed the greatest tissue survival per plant, 91.1% when averaged across all three cold-hardiness plots. At the infraspecific level, we compared plant and tissue survival of the three subspecies of *A. maritima* with genotypes from three populations of *A. serrulata*. All plants from both species survived the colder winters in Iowa and Minnesota, but

there was a difference in tissue survival among the six populations. *Alnus maritima* subsp. *maritima* showed the greatest percentage of tissue survival (96.1%), and the three subspecies of *A. maritima* each showed greater tissue survival than *A. serrulata* from their native areas (Table 2).

Although there were no differences in plant survival among the three subspecies or the three cold-hardiness plots, there were differences in tissue survival and canopy development at these levels (Table 3). At the plot near Ames (USDA zone 5a), winter damage was negligible for all three subspecies, but subsp. *oklahomensis* and *georgiensis* had greater growth (greater canopy volume) than subsp. *maritima* in this environment. At Chanhassen (USDA zone 4a), subsp. *maritima* showed greater tissue survival than subsp. *georgiensis*, but overall growth was not different for these two subspecies, and growth of subsp. *oklahomensis* was greater than that of subsp. *maritima*. At the plot near Lake George (USDA zone 3a), subsp. *maritima* again showed the greatest percentage of tissue survival (91.5%), but tissue damage and slow growth limited the size of plants from all three subspecies (Table 3). Means for subspecies across the three plots confirmed that subsp. *maritima* was the most cold hardy (96.1% tissue survival) of the three subspecies in climates of the Upper Midwest in 2000–01, but they also show that subsp. *maritima* grew more slowly than subsp. *oklahomensis*. Combining the results for the three subspecies, means for the three cold-hardiness plots show that tissue damage increased for *A. maritima* along with the severity of the winter environments at the three locations (Table 3). Yet, even in the severe environment of Lake George (USDA zone 3a), the 2-year-old seedlings showed 80.4% tissue survival overall.

Fig. 3. Growth of shoots from the three subspecies of *Alnus maritima* after they had received laboratory chilling treatments as low as -80°C . Shoots from all three subspecies are shown together because budbreak and growth were observed on all samples from all three subspecies under all treatment temperatures. Shoots were collected on 30 Jan. 2002 from 4-year-old trees near Ames, Iowa. This figure may be viewed in color at <http://www.hort.iastate.edu/graves/>.

The cold hardiness of *A. maritima*, projected from 2000–01 data, exceeded the daily minimum temperatures at all three sites for the entire winter of 2000–01 (Fig. 4) and the record minimum temperatures except for an extremely low temperature of -25.6°C for Lake George recorded for 27 Oct. (Fig. 4).

Table 2. Plant and tissue survival for 2-year-old trees from five species of *Alnus* after winter 2000–01. Winter survival is compared separately at specific and infraspecific levels by using combined data from cold-hardiness plots in Ames, Iowa (USDA zone 5a); Chanhassen, Minn. (USDA zone 4a); and Lake George, Minn. (USDA zone 3a).

Taxon	Plant survival ^z (%)	Tissue survival ^y (%)
Species level^x		
<i>Alnus maritima</i>	100 a ^w	91.1 a
<i>Alnus serrulata</i>	100 a	78.1 b
<i>Alnus glutinosa</i>	100 a	73.2 b
<i>Alnus nitida</i>	0 b	0 c
<i>Alnus nepalensis</i>	0 b	0 c
Infraspecific level^v		
<i>Alnus maritima</i>		
subsp. <i>oklahomensis</i>	100 a	87.2 bc
subsp. <i>georgiensis</i>	100 a	90.0 b
subsp. <i>maritima</i>	100 a	96.1 a
<i>Alnus serrulata</i>		
Arkansas	100 a	79.8 d
Georgia	100 a	73.4 d
Delaware	100 a	81.2 cd

^zPercentage of trees from each taxon that exhibited any new growth from any position on the existing shoot by mid-June, 2001.

^yPercentage of the existing shoot that broke bud and grew.

^x $n = 450$ for *A. maritima*; $n = 45$ for *A. serrulata*; $n = 15$ for *A. glutinosa*, *A. nitida*, and *A. nepalensis*.

^wMeans within columns and taxonomic levels followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's least significant difference test.

^v $n = 150$ for each subspecies of *A. maritima* and $n = 15$ for each population of *A. serrulata*.

Phenology of dormancy and active growth

The growth phenology of *A. maritima* varied among the three subspecies when plants were assessed in a common garden environment in Ames in 2001 (Fig. 5). Subspecies *maritima* was the earliest to break bud, followed by subsp. *oklahomensis* and then subsp. *georgiensis*. The first plants of subsp. *maritima* showed budbreak on 11 Apr., and the last plants from subsp. *georgiensis* broke bud on 23 Apr. For the remainder of April and the beginning of May, growth rates were consistent with the order of budbreak, but by the second week in May, subsp. *oklahomensis* had the highest rate of growth. By the second week in June, subsp. *georgiensis* had the highest growth rate, and it continued to grow faster than the other two subspecies until early September (Fig. 5). The timing of growth during the season was similar for subsp. *georgiensis* and *maritima*. Both of these taxa showed a peak in growth rate by late July, while subsp. *oklahomensis* maintained

its highest rate of growth from mid-July to mid-August. Subspecies *maritima* had the lowest growth rate throughout the season. It was the first of the three subspecies to enter dormancy and the first to undergo leaf abscission. Subspecies *oklahomensis* was the last to enter dormancy and the last to shed its leaves (Fig. 5). All three of the subspecies had entered dormancy by 5 Oct.

Table 3. Tissue survival and canopy development of 2-year-old trees of the three subspecies of *Alnus maritima* assessed after winter 2000–01. Means for subspecies are compared separately for trees from cold-hardiness plots in USDA zones 5a (Ames, Iowa), 4a (Chanhassen, Minn.), and 3a (Lake George, Minn.), followed by the means for subspecies across the three plots and a comparison of plot means for the survival and growth of the species *A. maritima*.

Plot location and subspecies ^z	Tissue survival ^y (%)	Canopy vol ^x (dm ³)
Ames		
<i>oklahomensis</i>	98.1 a ^w	40.7 a
<i>georgiensis</i>	98.7 a	38.8 a
<i>maritima</i>	99.4 a	29.2 b
Chanhassen		
<i>oklahomensis</i>	94.1 ab	43.3 a
<i>georgiensis</i>	91.0 b	29.5 ab
<i>maritima</i>	97.3 a	27.6 b
Lake George		
<i>oklahomensis</i>	69.4 c	3.8 b
<i>georgiensis</i>	80.2 b	5.5 a
<i>maritima</i>	91.5 a	5.3 a
Subspecies means across plots		
<i>oklahomensis</i>	87.2 c	29.3 a
<i>georgiensis</i>	90.0 b	24.6 ab
<i>maritima</i>	96.1 a	20.7 b
Plot means for <i>A. maritima</i>^v		
Ames	98.7 a	36.2 a
Chanhassen	94.1 b	33.4 a
Lake George	80.4 c	4.9 b

^z $n = 50$ for subspecies means within each plot; $n = 150$ for subspecies means across plots.

^yPercentage of the existing shoot that broke bud and grew.

^xCanopy size calculated from three dimensional shoot measurements taken in mid-June 2001.

^wMeans within each group of three followed by the same letter are not significantly different at $P \leq 0.05$ according to Fisher's least significant difference test.

^vPlot means for the species *A. maritima* (main effect of plots, $n = 150$).

Fig. 4. Daily minimum temperatures for 2000–01 and 50- or 100-year record daily minimum temperatures in Ames, Iowa; Chanhassen, Minn.; and Lake George, Minn., for September through April are plotted along with the LST (lowest survival temperature) of 3-year-old *Alnus maritima* in Ames, Iowa, assessed on seven dates during 2000–01 (25 Sept., 30 Oct., 4 Dec., 8 Jan., 12 Feb., 19 Mar., and 23 Apr.). Data points are mean values $\pm$ SE.

Discussion

It has been proposed that the three disjunct provenances of *A. maritima* were once part of a continuous distribution that covered much of North America, including colder regions in the Pacific Northwest, and that the ancestors of *A. maritima* migrated from Asia into North America across the Bering land bridge (Furrow, 1979; Schrader and Graves, 2000; Schrader and Graves, 2002). Our results fit this model well. *Alnus maritima* of all three subspecies is extremely cold hardy when compared to the mild winter conditions found in its natural habitats. It is clear that this level of cold hardiness is not required for survival of plants in Oklahoma, Georgia, and on the Delmarva Peninsula, but plants from the three subspecies express this strong cold hardiness (mean LST = -64.1°C on 8 Jan.) even when growing in their natural environments (Table 1). Based on the similar patterns of cold acclimation and the extreme cold hardiness of the three subspecies of *A. maritima* when

compared to the hardiness required for plants to exist in their native habitats (Table 1), we propose that the genotypic capacity for this strong cold hardiness was evolved in a much colder climate and was acquired before the divergence of the three subspecies. While some of the genotypes from *A. maritima* can withstand -80°C in mid-winter (Table 1, Fig. 3), we know that the other two members of subg. *Clethropsis*, *A. nitida* and *A. nepalensis* from southern Asia, cannot survive even -31°C (the coldest temperature recorded in Ames for Winter 2000–01), because all plants of *A. nitida* and *A. nepalensis* were killed at the Ames plot during Winter 2000–01 (Table 2, Fig. 4). The model of ancestral migration described above explains these data well. *Alnus maritima* appears to have acquired its extreme cold hardiness after its divergence from the other two members of *Clethropsis* and during its ancestral migrations through the area of the Bering Strait, but cold hardiness is lacking in *A. nitida* and *A. nepalensis* because their ancestral lines have never ventured beyond eastern and southern Asia (Furrow, 1979; Schrader, 2002; Schrader and Graves, 2002).

Alnus serrulata is the only species of *Alnus* with a distribution that overlaps that of *A. maritima*. *Alnus serrulata* is sympatric with *A. maritima* in Georgia and on the Delmarva Peninsula, and its continuous distribution begins as far north as Quebec, Canada (USDA zone 4a), and reaches into eastern Oklahoma, within 150 km of *A. maritima* (Furrow, 1979; Stibolt, 1981). Results from our cold hardiness plots reveal that genotypes of *A. maritima* and *A. serrulata* from the same localities have similar cold hardiness, with *A. maritima* being slightly more cold hardy. The similar pattern of cold hardiness in the two species supports the notion that *A. maritima* also once occurred over a large continuous distribution, which may have included areas with colder winter temperatures.

The phenology of dormancy and active growth reveals another area where the three subspecies of *A. maritima* have diverged somewhat. It has been demonstrated in several species that autumnal phenological events such as cessation of growth, leaf senescence, and leaf abscission are genetically controlled in response to shortening photoperiod, while spring phenological events (cold deacclimation, budbreak, and commencement of growth) appear to be less genetically controlled because they proceed in response to warming spring temperatures (Flint, 1972; Smithberg and Weiser, 1968). Our findings with the subspecies of *A. maritima* appear to follow this pattern. While there were only slight differences in the timing of budbreak among the three subspecies growing in our common garden (all 355 plants broke bud within a span of 12 d), midseason growth rate, timing of the cessation of growth, and leaf abscission in the autumn showed greater variation (Fig. 5). Although genetic differences were evident for growth rate and autumnal phenology among the three subspecies (Fig. 5), no differences were resolved among subspecies for the timing of cold acclimation (Table 1), a phenomenon possibly explained by the observation of Flint (1972) that cold hardiness in established trees of *Quercus rubra* L. did not appear to be a factor important in natural selection.

The results of our cold-hardiness assessments demonstrate

Fig. 5. Phenology of budbreak, shoot growth, and leaf abscission of two-year-old *Alnus maritima* growing near Ames, Iowa, in 2001. Data were collected from 125 plants each from subsp. *maritima* and *georgiensis*, and 105 plants from subsp. *oklahomensis*. Percentage budbreak was the percentage of plants that had broken bud on or before each assessment date, shoot growth was a simple growth rate (increase in shoot volume divided by the number of days of growth), and leaf abscission is shown as the mean percentage of leaves remaining on a plant. Data points are mean values $\pm$ SE except for budbreak, which values are the percentage of the total number of plants and no SE is possible.

conclusively that *A. maritima* can be safely planted in landscapes of areas with winters colder than those of its native habitats. Our assessment of cold acclimation shows that mean cold hardiness of *A. maritima* exceeds even the 50- and 100-year record daily minimum temperatures for sites in USDA zones 5a, 4a, and 3a, from 25 Sept. to 23 Apr. (Fig. 4). While there was some tissue damage on 2-year-old seedlings at cold-hardiness plots in zones 4a and 3a (Table 3), laboratory assessments show that stems from mature plants can survive temperatures as low as -80°C in midwinter (Table 1, Fig. 3). Along with these results, we report our successful use of *A. maritima* subsp. *oklahomensis* in the landscapes of central Iowa (zones 4b and 5a) since 1998 and use of subsp. *georgiensis* and *maritima*, since 1999. These plants (≈ 700) have thrived in Iowa through four and three winters, respectively.

Trends for 2000–01 suggest two potential periods during the season when the tissue damage may have occurred on seedlings in zones 4a and 3a. The cold hardiness of *A. maritima* follows the overall curve of the daily minimum temperatures well, except during the early part of the season, when cold hardiness is closest to the recorded minima for all three sites (Fig. 4). The tissue damage may have taken place in Sept. or early Oct. as cold acclimation lagged behind the lowering minimum temperatures in 2000–01. The other possibility is that tissue damage took place after the unseasonably warm temperatures shown for mid-January, when daily minima approached 0°C (16 to 20°C warmer than average). This warming trend may have caused the seedlings to deacclimate slightly before daily temperatures returned to normal.

Although some plants from all three subspecies have been shown to survive -80°C in our laboratory assessments with no statistical difference between the taxa (Table 1, Fig. 3), and our field trials showed 100% plant survival for all three subspecies, our results for percentage tissue survival suggest that subsp. *maritima* may be better suited for extremely cold regions like USDA hardiness zone 3a. Two-year-old seedlings of subsp. *maritima* from 10 half-

sibling groups showed mean tissue survival of 91.5% after winter in Lake George (zone 3a) (Table 3), and two of these half-sibling groups showed 100% tissue survival at this location. It is likely that selections taken from subsp. *maritima* will include genotypes hardy to zone 3a. Even though genotypes from subsp. *maritima* appear to be slightly more cold hardy than those of the other two subspecies, rate of plant growth should also be one of the essential criteria for matching the proper genotype to local climates. Both subsp. *oklahomensis* and *georgiensis* grew faster than subsp. *maritima* in Ames (zone 5a) and Chanhassen (zone 4a) (Table 3). This greater capacity for growth may offset any added risk of tissue damage and may justify choosing one of these two subspecies over subsp. *maritima* for landscape applications in zones 5a and 4.

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