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Propagation of *Alnus maritima* from Softwood Cuttings

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Abstract. ***Alnus maritima*** **(Marsh.) Nutt. (seaside alder) is a rare, woody-plant species with potential for use in managed landscapes. Information on the propagation and production of this species is not available. Our objective was to evaluate the use of softwood cuttings to propagate *A. maritima*, with emphasis on how indole-3-butyric acid (IBA), plant provenance, and time of collection affect cutting survival, rooting percentage, the number of roots produced, and their length. Propagation trials were conducted with cuttings from seven trees on the Delmarva Peninsula (Eastern Shore of Maryland and southern Delaware) and seven trees in Oklahoma. Cuttings from mature plants in both provenances were collected on 14 June and 23 Aug. 1998; wounded; treated with IBA at 0, 1, or 8 g·kg^{-1}; and placed under intermittent mist in a greenhouse for 9 weeks. Use of IBA at 8 g·kg^{-1} caused a greater rooting percentage (68%), root count (7.2), and root length (39.2 mm) than did the other IBA rates when applied to cuttings from Oklahoma in June, but IBA had little effect on cuttings from the Delmarva Peninsula. Across IBA treatments, rooting of cuttings from Oklahoma (55% in June and 12% in August) was greater than the rooting of cuttings from Delmarva (27% in June and 3% in August). Cuttings from Oklahoma had greater survival, callus development, root length, and root count than did cuttings from the Delmarva Peninsula during June and August trials. Averaged over IBA treatments and provenances, cuttings collected on 14 June rooted more frequently (41%) than did cuttings collected 23 Aug. (8%). We conclude that softwood cuttings from mature plants are an effective way to multiply clones of *A. maritima*, particularly when cuttings are collected early in the season and treated with IBA at 8 g·kg^{-1}.**

Demand is increasing for woody plants that are ornamental and resistant to environmental stresses. *Alnus maritima* (seaside alder) is a rare species from North America found naturally in three provenances (south-central Oklahoma, northwestern Georgia, and the Delmarva Peninsula) that are separated by ≥1000 km (B. Dickman, personal communication; Stibolt, 1981). Its appealing ornamental characteristics and physiological attributes have generated interest in *A. maritima* as a nursery crop. Plants attain a height of 10 m with several trunks ≤15 cm in diameter and produce glossy, dark green foliage and pendulous, male catkins that are yellow in late summer and early autumn (Furlow, 1979; Mazzeo, 1986). The species occurs almost exclusively in soils saturated with fresh water (Schrader, 1999; Stibolt, 1981), yet is less affected by drought than are plants of *Alnus glutinosa* (L.) Gaertn. (Hennessey et al., 1985). The capacity of *A. maritima* to associate with nitrogen-fixing bacteria (Furlow, 1979; Stibolt, 1978) may give it an advantage over other woody taxa on sites with soils low in nitrogen.

Alnus maritima is propagated easily from seed (Schrader and Graves, 2000), but vegetative propagation will be required to produce clones of genotypes selected for superior horticultural traits. Information that describes vegetative propagation of this species is not available, but work has been done with other species of alder. Softwood cuttings have been used to propagate clones of *A. glutinosa*, *Alnus rubra* Bong., and *Alnus incana* (L.) Moench. Root development on cuttings varies among these alders and is influenced by exogenous growth regulators and age of the ortets (Huss-Danell et al., 1980; Monaco et al., 1980; Radwan et al., 1989; Saul and Zsuffa, 1982).

Our objective was to evaluate the use of softwood cuttings from mature plants to propagate *A. maritima*. Pilot studies in 1997 with cuttings collected in June from plants on the Delmarva Peninsula resulted in 44% rooting when cuttings were treated with IBA at 1 g·kg^{-1}. Cuttings collected from plants in Oklahoma in August rooted at 17% with IBA at 8 g·kg^{-1}. Based on these observations, we designed trials with cuttings from both provenances to examine how IBA concentration and provenance affect survival and rooting of softwood cuttings collected on two dates in 1998. Variation in rooting among cuttings taken from different plants within each provenance also was examined.

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Materials and Methods

On two dates in 1998 (14 June and 23 Aug.), we collected terminal, softwood stem cuttings from mature plants (plants that had bloomed and were ≥10 years old) in Oklahoma and on the Delmarva Peninsula. The same plants were sampled on both dates. In Oklahoma, cuttings were collected from three plants along the Blue River (latitude 34°19’40”N; longitude 96°35’30”W) and four plants along Pennington Creek (latitude 34°19’20”N; longitude 96°42’20”W) in Johnston County. On the Delmarva Peninsula, cuttings were taken from one plant along Broad Creek (latitude 38°34’03”N; longitude 75°37’06”W), one plant along Plum Creek (latitude 38°31’51”N; longitude 75°43’57”W), four plants along the Nanticoke River (latitude 38°32’55”N; longitude 75°43’25”W), and one plant along Marshyhope Creek (latitude 38°34’45”N; longitude 75°47’34”W). *Alnus maritima* sustains shoot elongation late into the growing season, which allowed us to collect softwood cuttings on both dates.

Collections began at sunrise and were completed within 3 h. Cuttings from the 14 plants were placed in polyethylene bags that were held in insulated containers with ice and were transported to Ames, Iowa. After transport (≤12 h after collection), the bags were placed in a dark cooler (≈4 °C) until 0800 HR the next morning. The bags of cuttings were removed from the cooler in random order, and stems were re-cut to between 12 and 20 cm long with a razor blade. Leaves were removed from the basal half of each stem, and cuttings were wounded by scraping off the epidermis and phloem of the basal 2 cm on opposite sides of the stem. The basal 3 cm of each stem received one of two concentrations of talc-based IBA, 1 g·kg^{-1} [Hormodin® #1 (MSD-Agvet, Merck & Co., Rahway, N.J.)] or 8 g·kg^{-1} [Hormodin® #3 (MSD-Agvet)], by first misting the wounded stems with tap water and then dipping them in the treatment compound. Control cuttings were misted but not treated with talc. Cuttings were inserted vertically to a depth of 6 cm in wooden flats (inside = 12.8 cm wide × 41 cm long × 7 cm deep; volume = 3.67 dm^3) filled with moist horticultural perlite (Krum, Silbrico Corp., Hodgkins, Ill.). All cuttings were stuck within 40 h of collection.

An experimental unit consisted of five cuttings from one of the 14 plants, spaced uniformly in a row ≈3 cm from the long side of a wooden flat. Each flat contained two experimental units. Units from the seven plants of each provenance were arranged in a completely randomized, split-plot design, with IBA treatments randomly assigned to whole plots (84 wooden flats) and provenances randomly assigned to sub-plots (the two rows of five samples in each flat). This design allowed for 168 experimental units from the two provenances to be assigned to three treatments, resulting in 84 replications per provenance, 56 replications per IBA treatment, and 12 replications per ortet.

The flats of cuttings were held on a greenhouse bench under intermittent mist controlled by a screen balance (Mist-A-Matic®, E.C. Geiger, Harleysville, Pa.), which provided ≈10 s of mist every ≈10 min depending on environmental conditions in the greenhouse. One layer of black polyethylene was placed on the south wall of the greenhouse to block direct solar radiation, and supplementary irradiance was provided by three 100-W incandescent lamps between 0600 and 2200 HR. Environmental conditions were monitored twice each week between 1200 and 1300 HR with a model 1600 steady-state porometer (LI-COR, Lincoln, Nebr.). During the trial that began in June, air temperature averaged 27.2 °C, mean relative humidity was 68.1%, and mean photosynthetically active radiation was 122 $\mu mol \cdot m^{-2} \cdot s^{-1}$. During the trial that began in August, the corresponding means were 25.5 °C, 59.2%, and 103 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

After 9 weeks, cuttings were harvested, and experimental units were evaluated for the percentages of cuttings that were alive and had at least one viable leaf, had formed callus, and had formed at least one adventitious root >5 mm long. The number of primary roots >5 mm long (root count) and the length of the longest root per cutting were measured. Each cutting was given a score of 0 if dead, or 1 to 5 if alive (1= no callus; 2 = leaves and callus; 3 = one to four primary roots; 4 = five to nine primary roots; and 5 = ≥10 primary roots). Root count, length of longest root, and cutting score for the five cuttings in an experimental unit were averaged to obtain a value for each unit.

Data were analyzed by using the general linear model (GLM) procedure and the least significant difference (LSD) option of Statistical Analysis System software (SAS Institute, 1988). Sources of error, along with their degrees of freedom for the statistical model were: treatment, 2; flat (treatment), 81; provenance, 1; ortets, 12; and provenance × treatment, 2. Flat (treatment) was the error term used to determine significance for treatment effects, and error from ortets was used to analyze provenance effects. Data from cuttings collected in June and August were analyzed separately because environmental conditions were not identical during the two trials, and the trials were randomized separately.

Results

There was no interaction between provenance and IBA concentration for cutting survival and percentage of cuttings with callus during the trial that began in June. With cuttings from both provenances, IBA at 1 $g \cdot kg^{-1}$ had no effect, whereas IBA at 8 $g \cdot kg^{-1}$ reduced survival and callus development (Table 1). Provenance × IBA treatment interactions were found for rooting percentage, root count, length of longest root, and cutting score (Table 1). Means for these variables increased with increasing IBA concentration for cuttings from Oklahoma, and increased only slightly or not at all for cuttings from Delmarva (Table 1). Responses for cuttings from Oklahoma were consistently higher than those from the Delmarva Peninsula (Table 1). The greatest rooting percentage (68%), root count (7.2), root length (39.2 mm), and cutting score (3) among provenance and IBA treatment combinations resulted with cuttings collected in June from Oklahoma that received IBA at 8 $g \cdot kg^{-1}$.

No significant interactions were found for cuttings collected in August, and no IBA effects existed when means were combined over provenances (data not presented), except that the root count was greater for cuttings that received IBA at 8 $g \cdot kg^{-1}$ (0.4) than for cuttings not treated with IBA (0.1). Averaged over IBA treatments, all measures of rooting were greater for cuttings from the Oklahoma provenance than for those from the Delmarva Peninsula (Table 2).

Responses varied among cuttings from the seven ortets within both provenances (data not shown). Means over IBA treatments by ortet for rooting percentage ranged from 25% to 95% (Oklahoma) and from 7% to 88% (Delmarva) in the first trial and from 0 to 33% (Oklahoma) and from 0 to 12% (Delmarva) during the trial that began in August. Cuttings collected in June from one plant in Oklahoma had the greatest rooting percentage (95%) and root count (11), the longest roots (52 mm), and the greatest cutting score (4). In contrast, no cuttings from one plant on the Delmarva Peninsula were alive when the trial that began in August ended.

More of the cuttings collected in June survived (72%), developed callus (67%), and rooted (41%) than did cuttings collected in August (31% survived, 29% developed callus, and 8% rooted) when means were combined over provenances and IBA treatments. Cuttings collected in June developed longer roots (19.5 mm) and had a greater root count (2.4) and cutting score (2.2) than did cuttings collected in August (1.9 mm long, root count = 0.2, and cutting score = 0.6).

Table 1. Effects of IBA concentration on softwood cuttings of *Alnus maritima* collected on 14 June from plants in Oklahoma and the Delmarva Peninsula.[z]

	IBA applied ($g \cdot kg^{-1}$)			
Provenance	0	1	8	Mean
		Cutting survival (%)		
Oklahoma	93 a[y]	91 a	79 b	88
Delmarva	58 ab	64 a	48 b	57
IBA × provenance				NS
		Cuttings with callus (%)		
Oklahoma	93 a	87 a	71 b	84
Delmarva	56 a	56 a	39 b	50
IBA × provenance				NS
		Rooting percentage (%)		
Oklahoma	39 c	57 b	68 a	55
Delmarva	20 b	32 a	29 ab	27
IBA × provenance				*
		Root count		
Oklahoma	1.4 c	2.9 b	7.2 a	3.8
Delmarva	0.6 b	1.1 b	1.8 a	1.2
IBA × provenance				***
		Length of longest root (mm)		
Oklahoma	16.3 c	30.9 b	39.2 a	28.8
Delmarva	6.4 b	13.1 a	11.6 ab	10.4
IBA × provenance				**
		Cutting score[x]		
Oklahoma	2.4 b	2.7 a	3.0 a	2.7
Delmarva	1.4 a	1.6 a	1.4 a	1.5
IBA × provenance				*

[z]Values are means of 28 experimental units from each provenance.
[y]Mean separation within rows by Fisher's least significant difference test, $P \leq 0.05$.
[x]Each cutting was given a score of 0 if dead, 1 to 5 if alive (1 = no callus; 2 = leaves and callus; 3 = one to four primary roots; 4 = five to nine primary roots; and 5 = ≥10 primary roots). Scores for the five cuttings in an experimental unit were averaged to obtain a composite value for each unit.
[NS, *, **, ***]Nonsignificant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively.

Discussion

Propagation from softwood cuttings of mature ortets is a viable method for obtaining clones of *A. maritima*. Cuttings from plants in

Table 2. Effects of plant provenance on survival, callus development, rooting percentage, root count, root length, and cutting score of softwood cuttings of *Alnus maritima* during the late-season propagation trial that began on 23 Aug.[z]

Dependent variable	Oklahoma	Delmarva
Cutting survival (%)	46 a[y]	16 b
Cuttings with callus (%)	45 a	12 b
Rooting percentage (%)	12 a	3 b
Root count	0.4 a	0.1 b
Length of longest root (mm)	3.1 a	0.6 b
Cutting score[x]	1.1 a	0.3 b

[z]Values are provenance means for 84 experimental units.
[y]Mean separation within rows by Fisher's least significant difference test, $P \leq 0.05$.
[x]Each cutting was given a score of 0 if dead, 1 to 5 if alive (1= no callus; 2 = leaves and callus; 3 = one to four primary roots; 4 = five to nine primary roots; and 5 = ≥10 primary roots). Scores for the five cuttings in an experimental unit were averaged to obtain a composite value for each unit.

Oklahoma were more suitable for this method of propagation and responded more favorably to exogenous IBA than did cuttings from the Delmarva Peninsula. Treatment with IBA improved the rooting of softwood cuttings, but effects of IBA concentration varied depending on provenance. Identification of amenable ortets is crucial for successful propagation from cuttings taken from mature stock plants, and collection of cuttings in June leads to greater success than collection in August.

Softwood cuttings have been used to produce clones of other alder species. Cuttings from 2-year-old seedlings of *A. glutinosa* rooted 60% to 100% after treatment with IBA at 3 $g \cdot kg^{-1}$ (Saul and Zsuffa, 1982). Stem sections from 2-year-old seedlings of *A. rubra* rooted at 78% under intermittent mist after application of IBA at 4 $g \cdot kg^{-1}$ (Monaco et al., 1980). Rooting of *A. rubra* was 80% when cuttings were taken from epicormic sprouts on mature trees (Radwan et al., 1989). Rooting percentages from 93% to 100% can be achieved for cuttings from 5- to 8-year-old *A. incana* held in a nutrient solution with and without application of IBA (Huss-Danell et al., 1980). Compared with these species, *A. maritima* seems more difficult to root. Yet, variation in rooting percentage among provenances, IBA treatments, ortets, and collection dates shows the potential for improving rooting success. The greater rooting percentage and root count for cuttings from Oklahoma (Table 1, Table 2) suggest that plants of this provenance are more suitable as sources of softwood cuttings than are plants of the Delmarva Peninsula.

Treatment of cuttings from *A. maritima* with IBA increased rooting percentage and the number of roots produced (Table 1). Of the IBA concentrations used in our study, 8 $g \cdot kg^{-1}$ was most effective for the rooting of cuttings from Oklahoma, whereas 1 and 8 $g \cdot kg^{-1}$ were similarly effective for cuttings from the Delmarva Peninsula (Table 1). The most notable difference was the greater increase in root count evoked by 8 $g \cdot kg^{-1}$ (increased 5.1 times) compared with that evoked by 1 $g \cdot kg^{-1}$ (increased 2.1 times) for cuttings from Oklahoma. The higher concentration produced a greater number of well-rooted cuttings.

The variation in survival and rooting among ortets from each provenance showed that successful production of new plants from mature stock will depend on the identification of amenable ortets. Use of juvenile stock plants can reduce variation and increase rooting success (Couvillon, 1988; Hartmann et al., 1990), and this may be true for *A. maritima*. In a preliminary study, we found that all cuttings from one greenhouse-grown seedling from Oklahoma rooted following treatment with IBA at both 1 and 8 $g \cdot kg^{-1}$.

The time of collection is important for successful propagation of *A. maritima* cuttings from mature ortets. Early-season collection (≈15 June) should be favorable for propagation when using mature plants in their native habitats. Observations from our early-season trial indicate that newly elongated stems may not be of sufficient length and rigidity if collected earlier than this.

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