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Propagation of *Rhamnus alnifolia* and *Rhamnus lanceolata* by Seeds and Cuttings[1]

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Abstract

Rhamnus alnifolia L'Her (Alderleaf Buckthorn) and *Rhamnus lanceolata* Pursh ssp. *lanceolata* (Lanceleaf Buckthorn) are native shrubs that are uncommon in the wild. Seed germination of both species and vegetative propagation of *R. alnifolia* was studied. Germination was low (≤ 13%) among moist-prechilled seeds of *R. alnifolia* collected in mid-season, but 48% germination was observed among seeds collected late in summer and moist-prechilled for 30 days. Nontreated seeds did not germinate, nor did seeds first scarified with sulfuric acid or hot water followed by moist-prechilling. Germination was ≤ 5% among seeds of *R. lanceolata* and occurred only after seeds were moist-prechilled for at least 90 days. Seedling survival among both species ranged from 90 to 100% and was not influenced by pregermination treatment. Rooting among softwood cuttings of *R. alnifolia* was 85% within 35 days after application of 3 g/kg (3000 ppm) or 8 g/kg (8000 ppm) indole-3-butyric acid (IBA) in talc. When IBA was applied in acetone solution at 3 and 8 g/liter (3000 and 8000 ppm), rooting was ≤ 15%. While 75% of the nontreated cuttings rooted, these cuttings had fewer roots than those treated with IBA. Rooting was more extensive in vermiculite compared to a medium of equal volumes of vermiculite and perlite. Talc-based IBA and vermiculite should be used to induce root formation on softwood stem cuttings of *R. alnifolia*. Both *R. lanceolata* and *R. alnifolia* can be propagated from moist-prechilled seeds, but *Rhamnus lanceolata* is recalcitrant and merits further assessment of drupe phenology, timing of seed collection, and barriers to germination.

Index words: seed germination, dormancy, drupe phenology, softwood cuttings, vegetative propagation, IBA, *Aphis glycine*, buckthorn.

Significance to the Nursery Industry

Rhamnus alnifolia (Alderleaf Buckthorn) and *Rhamnus lanceolata* (Lanceleaf Buckthorn) are small or medium shrubs that bear attractive, lustrous green foliage in summer and dark blue drupes (fruits) in the autumn. Plants of *R. alnifolia* grow in shaded, wooded areas and might be valued as shade-tolerant species. Both species may also present an ecological and sustainable alternative to use of European species of *Rhamnus*, which have escaped cultivation to become noxious weeds in some parts of the United States. Our research shows that moist-prechilling for 30 days is required to induce germination among seeds of *R. alnifolia* that are collected in late summer, and moist-prechilling for 90 days is required to break dormancy among seeds of *R. lanceolata* collected in mid-summer. Drupes should be collected after they have ripened fully while attached to the mother plant. Terminal and subterminal softwood cuttings of *R. alnifolia* rooted within 35 days, and 3 or 8 g/kg (3000 or 8000 ppm) IBA in talc induced more roots per cutting.

Introduction

Several Eurasian species of *Rhamnus* L. (Buckthorn) are considered invasive in some parts of the United States, whereas the native *Rhamnus* often are rare in the wild (6, 12, 16). Many of the native species have landscape attributes and are adapted to environmental conditions that make them suitable for horticultural use. *Rhamnus alnifolia* is a simple or slightly forking low shrub [up to 1 m (3 ft)] that is polygamodioecious and is the only member of the genus that has five sepals (6). This understory taxon typically is distributed sparsely in calcareous streambeds, swamps, low woods and meadows lying to the east of Cascades, from British Columbia east to Quebec and Maine, and south to California, Iowa, Illinois, and West Virginia (6, 13). Plants are low-growing with a prostrate habit, and tolerate shaded, moist growing conditions. *Rhamnus lanceolata* is a medium [up to 3 m (10 ft)], widely branched shrub occurring in a variety of habitats including open wooded slopes, thickets and borders of woods, or on rocky limestone or dolomite glades (7). Its natural distribution extends from Alabama to Texas, north to central Pennsylvania, West Virginia, Ohio, Indiana, southern Wisconsin, southern Iowa, Nebraska, and southeastern South Dakota (6, 12). This shrub would be suitable for open, relatively dry conditions. Despite their merits, neither species is used extensively in landscapes. Whether these native *Rhamnus* should be promoted for landscape use depends on many factors, including how easily they can be propagated.

Propagation of native *Rhamnus* species also is of interest because the nonnative members of the genus serve as alternate hosts for an introduced and devastating pest of *Glycine max* (L.) Merr. (soybean) in North America. *Aphis glycine* Matsumura (soybean aphid) overwinters and oviposits on Eurasian buckthorns, including *Rhamnus cathartica* and *Rhamnus davurica*. Although Voegtlin et al. (17) have observed the aphid's affinity for *R. alnifolia* and *R. lanceolata* under experimental conditions, this relationship remains to be tested in the wild. Also, soybean aphid does not occur throughout the United States, and therefore, the native *Rhamnus* still may be used in landscapes where the aphid is not a threat.

Propagation of plants by using seeds is advantageous when genetic variability in propagules is desired, but many biochemical, physical, or morphological obstacles to germination often are present in seeds of temperate origin. Approximately two-thirds of North American woody species have some form of seed dormancy (15), and inhibitors to germination can develop at various times during seed development

[1]Received for publication on November 1, 2004; in revised form March 10, 2005. Florida Agricultural Experiment Station Journal Series No. R-10517. We extend our gratitude to Darrell Kromm for collecting seeds and to The Nature Conservancy for allowing collection of stems of *Rhamnus alnifolia*.

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(3). Although experimental methods and results are not available for germinating seeds of *R. alnifolia* and *R. lanceolata*, some non-statistical reports indicate that fresh seeds of *R. alnifolia* (11), *Rhamnus caroliniana* Walter (Carolina Buckthorn) (14), *Rhamnus crocea* var. *ilicifolia* (Kellogg) Greene (Hollyleaf Redberry) (11), and *Rhamnus californica* Eschsch. (Coffeeberry) (19) germinate without pre-treatment. This might occur because physiological dormancy develops during after-ripening, and consequently, stored seeds require moist-prechilling before germination commences (2, 3). While seeds of *R. alnifolia* can remain viable for 5 to 7 years if placed in sealed containers and stored between 3C (37.4F) and 5C (41F) (13), moist-prechilling for 90 days typically is required for seeds to germinate (2; Darrell Kromm, personal communication). Dirr and Heuser (5) have reported 'excellent' germination after moist-prechilling seeds of *R. alnifolia* for 3 months, and seventy five percent germination was reported for *R. alnifolia* seeds that were stratified outdoors for five months (13). While these reports provide some useful descriptive information, they do not include replicable experimental methods, quantitative data, or statistical analysis of results.

In other studies, scarification treatments have improved seed germination in some species of *Rhamnus*, including *Rhamnus davurica* and *Rhamnus frangula* (20); however, a similar treatment inhibited germination among seeds of *Rhamnus cathartica* (5). When Gourley (8) compared the effects of duration of scarification with sulfuric acid (H_2SO_4) on seeds of *R. cathartica*, mean time to germination was lower among scarified seeds; germination percentage, however, was not affected by these treatments. In nature, seeds may be exposed to similar conditions when passing through an animal's digestive system. For example, compared to non-passage, passage through the digestive system of gray fox resulted in faster germination among seeds of *R. californica* and *Rhamnus ilicifolia* (synonym: *Rhamnus crocea* var. *ilicifolia*) (18). Effects of scarification are not known for seeds of *R. alniflolia* and *R. lanceolata*.

Softwood stem cuttings often are used to propagate many woody species vegetatively, although other types of cuttings such as hardwood or semi-hardwood stem cuttings might be more suitable for some taxa (10). Rooting also can be influenced by the position of a cutting on a branch (proximity of the wood to a primary apex) (9, 10). Both terminal and subterminal cuttings of *R. caroliniana* produced adventitious roots in response to 3 and 8 g/kg (3000 ppm and 8000 ppm) IBA, but highest rooting was obtained when 3 g/kg (3000 ppm) IBA was applied to terminal cuttings collected from juvenile, sibling plants (9). Experimental methods for vegetative propagation of *R. alnifolia* have not been reported, however. In light of this, the objectives of this study were to evaluate protocols for sexual and asexual propagation of two uncommon North American species of *Rhamnus*. We evaluated germination among seeds of *Rhamnus alnifolia* and *Rhamnus lanceolata* following scarification, moist-prechilling, or a combination of the two, and tested the effect of cutting position, rooting substrate, and IBA application on adventitious root formation among softwood stem cuttings of *R. alnifolia*.

Materials and Methods

Two- to three-seeded drupes (fruits) were collected from a natural population of *R. alnifolia* in eastern Vilas County, WI, on July 27, 2002 (Expt. 1 described below). Another collection was made on September 6, 2002, from plants growing in Marinette County, WI (Expt. 2 described below). Two-seeded drupes of *R. lanceolata* were collected on August 10 and 15, 2002, from plants growing in a calcareous fen in Kendall County, IL. All fruits were stored in the dark at 5C (41F) until they were used in respective germination experiments, which were conducted in a glasshouse at Iowa State University in Ames, IA.

Experiment 1. Summer germination trial. Seeds were extracted before pretreatment by removing the surrounding fruit tissues by hand and were subjected to the following pregermination treatments by applying each treatment to 75 seeds: (1) moist-prechilling at 5C (41F) in growing medium (LC-1; Sun Gro Horticulture Canada Ltd., Seba Beach, Alta., Canada) for 120 days; (2) moist-prechilling at 5C (41F) in growing medium for 90 days; (3) scarification for 10 min in 96% H_2SO_4 followed by moist-prechilling at 5C (41F) in growing medium for 90 days; and (4) control conditions, i.e., storage at 5C (41F). An 11 cm (4.5 in) square pot [Kord Products, Brampton, Ont., Canada; volume = 81 in^3 (1327 cm^3)] containing five seeds [placed at the depth of 0.5 cm (0.2 in)] was an experimental unit in a randomized complete block design that contained five blocks. Consequently, each block contained 15 seeds per treatment combination. After pre-germination treatments were applied, seeds were placed in a glasshouse where day/night temperatures averaged 22/20C (72/68F). Supplemental irradiance was not provided during this experiment. The substrate was kept moist with tap water, and germination was recorded every 7 days until the test was concluded 35 days after seeds were placed in the glasshouse. Because seeds of both species exhibit epigeous germination, emergence of the hypocotyl hook was considered an indicator of germination in all germination experiments (10).

Experiment 2. Fall germination trial. *Rhamnus alnifolia* drupes collected on September 6, 2002, from plants growing in Marinette County, WI, were used. Seeds were extracted by removing the surrounding fruit tissues by hand. On September 12, 2002, 50 seeds were submerged for 1 hr in water that had been boiled at 100C (212F) and then were placed in germination substrate (as described for Expt. 1); an additional 50, nontreated seeds also were placed in germination medium. Pots containing the seeds were moist-prechilled at 5C (41F) for 30 days. The experimental units and cultural practices in the glasshouse were similar to those described for Expt. 1, and data also were collected similarly.

Experiment 3. Propagation by rooting. Fifty, terminal, 25-cm-long (10 in), actively growing shoots were collected on June 7, 2003, from plants of *Rhamnus alnifolia* growing on a slope along a stream in Clayton County, IA, after appropriate permits were obtained from The Nature Conservancy. Stems were transported on ice to Iowa State University in Ames, IA, where they were stored at 5C (41F) until they were processed the next day. Shoots were then cut mid-length to obtain two types of cuttings: 1) terminal cuttings and 2) subterminal cuttings. A factorial arrangement was established in a completely randomized design by using two cutting types, two rooting substrates, and five indole-3-butyric acid (IBA) treatments. Ten cuttings were assigned randomly to each IBA

treatment: 1) nontreated control; 2) 3 g/kg (3000 ppm) IBA in talc [Rhizopon® # 2 (Phytotronics, Inc., Earth City, MO)]; 3) 8 g/kg (8000 ppm) IBA in talc (Rhizopon® # 3); 4) 3 g/liter (3000 ppm) IBA in a solution containing one part acetone (2-propanone [$(CH_3)_2$ CO]) and three parts deionized water (by vol); and 5) 8 g/liter (8000 ppm) IBA in a solution containing one part acetone and three parts deionized water (by vol). Bases of cuttings were dipped for 10 sec into the IBA solution and were then dried for 1 min to allow absorption of the solution (10). To apply the talc, cuttings were dipped in the powder (2-cm-deep) and then were tapped slightly to remove excess powder. Five of the 10 cuttings assigned to each IBA treatment were selected for placement in medium grade vermiculite (Therm-O-Rock, New Eagle, PA), and the other five were placed in a mixture (by vol) of one part coarse grade perlite (Silbrico Corporation, Hodgkins, IL) and one part vermiculite. The basal 2.5 to 4 cm (1 to 1.5 in) of each cutting was inserted into a previously created hole in moist substrate contained in a plastic container [SR225, The Lerio Corp, Mobile, AL; vol = 14 in^3 (227 cm^3)]. One cutting per container was an experimental unit. Potted cuttings were placed under a mist system controlled by an evaporative sensor (Mist-a-Matic™, E.C. Geiger, Harleysville, PA). Ambient temperature in the glasshouse was recorded every 20 min by using a HOBO datalogger (Onset Computer, Bourne, MA). The day/night temperature averaged 22/18C (72/64F). Data were recorded 35 days after cuttings were placed under mist. At this time, we recorded the presence of callus, condition of the shoots (whether or not the leaves remained green), number of roots, and length of the longest root. A cutting was considered rooted if it had at least one root measuring ≥ 2.54 cm (1 in).

Data analysis. Seed germination data were subjected to analysis of variance (ANOVA) to determine differences among treatments. Proportions were arc-sin transformed before statistical procedures (GLM) were conducted by using SAS/STAT (version 8.02, SAS Institute, Inc., Cary, NC). Nontransformed means were converted to percentages for presentation. Germination value as described by Czabator (4) was calculated to compare the speed and completeness of germination. Root formation on softwood stem cuttings of *Rhamnus alnifolia* also was assessed by using analysis of variance models. Rooting proportions were arc-sin transformed before analyses, but non-transformed, percentage data are presented. When appropriate, means were separated by using Fisher's least significant difference ($\alpha = 0.05$).

Results and Discussion

Experiment 1. Summer germination trial. There was no effect of blocking, but interactions were observed between species of *Rhamnus* and pregermination treatments. Highest germination percentage (13%; germination value = 0.15) was observed among *R. alnifolia* seeds moist-prechilled for 90 days (Fig. 1A). Approximately 5% germination (germination value = 0.04) was observed among seeds moist-prechilled for 120 days. Scarified seeds and nontreated seeds did not germinate. While softening of outer tissues of seeds may benefit germination, scarification for 10 min in sulfuric acid likely damaged the embryos of both species. It is conceivable that shorter scarification periods might be more beneficial for abrasing the seed coat without damaging the embryonic tissues. Assessment of moist-prechilling duration also merits further consideration. Shorter germination delays are reported for shade-tolerant taxa than for shade-intolerant taxa (1); however, germination percentages for *R. alnifolia* (a reportedly shade-tolerant species (16) were low in this experiment after 90 or 120 days of moist-prechilling. Although the outer tissues of fruits had changed from green to dark blue, it is possible the seeds had not ripened fully at the time of collection in late-July. Higher germination percentages often are obtained among seeds that are collected after they have dried while attached to the mother plant than when germination is attempted with immature seeds collected in mid-season (2). Germination was ≤ 5% among *R. lanceolata* seeds subjected to 90 or 120 days of moist-prechilling (data not presented). Germination values ranged from 0.0 to 0.04 and were similar across treatments. Seeds for our experiments were collected from shrubs in their natural habitat, and it is possible that further maturation on the mother plant was required or that the seeds were non-viable at the time of collection. Although we did not test viability of seeds used in this study due to a limited supply of seeds, viability does not necessar-

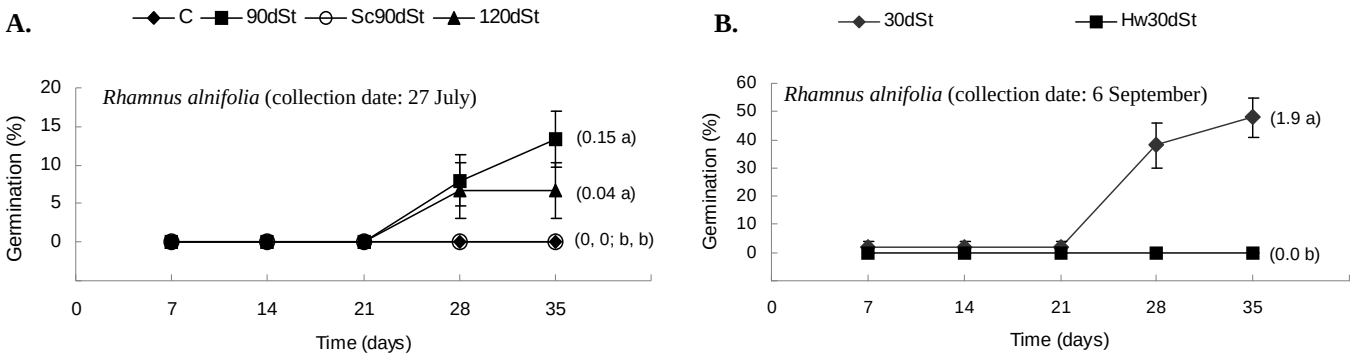

Fig. 1. A. Drupes of *Rhamnus alnifolia* were collected in late July and were subjected to a 35-day germination test after moist-prechilling for 90 days (90dst), scarifying seeds for 10 min in 96% H_2SO_4 and subsequent moist-prechilling for 90 days (Sc90dSt), cold-treating seeds for 120 days (120dSt), or sowing nontreated seeds (C). B. Seeds of *Rhamnus alnifolia* were collected again in September and were subjected to a 35-day germination test after seeds were cold-treated for 30 days (30dSt) or were scarified for 1 h in hot water followed by a cold-treatment for 30 days (Hw30dSt). Vertical bars about the data symbols represent ± the standard error of mean germination percentages. Germination value, a composite measure of the speed and completeness, was determined, and values are presented in parentheses. Germination values followed by the same letter are not significantly different according to Fisher's LSD test ($\alpha = 0.05$).

Table 1. Bases of cuttings of *Rhamnus alnifolia* were treated with IBA, and cuttings were kept under intermittent mist for 35 d. Because interactions among treatments were not detected, data for type of substrate and for position of stem cutting on ortet were pooled (n = 20).

IBA g/kg (ppm)	Cuttings with leaves remaining green (%)	Callus (%)	Rooting (%)	Root no.	Length of longest root (cm)
0	0a[x]	80a	75a	7c	6a
3 (3000) [talc][z]	10a	90a	85a	15b	8a
8 (8000) [talc]	30a	85a	85a	30a	5a
3 (3000) [solution][y]	5a	15b	15b	4c	2b
8 (8000) [solution]	0a	0b	0b	0c	0b

[z]Rhizopon® # 2 talc and # 3 talc were used to apply 3 and 8 g/kg (3000 and 8000 ppm) IBA, respectively.

[y]Liquid formulations of IBA were prepared by dissolving the chemical in a 1:3 mixture (by vol) of acetone (2-propanone [$(CH_3)_2$ CO]) and deionized water.

[x]Means with the same letter within columns are not significantly different (Fisher's LSD test; $\alpha = 0.05$).

ily indicate germinability. Physiological or chemical barriers often prevent germination of viable seeds (2). Our results indicate that moist-prechilling is necessary to germinate seeds of *R. alnifolia* and *R. lanceolata*, but additional experiments are needed to assess the timing of seed-collection further and to investigate the effects of physiological or chemical barriers to germination among seeds of *R. lanceolata*.

Experiment 2 Fall germination trial. Pregermination treatment influenced germination. Germination percentage was 48% (germination value = 1.9) among fresh *R. alnifolia* seeds harvested in September and then moist-prechilled for 30 days (Fig. 1B). In comparison, scarification of seeds in hot water and subsequent moist-prechilling at 5C (41F) for 30 days inhibited germination. Scarification with acid (Expt. 1) may have damaged the embryos regardless of seed maturity. In light of our results from Expt. 1, where the highest germination was 13% among seeds that were collected in July, it appears that seeds of *R. alnifolia* germinate to higher percentages if they are collected later in the growing season. Drying of the seeds while they are attached to the mother plant might be a prerequisite for *R. alnifolia* seeds to germinate. Results from this study also support the observation by Arevalo and Fernandez-Palacios (1) that shade-tolerant taxa may germinate relatively soon, because 30-day moist-prechilling induced 48% germination among seeds of *R. alnifolia*.

Experiment 3. Propagation by rooting. Interactions did not occur among types of cuttings, IBA treatment, and substrate. Main effect of IBA treatment was significant, and rooting frequency of softwood stem cuttings of *R. alnifolia* ranged from 0% [all cuttings that received 8 g/liter (8000 ppm) IBA in solution] to 85% [cuttings treated with 3 g/kg (3000 ppm) or 8 g/kg (8000 ppm) IBA in talc]. Seventy-five percent (75%) of the nontreated cuttings also rooted, and the frequency of rooting was similar statistically to that obtained among cuttings that received IBA in talc (Table 1). Fewer (mean = 7) roots formed on cuttings to which IBA was not applied (Table 1 and Fig. 2). Among the cuttings to which IBA was applied in talc, mean number of roots increased from 15 to 30 as the concentration of IBA increased from 3 g/kg (3000 ppm) to 8 g/kg (8000 ppm). Application of IBA via an acetone solution apparently inhibited development of roots (Table 1). Fewer cuttings developed roots when IBA was applied in an acetone solution as opposed to in talc, and this was true for both concentrations. Cumulatively, fewer roots formed on cuttings treated with IBA in solution than were produced on cuttings treated with talc formulations of IBA; roots also were shorter on cuttings treated with IBA in solution than were roots on their talc-treated counterparts (Table 1). Sensitivity to alcohol formulations have been reported for stem cuttings of several species (10), and potassium salt formulations, which are dissolved in water, might be a better method of applying IBA in solution to *R. alnifolia*. Nonetheless, our results show that softwood stem cuttings of *R. alnifolia* root when they are treated with IBA in talc. Trends in callus production were similar across treatments to those observed in rooting (Table 1). Although some (up to 6%) cuttings that produced callus did not develop roots, callus production generally was followed by root initiation and development. However, IBA treatments did not affect the retention of green leaves on stem cuttings (Table 1), whereas the type of substrate did (Table 2). Seventeen percent of the cuttings that were placed in vermiculite alone retained green leaves in comparison to 3% of the cuttings in a 1:1 mixture (by vol) of perlite and vermiculite. A main effect of type of substrate also was observed on the number of roots on cuttings, whereby more (mean = 15) roots developed on cuttings in vermiculite alone in comparison to the mean number of roots

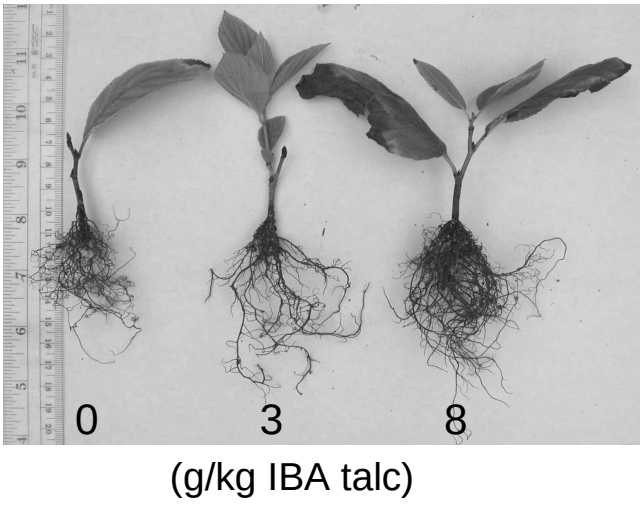

Fig. 2. Roots developed on stem cuttings of *Rhamnus alnifolia* within 5 weeks after application of IBA to bases of cuttings. Rooting was higher (85%) when IBA was applied in talc in comparison to rooting (≤ 15%) after IBA was applied via an alcohol-based solution; 75% of the nontreated cuttings also produced roots. More roots formed on cuttings treated with 8 g/kg IBA in talc than on cuttings treated with other formulations of IBA.

Table 2. Bases of cuttings of *Rhamnus alnifolia* were treated with IBA, and cuttings were kept under intermittent mist for 35 d. Main effect of substrate (n = 50) is reported because interactions were not observed among substrate, position of cutting, and hormone treatment.

Medium	Cuttings with leaves remaining green (%)	Callus (%)	Rooting (%)	Root no.	Length of longest root (cm)
Vermiculite	17a[z]	60a	58a	15a	5a
perlite:vermiculite (1:1 by vol)	3b	53a	52a	10b	3b

[z]Means with the same letter within columns are not significantly different (Fisher's LSD test; $\alpha = 0.05$).

on cuttings in a 1:1 mixture (by vol) of perlite and vermiculite (mean = 10; Table 2). Mean length of the longest root was 5 cm (2 in) on cuttings in vermiculite alone, whereas the longest root on cuttings in the blended medium averaged 3 cm (1.2 in). These effects could be attributed to the higher moisture holding capacity and finer texture of vermiculite compared to perlite. Substrate composition, however, did not affect rooting, nor callus production. In summary, we recommend talc-based IBA and vermiculite to induce root formation on terminal or subterminal softwood stem cuttings of *R. alnifolia.*

This study demonstrates methods to propagate *Rhamnus alnifolia* and *Rhamnus lanceolata*, which are native shrubs with ornamental and ecological merit. Both species are uncommon in nature, and our assessment of their reproductive capacity will benefit horticulturists and plant ecologists alike. While moist-prechilling appears to be necessary for seeds of both species to germinate, germination among seeds of *R. alnifolia* improved when seeds were allowed to dry (mature) while attached to the mother plant. *Rhamnus lanceolata* seeds might exhibit a similar pattern with regard to seed maturity and merit further study. Viability among seeds of both species also could be investigated. Although it was reported that seeds of *R. alnifolia* can remain viable for 5–7 years if stored in sealed containers between 3C (37F) and 5C (41F) (13), the viability of seeds at the time of harvest and changes that may occur during storage remain unknown.

While it is important to have in place methods to regenerate uncommon plants, widespread use of any species outside of its natural range must be recommended with caution. For example, until recently, invasive, Eurasian species of *Rhamnus* (e.g., *Rhamnus cathartica*) were the only known overwintering hosts of the soybean aphid in the United States. Recent research (17) showed that *R. alnifolia* and *R. lanceolata* as suitable overwintering hosts under experimental conditions. While plants of *R. alnifolia* and *R. lanceolata* have not been observed as suitable hosts in their natural habitat, horticultural use of these native species of *Rhamnus* should be tempered by considering their role in the life cycle of the aphid.

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