Propagation

Cecil Pounders
Section Editor and Moderator

Use of a Thickening Agent for Application of Auxin to Cuttings of Hibiscus and Rose

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Index Words: Adventitious Rooting, Cutting Propagation, Root-promoting Compounds, Sodium Cellulose Glycolate, Sodium Carboxymethylcellulose

Nature of Work: Auxins have been known to promote rooting of stem cuttings of ornamental plants since the 1930s (Chadwick and Kiplinger, 1938). Commercial propagators typically apply auxin to the base of stem cuttings using either a quick-dip in a liquid formulation or a dry talc formulation. An extended basal soak in a dilute auxin solution has also been used in the past (Hartmann et al., 2002). Our recent research with auxin applied to cuttings via a stabilized organic substrate (Blythe et al., 2003, 2004) has indicated that, depending upon the species and cultivar, an extended period of exposure can result in rooting that is equal to, or better than, results with a conventional basal quick-dip, while allowing use of a lower auxin rate.

The objective of this experiment was to determine whether the inclusion of the sodium cellulose glycolate (SCG) product Dip-Gel (Dip 'N Grow, Inc., Clackamas, OR) as a thickening agent for solutions of Dip 'N Grow [indole-3-butyric acid (IBA) + 1-naphthaleneacetic acid (NAA); Dip 'N Grow, Inc., Clackamas, OR] would affect the rooting response of stem cuttings of two selected woody ornamentals. Beeson (2000) and Childs and Beeson (2001) had previously tested the SCG product Cell-U-Wett for treating cuttings using moderate to high rates of auxin and found no improvement in rooting response. SCG, also known as sodium carboxymethylcellulose, is used as a thickener, binder, emulsifier, stabilizer and colloidal suspending agent in salad dressing, fruit pie fillings, baked goods, dietetic foods, and other products (Joint FAO/WHO Expert Committee on Food Additives, 1966).

Auxin solutions were prepared at 0 + 0, 50 + 25, 250 + 125, 500 + 250, 750 + 375, and 1000 + 500 mg/L IBA + NAA in combination with 0 and 13.5 g/L SCG. Semi-hardwood stem cuttings of *Rosa* 'Red Cascade' were prepared as 0.75-inch, single-node cuttings, dipped to a depth of 0.25 inch into their respective auxin/SCG treatments, and inserted to a depth of 0.5 inch into Fafard 3B mix (peat/perlite/vermiculite/pine bark) (Conrad Fafard, Inc., Agawam, MA) in individual cells of 72-cell plug trays (28 cm^3 per cell; Landmark Plastics, Akron, OH). Semi-hardwood stem cuttings of *Hibiscus syriacus* 'Collie Mullens' were prepared as 3-inch, 3-node cuttings with the leaf removed from the lowest node, dipped to a depth of 0.5 inch into their respective auxin/SCG treatments, and inserted to a depth of 0.75 inch into Fafard 3B mix in individual cells of 50-cell plug trays (85 cm^3 per cell; Landmark Plastics, Akron, OH).

All cuttings were prepared on May 23, 2003 and placed under a greenhouse mist system providing overhead mist for 6 seconds every 20 minutes during daylight hours for a rooting period of 28 days (rose) or 35 days (hibiscus).

Rooting responses (number of primary roots and total root length per cutting) were analyzed using a general linear model with SAS Version 9.1 (SAS Institute, Cary, NC).

Results and Discussion: Cuttings of *Hibiscus syriacus* 'Collie Mullens' showed an increasing trend in both number of roots and total root length with increasing auxin concentration; a maximum response may have been reached at around 750 mg/L IBA + 375 mg/L NAA. Number of roots was greater at all rates of auxin with 13.5 g/L SCG than with no SCG, while total root length tended to be more notable for solutions containing SCG with increasing auxin concentration.

Cuttings of *Rosa* 'Red Cascade' also showed a generally increasing response in number of roots with increasing auxin concentration, with a possible maximum response around 750 mg/L IBA + 375 mg/L NAA. Cuttings treated with solutions containing 13.5 g/L SCG showed a greater number of roots and total root length across all rates of auxin.

The cause of the increased rooting responses of cuttings with the addition of SCG to the liquid auxin solutions has not been determined. Further studies have shown that the increased rooting response does not extend to all species.

Significance to the Industry: Experimental results indicate that the inclusion of SCG in liquid auxin solutions can improve the rooting response of *Hibiscus syriacus* 'Collie Mullens' and *Rosa* 'Red Cascade'. Further research will be conducted to determine if less-easily-rooted species also show such a response, and whether any improvement in rooting is due to the SCG alone or the combination of SCG and auxin. On species where the SCG treatments are more effective than with auxin alone, use of SCG may allow lower rates of auxin to be used without a decrease in rooting response.

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Table 1. Rooting response of stem cuttings[z] of *Hibiscus syriacus* 'Collie Mullens' and *Rosa* 'Red Cascade' treated with Dip 'N Grow[y] rooting compound solutions prepared with 0 and 13.5 g/L sodium cellulose glycolate (SCG).

Auxin (IBA + NAA) rate (g/L)	Roots/rooted cutting (no.)		Total root length/ rooted cutting (mm)	
	0 g/L SCG	13.5 g/L SCG	0 g/L SCG	13.5 g/L SCG
	Hibiscus syriacus 'Collie Mullens'			
0 + 0	13.5	14.7	1221	1231
50 + 25	14.9	17.7	1331	1364
250 + 125	18.1	18.3	1548	1534
500 + 250	17.9	19.1	1519	1821
750 + 375	20.5	21.3	1566	1924
1000 + 500	18.3	24.1	1398	2049
Significance[x]:				
Auxin rate	<0.0001		<0.0001	
SCG	0.0031		0.6366	
Auxin rate x SCG	NS		0.0002	
	Rosa 'Red Cascade'			
0 + 0	5.9	7.2	266	345
50 + 25	5.7	7.0	247	336
250 + 125	6.3	6.3	347	413
500 + 250	6.5	6.8	399	423
750 + 375	7.7	7.5	410	485
1000 + 500	6.6	7.6	411	502
Significance:				
Auxin rate	0.0200		<0.0001	
SCG	0.0427		0.0011	
Auxin rate x SCG	NS		NS	

[z]Fifteen cuttings per treatment combination per species.

[y]Indole-3-butyric acid (IBA) + 1-naphthaleneacetic acid (NAA)

[x]*P*-value for main effects (auxin rate and SCG) and interaction. If the interaction term was not significant, significance is shown for a model with main effects only.

Effects of Seedling Age, Basal Medium, and Explant on Shoot Production on Eastern Redbud (*Cercis canadensis*) In vitro

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Index Words: Redbud, Micropropagation, Tissue culture

Significance to Industry: Our findings indicate that first and second leaf nodal explants are more effective than cotyledonary nodes for redbud shoot production. Also, regeneration of shoots from immature nodal segments of redbud may provide an opportunity to introduce disease resistance genes into redbuds through particle bombardment or *Agrobacterium*--mediated transformation.

Nature of Work: Eastern redbud (*Cercis canadensis*) is a popular tree found both in natural landscapes and cultivation. It is commercially important because of its brilliant lavender-pink spring flowers, attractive glossy foliage, and ability to grow in a variety of tough conditions. It belongs to the legume family, is a member of the caesalpinoid sub-family and is native to the eastern United States. Traditional propagation of redbud includes seeding and grafting. However, these methods are difficult and inefficient (1). The difficulty of propagation has limited large-scale production and led to an exploration of tissue culture as a method of mass propagation. *In vitro* studies of *Cercis* spp. have been conducted using shoots (1), cotyledonary node segments (3), axillary buds and leaf tissue (2), and somatic embryos (4). In Distabanjong and Geneve's study (3) of cotyledons and cotyledonary node segments, 4 to 10-day-old seedlings were used as the source of explants, but the effects of different seedling ages was not tested. The objective of our study is to clonally propagate eastern redbud and determine the effects of seedling age, basal medium, and explant source on shoot proliferation *in vitro*. By investigating the factors leading to shoot formation in eastern redbud, we hope to develop or further improve efficient, multiple shoot formation systems for large-scale production of this valuable ornamental tree.

Cercis canadensis seeds were harvested in September 2004 from a six-year-old tree on the Kansas State University campus, Manhattan, Kansas. Seeds were acid scarified for 30 min in concentrated sulfuric acid, surface disinfested for 15 min in 20% Clorox bleach (Clorox Co. Oakland, Calif.) solution containing 1 drop Tween 20 (polyoxyethylenesorbitan) per 100 mL, and rinsed 3 times for 5 minutes in sterile deionized water (dH_2O). Following disinfestation, seeds were imbibed for 48 h in dH_2O, then mixed with moistened, autoclaved 1 peat:1 perlite medium and refrigerated at 37 ºF for 60 d. After the cold stratification, seeds were removed from the peat/perlite medium and surface disinfested as described above. Seeds were split longitudinally with a scalpel and embryos excised. Embryos were germinated in GA-7 containers (Magenta Corp., Choicago, Ill.) containing ½ strength Murashige and Skoog (7) basal medium with vitamins (MS) (PhytoTechnology Labratories, Shawnee Mission, Kansas) in a growth chamber

under a 16 h photoperiod at 73 ºF. Explants were harvested from seedlings at cotyledonary, first true leaf or second leaf stage and were 2-, 3-, or 4-weeks old, respectively, at the time of harvest. Each seedling was dissected and separated into stem explants (hypocotyl, cotyledonary node, first node, and second node depending upon seedling stage) 0.4 inches in length and leaf explants (cotyledons, first, and second leaf depending upon seedling stage) that were cut in half laterally. Stem and leaf explants were placed in separate GA-7s on MS or Lloyd and McCown Woody Plant Medium (6) with vitamins (WPM) amended with 6.6 mg per liter Thidiazuron (TDZ) plus 0.2 mg per liter indole-3-butyric acid (IBA). Cultured explants were maintained in a growth chamber under conditions described above in a completely randomized design. Ten seedlings per growth stage per basal medium were used. After 4 weeks in culture, explants were sub-cultured to MS or WPM medium supplemented with 2 mg/L 6-benzyladenine (BA) plus 1 mg/L IBA and again after an additional 4 weeks to the respective medium. Data were collected on number of shoots per explant and subjected to analysis of variance and means separation by $LSD_{0.05}$.

Results and Discussion: *Effects of seedling age on shoot production.* Two-week-old seedlings produced significantly more shoots per cotyledonary node explant than 3 or 4-week-old seedlings, 2.9 compared to 2.2 and 2.4, respectively (Fig. 1). These results agree with findings of Distabjong and Geneve (3) that 4 to 10-day-old redbud seedling explants produced more shoots than 0 and 2-day-old seedlings. They also agree with results from soybeans (5). Shoot production per explant in our study was about half that of Distabjong and Geneve (3), however, we removed the apical meristem while they did not.

Effects of stem section on shoot production. Nodal explants from the first true leaf and second leaf of 4-week-old seedlings produced significantly more shoots than hypocotyls and cotyledonary nodes, averaging 2.9 shoots per explant compared to 0 and 2.4, respectively (Fig. 2). These results indicate that although the cotyledons produce slightly fewer shoots per explant at 4-weeks-of age compared to 2 weeks, there is a significant gain in total number of shoots produced per seedling by using the first and second nodes as well as the cotyledonary nodes.

Adventitious shoot production on cotyledons and leaf tissue. No shoots formed on leaf tissue and only 2 shoots total were produced on 120 cotyledons in this study. These results differ from results with Chinese redbud (*Cercis yunnanensis*) where 42% of the leaf explants produced shoots (2). Additionally, no significant differences for shoot production were found between MS and WPM basal medium.

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Figure 1. Shoot production on eastern redbud (*Cercis canadensis*) cotyledonary nodes *in vitro* as influenced by seedling age.

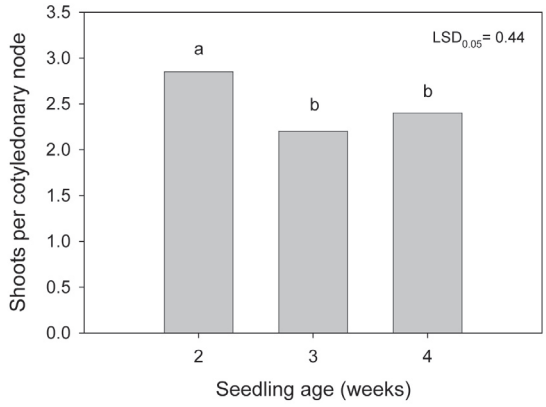
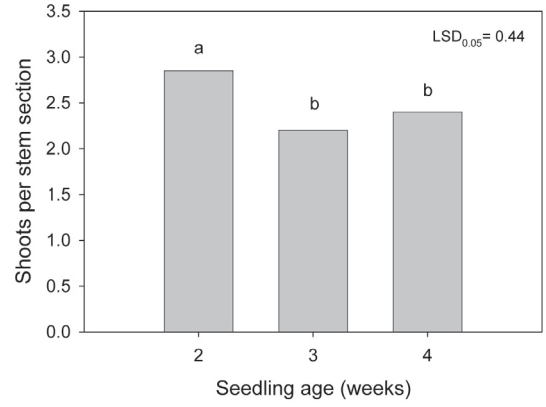


Figure 2. Shoot production on eastern redbud (*Cercis canadensis*) seedlings *in vitro* as influenced by stem section.



Micropropagation of Two *Lonicera* Species for Genetic Engineering for Sterility

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Index Words: honeysuckle, *Lonicera*, micropropagation, invasive plant

Significance to Industry: Japanese and Amur honeysuckles have many good attributes for use in the landscape; however, their invasiveness makes them undesirable. We developed an efficient micropropagation system for both species. It can be used to produce consistent plant materials for developing a genetic engineering system, and later for a biotechnological approach for genetic manipulation. The micropropagation system will provide consistent and quality materials for genetic engineering for female sterility and seedlessness.

Nature of Work: Many invasive plants produce massive amounts of seed that are dispersed by birds and other means to far distances. Other invasive species invade nearby territory by vegetative means. Approximately $35 billion has been spent annually in controlling invasive plants and the associated economic and environmental damages in the United States (Hall, 2000; Pimentel *et al*., 1999). One of the strategies to stop the spread of invasive species is to generate seedless plants with fruits birds can still eat but which have no viable seed to facilitate undesirable spread.

Japanese honeysuckle (*Lonicera japonica*) is a climbing vine introduced to North America from Asia as an ornamental plant for its lovely fragrance and pretty flowers. Several exotic bush honeysuckles, Amur honeysuckle [*L. maackii* (Rubr.) Herder], Morrow's honeysuckle [*L. morrowii* Gray], Tatarian honeysuckle [*L. tatarica* L.], Sweet-breath-of-spring [*L. fragrantissima* Lindl. & Paxton], and Belle honeysuckle [*L.* X *bella* Zabel] (a hybrid cross between Tatarian and Morrow's honeysuckles) have also been frequently used for landscaping to improve wildlife habitats and for soil erosion control. Their characteristic vigorous growth habit, shade-tolerance, wide adaptability and ease of establishment make them aggressive competitors against native flora in all forest types over a wide range of sites, posing a significant threat to the ecosystems (Schierenbeck, 2004). Recent advances in genetic engineering and molecular biology have opened new avenues to solving or potentially solving many biological problems that otherwise might be impossible. For example, male sterility and female sterility genes can neutralize the seed-mediated invasiveness of invasive plants (Li et al., 2004) and self-destruction of grasses can be obtained by environmentally regulated gene expression (Stanislaus and Cheng, 2002). Regardless, for most genetic engineering approaches, a dependable tissue culture protocol is necessary for reliably providing consistent experimental plant materials and for bringing up potential genetically-engineered plants to ambient environment. The objective of this research was to develop an efficient, reliable micropropagation protocol for Japanese honeysuckle and for one of the bush-type honeysuckles, Amur honeysuckle.

Vigorously growing shoot tips about 5 cm of Japanese and Amur honeysuckles were collected in Knoxville, TN. They were disinfected by soaking in a 1% Tween 20 solution for five min, in 70% ethyl alcohol for one min, and in 10% commercial bleach containing 5.25% sodium hypochlorite for 15 min. Explants were washed three times (5 min each) with sterile water. A 2-cm portion of the stem apex was excised from the explant and placed on medium containing Murashige and Skoog (MS) salts (Murashige and Skoog, 1962) with the addition of 100 mg/L myo-inositol and 3.0% sucrose. The pH of the medium was adjusted to 5.8 and solidified with 0.7% (w/v) agar prior to being autoclaved. In the initial *in vitro* culture establishment, three shoots were placed per 100 mL baby food jar containing 30 mL of medium. All cultures were subcultured to fresh medium every 4 weeks. Cultures were maintained in a growth room at 25ºC under a 16 h photoperiod (16 h light/ 8 h dark) with illumination of 125 μmol•m^{-2}•s^{-1}. Five growth regulator combinations used in the experiment included (in _M): 0, 2.5 6-benzyladenine (BA), 5.0 BA, 2.5 BA + 1.25 indole-3-butyric acid (IBA), and 5 BA + 2.5 IBA. Three different basal media were also tested: MS, DKW (Driver & Kuniyuki, 1984) and Woody Plant Medium (WPM) (Lloyd and McCown, 1981). Shoot tips were subcultured every four weeks for three months, and the average shoot numbers were recorded. Each treatment contained four jars and each jar contained four shoots. The experiment was repeated once. Microshoots were harvested from the jars and rinsed thoroughly with distilled deionized water. They were then exposed to 0 or 200 μM of IBA by dipping the basal 1-5 mm section for five sec. Shoots were placed in a sterile potting mix (Premier Pro-Mix, Premiere Horticulture LTD, Red Tail, PA) in 5-cm diameter pots and placed in a covered container with a clear lid under fluorescent lighting. Explants were acclimatized to ambient conditions by progressively opening the lid and increasing size of opening over two weeks. At the end of one month, the plantlets were removed from the pots and the roots counted. Each treatment contained twenty microshoots, and the experiment was conducted twice.

Results and Discussion: Two species responded differently to plant growth regulators in axillary shoot proliferation. For Amur honeysuckle, an average of 2-3 shoots were produced per original shoot in most treatments. Japanese honeysuckle only produced one new shoot in a four-week period. Among five plant growth regulator treatments, there was no significant effect on shoot proliferation of Japanese honeysuckle. The treatment of 2.5 _M BA alone seems to be the best treatment for Amur honeysuckle. For the basal medium treatment, DKW and MS media were more suitable for Amur honeysuckle. Although there were no significant differences in shoot proliferation due to media type on Japanese honeysuckle, the plants growing on DKW appeared healthier. Microshoots rooted differently depending on the treatment and species. Amur honeysuckle rooted at 13% with the water treatment and rooted at 70% when treated with 200 _M of IBA. Japanese honeysuckle rooted at 78% with the water treatment and rooted at 87% with 200 _M of IBA. This research showed that two *Lonicera* species responded differently in culture. Amur honeysuckle was more prolific than Japanese honeysuckle in the conditions tested, while Japanese honeysuckle was easier to root.

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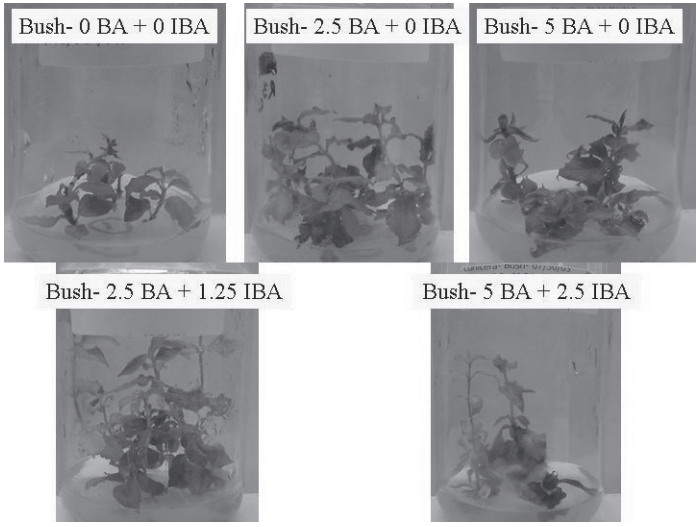

Redbud Germination Affected by Seed Treatment

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Index Words: *Cercis canadensis* L., propagation

Significance to Industry: Boiling water (212 F) poured over redbud seeds followed by soaking is a successful pre-germination seed treatment. Seeds treated with sulfuric acid had 30% lower germination compared to seeds treated with boiling water. Germination of seeds with mechanical scarification (sandpaper rub) was similar to seeds treated with acid, but was not a time efficient method of seed treatment. Boiling seeds for as little as 1 min killed the embryo and prevented germination.

Nature of Work: Eastern redbud, *Cercis canadensis*, is a popular native flowering tree. It is propagated from seed and cultivar selections are commonly budded onto seedling rootstock (3). Propagation has been successfully achieved with moist prechilling for 30 to 60 days and/or scarification with concentrated sulfuric acid (2,3). Concentrated sulfuric acid is caustic and safety measures must be adhered to prevent contact with skin or clothing. Seed coat thickness and hardness varies from year to year which requires adjustment in the immersion time of the seed in the acid. If immersion is too short, seed coats remain impermeable, if too long, the seeds are damaged. Hot water (190 F) soaks have been recommended but no details as to treatment duration are mentioned (1). Young and Young recommended boiling seeds for 1 minute to break dormancy (4). Our objective was to evaluate seed treatments with hot or boiling water and compare germination to conventional seed treatments such as mechanical scarification or exposure to sulfuric acid.

Eastern redbud seeds were collected, cleaned and put in moist cold storage (40 F) for at least six months prior to sowing. A series of experiments were conducted on 2 May 2004, 16 Aug 2004, 24 Jan 2005, and 2 March 2005 in which all or part of the following treatments were included at each date. Prior to the onset of each experiment, a float test was conducted in which seeds that floated were discarded. Units of 25 seeds were treated with a 5, 10, 15 or 30 min soak of 16.5% sulfuric acid, a 5, 10, 15 or 30 min soak with 32% sulfuric acid, or a 15 min soak with 66% sulfuric acid. After exposure to the acid, seeds were rinsed with tap water and sown immediately. Water treatments included bringing water and units of 25 seed to a boil for 1, 5, 10, 15, 30, or 60 min, or boiling water was poured over the units of seed and soaked in the water for 5 min or 24 hours. The last water treatment was hot tap water (133 F) poured over the units of seed and soaked for 24 hours. After all water treatments, water was drained from the seeds, and then seeds were sown. A mechanical scarification treatment was achieved by gently rubbing sandpaper on the seed coats. Untreated seed were used as a control. Seeds from each treatment were placed on a pine bark:peat (1:1, by vol) substrate in 4-inch square plastic containers. Seeds were covered with a thin layer of vermiculite then placed in a plastic covered greenhouse. Each seed treatment was replicated eight times (total of 200 seeds per treatment) and arranged in a completely randomized design. Seed germination was counted for

11 weeks (weeks 1-5 presented). Each week, seedlings were gently pulled from the substrate and discarded.

Results and Discussion: Like treatments from the separate experiments were not statistically different and therefore averaged together. Comparisons were then made between treatments. During the four experiments, the highest germination percentage (48%) was obtained by pouring boiling water (212 F) over the seeds followed by a 5 min or 24 hour soak that gradually cooled to ambient temperature (Fig. 1). Seeds soaked in 16% or 32% sulfuric acid for 5, 10 or 15 min germinated between 26% to 35%. Seed that were exposed to a 30 min soak in 32% sulfuric acid or a 15 min soak in 66% sulfuric acid had less than 15% germination. Mechanical scarification resulted in 30% germination, similar to many acid treatments, but was very time consuming to ensure all seeds were uniformly scarified. No seed germinated following boiled treatments, with the exception of seed that were boiled for one minute. Germination between 5 and 11 weeks was low and sporadic for all treatments.

As high as 90% redbud seed germination have been reported (3) in laboratory conditions, but extensive variability in seed lot characteristics influences germination. Germination percentage in these tests never exceeded 48%.

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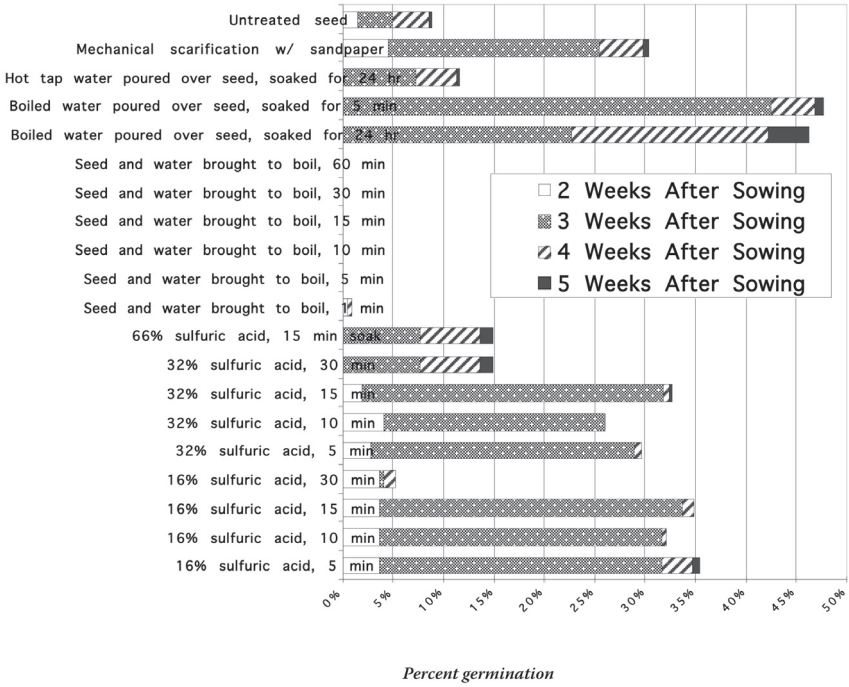
Fig. 1. Effect of seed treatment on germination of Eastern redbud, *Cercis canadensis*.



Auxin Affects Adventitious Rooting of Snowbells (*Styrax* L.)

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Index Words: adventitious rooting, indolebutyric acid, plant propagation, styrax, Styracaceae

Significance to Industry: The snowbells (*Styrax* L.) are a group of flowering shrubs and trees distributed throughout the warm-temperate regions of the northern hemisphere. There are approximately 120 species, of which, only *Styrax japonicus* Sieb. & Zucc. (Japanese snowbell) and its cultivars are currently of commercial significance. Increased awareness of more obscure taxa may lead to their eventual production and cultivation. For greater discussion on *Styrax* see 3. Asexual propagation of desirable plant material is necessary for production and selection of superior clones. Results herein demonstrate that propagation of a diverse assemblage of Styrax is possible by stem cuttings, although not all taxa can be treated similarly. For many taxa, percent rooting was improved when cuttings were treated with 3000 or 8000 ppm (0.3 or 0.8%) K-IBA. Rooting of other taxa, however, were unaffected by K-IBA treatment or were negatively affected by K-IBA.

Nature of Work: In an attempt to introduce and promote new and underutilized plants, information regarding asexual propagation is necessary. Although *Styrax* are not generally considered difficult to root, references almost exclusively refer to *S. japonicus*. Little to no information exists regarding asexual propagation of cultivars of *S. japonicus* or other species of *Styrax*.

In an attempt to expand information regarding asexual propagation of *Styrax*, terminal stem cuttings of 15 taxa (Table 1) were collected from plants growing at the JC Raulston Arboretum, (North Carolina State University, Raleigh) on July 26, 2004. Cuttings were placed in plastic bags and shipped, overnight, to the John C. Pair Horticultural Center, (Kansas State University, Wichita). The only exception was *S. formosanus* Matsum., of which cuttings were collected from containerized plants at the John C. Pair Horticultural Center on July 28, 2004. Cutting material was trimmed to 8 to 13 cm (3 to 5 in) terminal shoots and leaves were stripped from the lower half of the cuttings. The basal 1 cm (0.4 in) of each cutting was then dipped for 5 sec in 0, 3000 (0.3%), or 8000 ppm (0.8%) K-IBA dissolved in distilled water. The treated cuttings were inserted into flats 9 cm (3.5 in) deep containing a rooting substrate of perlite:peat (3:1 v/v). The flats were placed on a raised greenhouse bench under natural photoperiod and intermittent mist operating for 5 sec every 7 min during daylight hours. Greenhouse temperatures were set at days/nights of 28/24 °C (82/75 °F). For each taxon, the experimental design was a randomized complete block design with five blocks, and five

cuttings per treatment per block. Due to the number of taxa and variation in growth of each, cuttings from all species were not at the same physiological growth stage. Therefore, no statistical comparison was made between species, only between K-IBA treatments within a species.

At 9 weeks, cuttings were harvested and data recorded. Data included percent rooting and number of roots ≥ 1 mm (0.04 in) in length on rooted cuttings. A cutting was considered rooted if it had one adventitious root ≥ 1 mm (0.04 in) in length. Data were subjected to analysis of variance and where appropriate, means were separated by least significant difference (LSD). Due to a lack of cutting material, only three auxin treatments were utilized. Therefore, a means separation was employed rather than regression analysis.

Results and Discussion: Percent rooting or root number were affected by K-IBA application in 12 of the 15 taxa examined (Table 1). Application of K-IBA affected percent rooting in only five taxa: *S. dasyanthus* Perkins, *S. japonicus* 'Carillon', *S. japonicus* 'Pink Chimes', *S. japonicus* 'Snowfall', and *S. tonkinensis* (Pierre) Craib ex Hartwich. Percent rooting increased two-fold for *S. japonicus* 'Carillon', *S. japonicus* 'Pink Chimes' and *S. tonkinensis* when treated with 3000 ppm (0.3%) K-IBA. Nontreated cuttings of *S. dasyanthus* and *S. japonicus* 'Snowfall' failed to root. However, 3000 ppm (0.3%) K-IBA improved rooting in these two taxa to 36% and 25%, respectively, with a further increase in rooting when cuttings were treated with 8000 ppm (0.8%) K-IBA. The remaining four taxa that were affected by auxin application were affected negatively: *S. formosanus*, *S. japonicus* 'Issai', *S. japonicus* 'Pink Cascade', and *S. serrulatus* (clone B). Percent rooting of the remaining taxa was unaffected by K-IBA treatment.

Of the 15 taxa examined, auxin application increased the number of roots produced on a rooted cutting for five of the taxa: *Styrax calvescens* Perkins, *S. confusus* Hemsl., *S. japonicus* 'Pink Chimes', *S. serrulatus* (clone A), and *S. tonkinensis*. If the level of significance is relaxed to P ≤ 0.1, root number on *S. japonicus* 'Issai' and *S. serrulatus* (clone B) was also influenced by K-IBA application. Overall, when root production was improved by auxin application, numerous roots were produced (14 to 40 roots per rooted cutting). Taxa where K-IBA application failed to improve root production generally produced few roots overall (1 to 14 roots per rooted cutting).

Results herein suggest that clonal propagation of Styrax is possible although there is great variability in rooting potential between species, and between clones and cultivars within a species. In general, cultivars of *S. japonicus* rooted moderately well, which had been previously reported (2). However, *S. japonicus* 'Emerald Pagoda' rooted poorly, as has been noted previously for this cultivar (1). The two accessions of *S. serrulatus* demonstrated a very different response to auxin application, with "clone A" showing no response to auxin and "clone B" showing a decrease in rooting in response to increasing K-IBA.

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Table 1. Percent rooting and number of roots per rooted cutting of various taxa of Styrax as affected by K-IBA treatment.

	Rooting (%)				Root no./rooted cutting			
	K-IBA concn. (ppm)				K-IBA concn. (ppm)			
Species or cultivar	0	3000	8000	Sig	0	3000	8000	Sig
S. calvescens	28	36	21	NS[z]	3.0 b	9.5 b	21.0 a	**
S. confusus	28	44	32	NS	3.1 b	14.1 a	12.0 a	**
S. dasyanthus	0 b	36 a	44 a	**		3.1	5.2	NS
S. formosanus	84 a	80 a	48 b	*	7.6	14.2	5.9	NS
S. japonicus ‘Carillon’	30 b	75 a	45 ab	*	1.7	3.9	5.0	NS
S. japonicus ‘Crystal’	12	28	16	NS	10.8	7.3	9.7	NS
S. japonicus ‘Emerald Pagoda’	0	4	16	NS		1.0	2.5	NS
S. japonicus ‘Issai’	60 a	68 a	24 b	*	7.7 b	23.0 a	17.9 a	*P=0.09
S. japonicus ‘Pink Cascade’	48 a	24 ab	8 b	*	3.9	1.5	1.5	NS
S. japonicus ‘Pink Chimes’	48 b	92 a	76 ab	*	5.4 b	19.3 b	38.8 a	**
S. japonicus ‘Rubra Pendula’	68	56	64	NS	2.8	4.9	3.6	NS
S. japonicus ‘Snowfall’	0 b	25 b	75 a	**		6.5	8.0	NS
S. serrulatus (clone A)	72	84	88	NS	13 b	38.2 a	37.4 a	**
S. serrulatus (clone B)	68 a	36 b	16 b	**	13.9 b	39.3 a	27.5 ab	*P=0.08
S. tonkinensis	32 b	68 a	36 ab	*	2.0 b	6.3 a	4.0 b	**

[z]Effect of K-IBA treatment on percent rooting or root number is nonsignificant (NS), or significant at P≤0.05 (*) or P≤0.01 (**). Means within a row and followed by the same letter are not significantly different based on a protected LSD (P≤0.05) (n=25). If significance is relaxed to P≤0.1, root number of *S. japonicus* ‘Issai’ and *S. serrulatus* (clone B) become significant.

Effects of Substrate Physical Properties on Rooting Stem Cuttings of the Endangered Species, *Clematis socialis* Kral

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Index Words: Alabama Leather Flower, rooting medium

Significance to Industry: Little information is known about the cutting requirements of *Clematis socialis* stem cuttings. Results of studies conducted in 2000 and 2004 show that sand was among the two highest performing substrates along with vermiculite for rooting *C. socialis* stem cuttings in both years. Sand is also readily available and inexpensive making it the overall best substrate for rooting *C. socialis* stem cuttings.

Nature of Work: *Clematis socialis* Kral, also known as the Alabama Leather Flower, is an endangered species with only a few known populations all in northeast Alabama and northwest Georgia (6). *C. socialis* is an erect, non-vining, herbaceous perennial with blue-violet urn shaped flowers (1, 3, 4). The most distinctive feature of this species is the formation of dense clones by underground rhizomes. *C. socialis* was federally listed as an endangered species by the United States Fish and Wildlife Service because of small population numbers and size, as well as their existence on sites subject to human disturbance (5, 6). Information on the successful rooting of its stem cuttings could be beneficial in establishing self-sustaining populations, providing genetic material for future hybridization, and potentially introduce a new flowering groundcover to popular commercial and native nurseries.

Stem cutting propagation of other *Clematis* sp. has varied in success dependent upon technique, rooting medium, and species or cultivar propagated. In the United States, sand is currently the primary substrate used in commercial clematis stem cutting propagation (2). The objective of studies conducted in 2000 and 2004 was to evaluate the effects of four non-amended substrates on root initiation and growth of *C. socialis* stem cuttings.

Stem cuttings of *Clematis socialis* were taken May 26th in 2000 and June 11th in 2004 from a roadside population in Cherokee County, Alabama. The cuttings were placed in plastic bags on ice and transported back to Auburn University. Later in the day, the cuttings were re-cut to leave an inch (2.54 cm) of stem below the bottom-most node and received a basal application of Dip 'N Grow (Dip 'N Grow, Inc. Clackamas, Oregon) with the bottom-most node submerged for approximately five seconds. The treated cuttings were stuck in 606 cell packs with a volume of 10 in^3 per cell (163 cm^3) containing non-amended sand, perlite, vermiculite, and 1:1:1 (by volume) sphagnum peat, pine bark, and sand (P:PB:S).

The 2000 study used 2-node cuttings treated with 1,000 ppm of indole-3-butyric acid (IBA) and 500 ppm of naphthalene acetic acid (NAA). Terminal 3-node

cuttings treated with 1,500 ppm IBA and 750 ppm NAA were used in the 2004 study. Trays containing the cell packs were placed under an intermittent mist system that ran from 7:30 A.M. to 6:30 P.M. In 2000, the cuttings were kept in a full sun double-poly greenhouse and misted for 6 seconds every 5 minutes. Cuttings in the 2004 study were kept in a shaded glass greenhouse and misted for 5 seconds at 15 minute intervals.

The study was a randomized complete block design using six plants with four replications in 2000 and four plants with six replications in 2004. Data including root ratings (0 to 4, with 4 highest in 2000; 0 to 5, with 5 highest in 2004), root number (roots > 5 mm (0.197 in) in length), and averages of the three longest roots were collected ten weeks after treatment. In 2004, data on substrate physical properties including porosity, air space, water holding capacity, and particle size distribution was collected. All data were subjected to Duncan's Multiple Range Test ($P = 0.05$).

Results and Discussion: Cutting survival, root length, root number, and root rating was greater in 2004 than 2000 regardless of substrate type. A cooler environment, decreased number and duration of misting cycles, and an increased IBA and NAA concentration in the rooting solution may have caused the increased cutting survival and growth observed in 2004 compared to 2000. Of the four substrates evaluated, cutting survival was highest in sand in both 2000 (70.8 %) and 2004 (100 %) (Table 1). Although it is the heaviest of the four substrates, it is the least expensive and one of the most readily available. Similar cutting survival rates were achieved using vermiculite or perlite which are lighter weight but more expensive. In 2000, sand also produced the longest roots and highest root rating (Table 1). Root length, root number, and root rating was highest in vermiculite followed by sand in the 2004 study (Table 1). The 1:1:1 P:PB:S substrate produced the lowest averages for all growth data collected in both years. Perlite and 1:1:1 P:PB:S had the highest percent of particles larger than 0.066 inches (1.68 mm) in diameter (Table 3). Over 85 % of sand and vermiculite particles were 0.066 inches (1.68 mm) or smaller in diameter (Table 3). In 2004, cuttings rooted in finer-particled substrates, sand and vermiculite, had higher cutting survival, root growth, root number, and root rating than those rooted in perlite and 1:1:1 P:PB:S (Table 1, 3). Although cuttings performed best in sand and vermiculite in 2004, there were no statistical similarities between the two substrates in percent porosity, air space, and water holding capacity (Table 2). Results of these studies justify sand as the primary substrate used in commercial clematis stem cutting propagation today and that it would be a suitable choice for rooting *Clematis socialis* stem cuttings.

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Table 1. Root length, number, and rating[ZY] for *Clematis socialis* stem cuttings rooted in four non-amended substrates 10 weeks after treatment.

	Length (mm)		Number		Rating		Cutting survival percentage (%)	
Substrate	2000	2004	2000	2004	2000	2004	2000	2004
Sand	28.8 a[Y]	49.1 b	4.2 a	39.6 b	2.2 a	3.4 b	70.8 a	100.0 a
Perlite	15.0 a	26.8 c	4.2 a	22.1 bc	1.5 ab	1.6 c	66.7 ab	95.8 a
Vermiculite	23.9 a	145.7 a	1.7 b	112.1 a	1.4 b	4.8 a	58.3 ab	91.7 a
1:1:1 Peat/ Pinebark/Sand	0.1 b	18.0 c	0.2 b	6.3 c	0.1 c	1.0 c	12.5 b	33.3 b

[Z]Roots were rated on a 0 to 4 scale, with 4 being highest in 2000, and 0 to 5 scale, with 5 being highest in 2004.

[Y]Similar letters within columns were not significantly different at P = 0.05 using Duncan's Multiple Range Test.

Table 2. Percent porosity, air space, and water holding capacity of four substrates in 2004.

Substrate	Porosity (%)	Air space (%)	Water holding capacity (%)
Sand	39.75 d[Z]	13.50 b	26.25 c
Perlite	66.25 b	16.25 b	49.75 a
Vermiculite	69.75 a	20.25 a	49.50 a
1:1:1 Peat/Pine bark/Sand	49.25 c	13.50 b	35.75 b

[Z]Similar letters within columns were not significantly different at P = 0.05 using Duncan's Multiple Range Test.

Table 3. Percent particle size distribution of four substrates in 2004.[z]

Substrate	> 4.75	4.75 - 3.36	3.35 - 2.01	2.00 - 1.69	1.68 - 0.86	0.85 - 0.44	0.43 - 0.25	0.25 >
Sand	0.09 b[y]	1.12 c	8.54 bc	3.54 c	21.67 b	33.32 a	19.12 a	12.71 b
Perlite	0.06 b	3.84 a	26.71 a	9.65 a	19.58 c	14.23 d	5.83 c	17.97 a
Vermiculite	0.00 b	0.003 d	7.15 c	6.89 b	52.17 a	26.89 c	3.85 d	2.63 d
1:1:1 Peat/Pine bark/Sand	5.79 a	2.82 b	9.04 b	3.37 c	19.7 c	28.99 b	17.83 b	11.98 c

[z]Percent particle size distribution within each substrate (mm in diameter).

[y]Similar letters within columns were not significantly different at P = 0.05 using Duncan's Multiple Range Test.

Seed Germination of Seabeach Amaranth (*Amaranthus pumilus*) in Response to Temperature, Light, and Gibberellin A_3 Treatments

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Index Words: Sexual Propagation, Beach Restoration, Seed Dormancy, Amaranthaceae, Recovery Plans, Threatened Species, Dune Species

Significance to Industry: Physiological seed dormancy of seabeach amaranth [*Amaranthus pumilus* Raf. (Amaranthaceae)] can be removed by soaking the seeds for 24 hr at 21C (70F) in a solution of 1000 ppm of the potassium salt (K-salt) of gibberellin A_3 (K-GA_3). Treatment of seeds with K-GA_3 eliminates the need for lengthy (84 to 120 days) stratification (moist-prechilling) to achieve the same results. These findings will also reduce the time required to produce seedling transplants of this species.

Nature of Work: Seabeach amaranth is a summer annual native to the beaches and barrier islands of the Atlantic Coast. The plant once ranged from Massachusetts to South Carolina (6). However, by 1990 it no longer occurred in six of the nine states of its original range and the only populations were in New York, North Carolina, and South Carolina (6). Elimination of two-thirds of its historic range, and vulnerability of the plant to various threats, both natural and human, caused seabeach amaranth to be listed as “threatened” by the U.S. Fish and Wildlife Service in 1993 (4). As a result of the threatened status of the plant a recovery plan was developed by Weakley et al. (6).

One concern regarding loss of the species is that seabeach amaranth plays a role in the initial stages of the development of sand dunes by trapping and binding sand on the beach (5, 6). Ecologists also view the plant as an indicator to evaluate the vitality and vigor of a beach ecosystem. Thus, various state and federal agencies are very interested in restoring the species to areas where it once grew. In addition, beach restoration and sand renourishment projects have created a demand for seedling transplants of seabeach amaranth that are currently unavailable.

To establish seabeach amaranth in locations where it was once endemic and to meet the demand for transplants will require development of protocols for propagation and culture of the species. One approach may involve production of seedlings that can then be planted in suitable beach environments. Some research has been reported regarding sexual propagation, specifically seed germination. However, more research is needed. If such protocols are developed, they may provide opportunities for growers to produce and sell plants to federal, state, and private agencies for recovery efforts.

Seed germination studies of seabeach amaranth have determined that freshly harvested seeds are physiologically dormant which require a period of stratification (moist-prechilling) to break dormancy (1, 3). Stratification of 84 to 120 days is necessary to completely remove physiological dormancy followed by germination at high temperatures [e.g., 8/16 hr thermoperiod of 30/20 (86/68F)] with light (e.g., a daily 16 hr photoperiod) to achieve maximum germination (1,3). Thus, to maximize germination, seeds must first be stratified for 84 to 120 days which poses the question whether the need for lengthy stratification could be eliminated by treating the seeds with a growth regulator such as gibberellic acid (GA)? Physiological seed dormancy of many species has been removed by treating the seeds with various gibberellins most notably GA_3 or GA_{4+7} (2). Therefore, the following research was conducted to study the influence of temperature, light, and GA treatment on seed germination of seabeach amaranth.

Fruits of seabeach amaranth were collected from plants growing on Oak Island, NC, on September 15, 2003. Following drying and seed extraction, seeds were stored in the dark at 4C on November 18, 2003. On November 4, 2004 seeds were removed from storage and graded. The graded seeds were soaked for 24 hr in darkness in solutions of K-GA_3 at 0 (nontreated), 100, 500, or 1000 ppm at 21C (70F). The solutions were prepared by dissolving K-GA_3 in distilled water and the 0 ppm K-GA_3 treatment consisted of seeds soaked in distilled water. Following treatment, seeds were placed in covered 9-cm diameter glass petri dishes (50 seeds/dish) each containing two germination blotters moistened with tap water. The dishes were placed in black sateen cloth bags and were maintained overnight at 21C (70F). The following day, the dishes were completely randomized within two growth chambers. The chambers were maintained at 25C (77F) or an 8/16 hr thermoperiod of 30/20C (86/68F). Within each temperature regime seeds were subjected to daily photoperiods of 0 (total darkness) or 16 hr. Growth chambers were equipped with cool-white fluorescent lamps that provided an irradiance of 40 µmol/m^2/sec (3.2 klx) as measured outside the dishes at dish level. The constant darkness treatment was imposed by keeping the petri dishes in black cloth bags throughout the experiment, and all watering and germination counts for this treatment were performed in a dark room utilizing a green safelight.

For each temperature, all photoperiod and K-GA_3 treatments were replicated four times with a replication consisting of a petri dish containing 50 seeds. Germination counts were recorded every 3 days for 30 days and germinated seeds were removed from the dishes. A seed was considered germinated when radicle emergence was ≥ 1 mm (0.04 in).

Percent germination was calculated as a mean of four replications per treatment and data were subjected to analysis of variance procedures and regression analysis. The regression analysis focused on the influence of the K-GA_3 treatments. The F-test was also employed to determine the influence of temperature and light.

Results and Discussion: Regardless of photoperiod and temperature, germination of nontreated seeds (0 ppm K-GA_3) was negligible. Total percent (30-day) germination of nontreated seeds at both temperatures and photoperiods

was < 1%. Total germination percentages increased linearly as the concentration of K-GA_3 increased from 0 to 1000 ppm. Germination of K-GA_3 treated seeds was also affected by temperature. Germination at 25C (77F) increased linearly from 0 to 1000 ppm K-GA_3 with maximum germination of 52% whereas germination at 30/20C (86/68F) increased quadratically from 0 to 1000 ppm K-GA_3 with maximum germination of 71%. A germination temperature of 30/20C (86/68F) had a greater affect on germination than 25C (77F). Photoperiod also influenced germination. Averaging over both temperatures, germination at 0 hr for 100, 500, and 1000 ppm K-GA_3 was 7%, 48%, and 69%, respectively. At 16 hr, germination percentages were significantly lower for seeds treated with 500 and 1000 ppm K-GA_3 with 35% and 55% germination, respectively, whereas germination at 100 ppm K-GA_3 was similar (7%).

In summary, greatest seed germination of seabeach amaranth can be achieved by soaking the seeds for 24 hours in 1000 ppm K-GA_3 prior to exposing them to an 8/16-hr thermoperiod of 30/20C (86/68F) without light. Results are similar to those achieved by Blazich et al. (3) and Baskin and Baskin (1) without having to stratify seeds of seabeach amaranth for 84-120 days.

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Seed Germination of Seabeach Amaranth (*Amaranthus pumilus*) as Influenced by Stratification, Temperature, and Light

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Index Words: Sexual Propagation, Beach Restoration, Seed Dormancy, Amaranthaceae, Recovery Plans, Threatened Species, Dune Species

Significance to Industry: Results demonstrate that seed germination of seabeach amanthus [*Amaranthus pumilus* Raf. (Amaranthaceae)], a species federally listed as "threatened," is relatively easy to accomplish provided the seeds are first stratified (moist-prechilled) and germinated at an 8/16 hr thermoperiod of 30/20°C (86/68°F) with a daily 16-hr photoperiod. As durations of stratification increase in 30-day increments from 0 to 120 days, both the rate and total germination will increase. Following stratification, light is not necessary for germination but subjecting seeds daily to light will result in greater germination than seeds maintained in darkness. Seeds will not germinate at 25°C (77°F) with light despite prior stratification.

Nature of Work: Seabeach amaranth is a summer annual native to the beaches and barrier islands of the Atlantic Coast. Its range once included nine states from Massachusetts to South Carolina (6). By 1990, it no longer occurred in Massachusetts, Rhode Island, New Jersey, Delaware, Maryland, and Virginia. The only populations remaining were located in New York, North Carolina, and South Carolina (6). However,` in recent years it has reestablished itself naturally in some states where it once occurred such as Delaware and Maryland suggesting that seeds can remain viable for many years (5).

The species is extremely vulnerable to various threats such as beach stabilization and restoration, structures, particularly use of hardened structured like seawalls and rip rap,beach erosion and tidal inundation, intensive recreational uses of areas where it grows, and herbivory by insects and feral animals (4, 6). The aforementioned coupled with the fact that by 1990 it had been eliminated from two-thirds of its historic range (6) caused seabeach amaranth to be listed as "threatened" by the U.S. Fish and Wildlife Service in 1993 (3). As a result of the threatened status of the plant, a recovery plan was developed by Weakley et al. (6).

Seabeach amaranth plays a role in the initial stages of the development of sand dunes by trapping and binding sand on the beach (4, 6). Ecologists also view the plant as an indicator to evaluate the vitality and vigor of a beach ecosystem. Thus, various federal and state agencies are very interested in restoring the species to areas where it once grew. In addition, beach restoration and sand renourishment projects have created a demand for seedling transplants of seabeach amaranth that are currently unavailable.

Reestablishing seabeach amaranth in locations where it was once endemic and

meeting the demand for transplants will require development of protocols for propagation and culture of the species. One approach may involve production of seedlings that can then be planted in suitable beach environments. Little research, however, has been reported regarding sexual propagation and culture. If such protocols are developed, they may provide opportunities for growers to produce and sell plants to federal, state, and private agencies for recovery efforts.

Although limited research has been reported for seed germination of seabeach amaranth, one report by Baskin and Baskin (2) provides some quantitative information regarding seed germination requirements. They reported that freshly harvested seeds of seabeach amaranth have physiological dormancy that can be removed completely by stratification at 5°C (4°F) for 12 weeks. They also reported that light and high temperatures [12/12 hr thermoperiod of 30/15°C (86/59°F)] are required for germination as opposed to lack of germination at 12/12 hr thermoperiods of 15/6°C (59/43°F) or 20/10°C (68/50°F). Their research, however, was based on small sample sizes for various treatments (three replications of 20 seeds each). Therefore, the following research was conducted to further investigate the influence of stratification, temperature, and light on seed germination of seabeach amaranth.

Freshly harvested seeds of seabeach amaranth were stratified for 0, 30, 60, 90, or 120 days. Following stratification, seeds were germinated at 25°C (77°F) or an 8/16 hr thermoperiod of 30/20°C (86/68°F) with daily photoperiods at each temperature of 0 (total darkness) or 16 hr. Germination was recorded every 3 days for 30 days.

Results and Discussion: Regardless of photoperiod or duration of stratification, germination at 25°C (77°F) was negligible. After 120 days of stratification, total (30-day) germination at 25°C (77°F) with photoperiods of 0 or 16 hr was 4% and 3%, respectively, whereas considerably germination occurred at 30/20°C (86/68°F). Germination increased linearly with increasing durations of stratification for both photoperiods when seeds were germinated at 30/20°C (86/68°F). In addition, germination was greater when seeds were exposed to light. After stratification of 30 days and germination in the dark at 30/20°C (86/68°F), germination of 20% was observed by day 12 and increased to 27% by day 30, whereas for seeds exposed to a 16 hr photoperiod, 16% germination was noted by day 12 increasing to 38% by day 30. Following stratification of 120 days and germination in the dark at 30/20°C (86/68°F), germination of 49% was observed by day 12 increasing to 50% by day 30, whereas for seeds exposed to light, 82% germination was realized by day 12 increasing to 85% by day 30.

In summary, seed germination of seabeach amaranth can be achieved provided the seeds are first stratified for 90 to 120 days and then germinated at an 8/16-hr thermoperiod of 30/20°C (86/68°F) with a daily 16-hr photoperiod (1). Results of this investigation provide new information regarding seed germination requirements of this species that should aid in the production of seedling transplants.

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Stabilizing Dogwood Seed Supply through Proper Storage of Excess Seed

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Index Words: *Cornus florida*, Seed Storage, Flowering Dogwood

Significance to Industry: Dogwood producers frequently face shortages of flowering dogwood seed. The results of this study indicate that dogwood seed can be successfully stored if they are dried to 6 to 14% moisture content, placed in air-tight containers and stored in a freezer (-20ºC; 4ºF). Seed stored in this manner maintained good viability after 4 years in storage. Use of this seed storage procedure will help stabilize dogwood seed supply.

Nature of Work: A reliable supply of seed is a necessity for the production of flowering dogwood; however, weather and disease problems often cause dogwood seed shortages (2, 5). The objective of this study was to develop a reliable method of storing flowering dogwood seed that will allow producers to overcome year-to-year fluctuations in seed production. Because seed moisture level and storage temperature are known to greatly influence the viability of stored seed (3), these two factors were examined.

Flowering dogwood seeds were collected in Fall 2000. Three 50-seed samples were used for determining initial seed moisture content (MC). Seed were weighed and then dried in a 105ºC (221 ºF) oven for 24 hours. Each sample of seed was weighed again and MC determined using the following formula: (fresh weight – dry weight)/fresh weight = MC. Mean MC of the three samples was used as the initial MC for the seed collection. An additional three 50-seed samples were stratified after collection and sown in Spring 2001. Percent germination of these control samples was determined 4 weeks after planting.

The remaining seed were divided into three equal batches and each batch was weighed. A target seed weight was calculated for each batch using the following formula: $x – ax = b(1-c)$, where x = target weight, a = desired MC, b = initial weight, and c = initial MC. Seed were allowed to dry in the laboratory until they had reached target weights corresponding to 6, 10 and 14% MC. Seed were then placed in 20-ml glass bottles fitted with a screw-top lid for storage, with each bottle containing 50 seed. For each MC, seed were stored at 22 ºC (72 ºF), 5 ºC (41 ºF), and -20 ºC (-4 ºF). A refrigerator/freezer was used for storing the 5 and -20 ºC samples, while the 22 ºC seed were stored in the laboratory. All seed were stored in the dark. Seed were stored for 1, 2, 3 and 4 years. Three samples were stored for each MC x storage temperature x length of storage combination. After seed were removed from storage they were stratified for 15 weeks and sown in the greenhouse. Percent germination was determined after 4 weeks.

Data for different lengths of storage were analyzed separately. Each experiment consisted of nine treatments, representing all seed MC x storage temperature combinations. When germination of a treatment dropped to 0%, that treatment was removed from the following year's statistical analysis. No transformation of germination percentages was required since the equal variance assumption was not violated. Where data were significant, Fishers' LSD procedure was used to separate means.

Results and Discussion: Initial MC of the seeds was 20.3%. Seed that were stratified immediately after collection had a mean germination rate of 80.0%.

Seed viability dropped rapidly if seed were stored at room temperature (22 ºC), regardless of seed moisture content (Table 1). Seed MC was critical for storage at 5 ºC, with seeds dried to 14% MC failing to germinate after 3 years and seeds dried to 6% MC having very poor germination after 4 years of storage.

The best results were obtained when seed was stored at -20 ºC. No differences were observed among seed dried to different MCs when storage was at -20 ºC for 2 or 3 years. After 4 years of storage at -20 ºC, seed dried to 6% MC germinated better than those dried to higher MCs. All three MCs, however, resulted in excellent germination after storage at -20 ºC for 4 years. Extended periods of storage at -20 ºC might result in greater differences in germination rates among the three MCs.

A previous study indicated that air-dried flowering dogwood seed will maintain good viability for 4 to 8 years if the seed are stored at 3-5 ºC (1, 4). The results of this study indicate that dogwood seed retain better viability if stored at -20 ºC than at 5 ºC. It is recommended that seed be dried to 6 to 14% MC prior to storage and stored in a -20 ºC freezer in air-tight containers. Use of these guidelines should allow growers to store flowering dogwood seed for at least 4 years without a significant loss in viability.

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Table 1. Effect of storage temperature and seed moisture on germination of flowering dogwood seed stored for up to 4 years[zy].

		% germination			
Storage temperature	Seed moisture	1 year storage	2 years storage	3 years storage	4 years storage
22 ºC (72 ºF)	6%	2.7 e	0 c	- - -	- - -
	10%	0 e	- - -	- - -	- - -
	14%	0 e	- - -	- - -	- - -
5 ºC (41 ºF)	6%	34.0 b	39.0 b	10.7 c	1.4 d
	10%	21.3 c	77.3 a	55.3 b	71.8 c
	14%	12.7 d	40.7 b	0 d	- - -
-20 ºC (-4 ºF)	6%	38.7 b	85.3 a	73.3 a	89.3 a
	10%	35.3 b	86.0 a	67.8 a	80.0 b
	14%	50.0 a	80.7 a	69.8 a	82.0 b

[z]Values within a column followed by the same letter do not differ significantly according to Fisher's LSD test (P≤0.05). n=3.

[y]Germination of control (seed stratified after collection in Fall 2000 and sown in Spring 2001) = 80% .

Stratification Improves Germination of *Camellia oleifera*

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Index words: *Camellia oleifera*, germination, stratification, tea-oil camellia

Significance to Industry: Open pollinated seed from *C. oleifera* 'Lu Shan Snow' germinated in high percentages (>96%). Cold stratification for 45 to 60 days appears ideal for this species, though seed germinates well with as little as 15 days of stratification.

Nature of Work: *Camellia oleifera*, tea-oil camellia is grown extensively in southern China for the production of a high quality edible oil (2,4). The physical and chemical characteristics of tea-oil are similar to those of olive oil. A research project has been initiated by the author to look at the feasibility of growing tea-oil camellia in the southeastern United States (2).

Little information exists regarding the requirements for germination of tea-oil seed. One study suggests that seed should be stored at a low temperature (0.0 to 2.0C) for 15 to 30 days (3). Germination was accelerated by keeping the seeds between 20.0C to 25.0C. Plants were suitable for transplanting 25 to 35 days after sowing. Bill Ackerman suggests that tea-oil camellias need a minimum of five weeks cold, moist stratification to ensure decent germination (personal communication). For camellias in general, Tourje (5) suggests that germination occurs within 10 to 30 days with an ideal temperature of 18.3C to 21.1C. Non-stratified seed of *Camellia oleifera* germinates slowly over a longer period compared to stratified seed (J. Ruter, personal observation).

Seed from open pollinated *Camellia oleifera* 'Lu Shan Snow' were collected, sorted, and placed in zip-lock plastic bags with moist pinebark for cold stratification periods of 15, 30, 45, and 60 days at 4.4C. Only seeds which sank during a float test were used. After the stratification period was complete, seed were planted in 60 cell trays (cell size - 4.5 cm wide by 10.0 cm deep) with a substrate of 8:1 pinebark and sand. Seeds were covered to a depth of ~1.0 cm. Trays were randomly placed in a growth chamber with set temperatures (12 hr) of 24C day and 18C night. Light intensity measured at the top of the germination trays was ~900 $\mu mol/m^2/s^1$ during the 12 hour day period. Germination was recorded daily for 60 days as the visual emergence of the shoot from the substrate.

Treatments consisted of four stratification periods with six replications consisting of 10 seeds per replication, for a total of 60 seeds per treatment. Replications were randomly assigned within germination trays. Data was analyzed using the non-linear regression function of SigmaPlot.

Results and Discussion: Days to germination decreased curvilinearly as cold stratification period increased from 15 to 60 days [(days to germination = 60.1 – 0.89 (days of cold stratification) + 0.0084 (days of cold stratification)2), r2 = 0.99]. Seed stratified for 15 days germinated in 49 days compared to 31 days for seed receiving 45 and 60 days of cold stratification. Seed germination exceeded 96% for all four treatments.

Seed from *C. oleifera* 'Lu Shan Snow' germinated in high percentages in this study. Cold stratification for 45 to 60 days appears ideal for this species. Differences in days to germination compared with other studies may have been due to different methods (3,5) or determining that germination had occurred when radicle extension was visible (5). Germination officially ends when the shoot protrudes from the substrate (1) as was thus the chosen method for this study.

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Rooting of Cuttings of Yellow-flowered Cultivars of *Magnolia*

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Index Words: propagation, IBA

Significance to Industry: Advances in propagation can increase the production of high-value, high-demand crops such as yellow-flowered cultivars of *Magnolia*. Experimental results showed rooting of 'Ivory Chalice' and 'Yellow Lantern' cuttings is maximized by collection of terminal cuttings within 5 to 11 weeks after budbreak and treatment with 16,000 or 30,000 ppm IBA in talc. Research still is needed to identify optimal IBA concentration and collection date for rooting cuttings of 'Butterflies,' 'Golden Sun,' 'Hot Flash,' and 'Maxine Merrill.'

Nature of Work: Yellow-flowered selections of *Magnolia* are becoming popular among consumers and are in demand. A large number of these selections have been derived from *Magnolia acuminata* L. and *Magnolia denudata* Desr. Additional selections also have incorporated *Magnolia kobus* DC., *Magnolia acuminata* L. var. *subcordata* (Spach) Dandy and *Magnolia _soulangiana* Soul.-Bod. Cuttings of *M. acuminata* and *M. denudata* do not root easily and many of their yellow-flowered progeny also share this trait. Most growers prefer to produce these plants from cuttings rather than by budding, grafting, or tissue culture.

Stage of physiological development or time of year cuttings are harvested influences root development on cuttings of many woody species, and cuttings of *Magnolia* generally have a narrow 'window of rootability' which varies among cultivars (1). Leafy softwood cuttings have been used for various deciduous *Magnolia* species and cultivars, but experimental methods and results with cuttings of yellow-flowered cultivars are not available. Auxin improves rooting, and ranges of 5,000 ppm to 20,000 ppm Indole-3-butyric acid (IBA) in talc have been used to propagate cuttings of *Magnolia*, but variation among taxa is known (1, 2, 3). Among parent taxa, there are no reports of rooting cuttings of *M. acuminata*. However for another parent, *M. denudata*, 85 to 100% of cuttings collected in early June in the northeastern U.S. developed roots after wounding and application of 3,000 to 8,000 ppm IBA (1). *M. xsoulangiana* cuttings produce roots after treatment with 8,000 ppm to 16,000 ppm IBA (1). Up to 80% of cuttings of *Magnolia kobus* DC. var. *borealis* Sarg. rooted after wounding and treatment with 20,000 ppm IBA in talc (1). Non-statistical reports describe propagation of a few cultivars and parent taxa of yellow-flowered selections (4). These reports are encouraging but do not fully describe influence of IBA, physiological stage, dates of collection and environmental conditions during the experiments. Because physiological developmental stage and IBA application influence rooting of *Magnolia* cuttings, we initiated a study to determine the effect

of cultivar, collection date, and IBA on rooting of cuttings from six yellow-flowered, deciduous cultivars of *Magnolia*.

Cuttings of six cultivars ('Butterflies,' 'Golden Sun,' 'Hot Flash,' 'Ivory Chalice,' 'Maxine Merrill,' and 'Yellow Lantern') were collected on four dates and given four IBA treatments in a factorial arrangement in a completely randomized design. Cuttings were collected from mature, field-planted trees at 5, 7, 9, or 11 weeks after vegetative budbreak (Table 1), corresponding to softwood through semi-hardwood stages of growth. Thirty-two terminal cuttings with 2 to 4 nodes were collected from each cultivar on each date. Cuttings were wounded (by scraping the basal 1 to 2 cm of stem), stripped of all but two fully expanded leaves, and the distal halves of remaining leaves were removed. Eight cuttings of each cultivar were assigned randomly to four hormone treatments: 1) 0 ppm; 2) 8,000 ppm; 3) 16,000 ppm; or 4) 30,000 ppm IBA. Hormex talc formulations #8, #16 and #30 (Brooker Chemical , Chatsworth, CA) were used to apply the IBA to the base of cuttings for treatments 2 through 4, respectively. The basal 5 cm of each cutting was inserted into a plastic container (5.7 cm by 5.7 cm at the top and 7.5 cm deep; SR 225, The Lerio Corp., Mobile, AL) holding a 1:1 mixture (by volume) of milled sphagnum peat and coarse perlite.

Treated cuttings were placed under a programmable intermittent mist system (Gemini 6A, Phytotronics, Inc., Earth City, MO) applying 6 s of mist every 6 min during daylight hours. Mist was emitted at 35 to 45 psi via brass deflector-type nozzles (Reed S. Kofford Co., Pleasant Hill, CA) with 1 mm orifice.

Rooting and visual rating of the root system were recorded 10 weeks after each group of cuttings was collected. The visual rating was based on a scale of 0 to 5, where 0 = neither callus nor roots were observed; 1 = presence of callus but roots absent; 2 = one or two roots ≥ 0.5 cm in length; 3 = few, relatively short roots; 4 = several, developed roots; and 5 = many, well-developed roots. Data were subjected to analysis of variance (ANOVA) by using the GLM procedure in SAS/STAT (version 8.02, SAS Institute, Inc., Cary, NC).

Results and Discussion: Among 128 cuttings of each cultivar, the number of cuttings forming at least one root was: 2 ('Butterflies'), 18 ('Golden Sun'), 17 ('Hot Flash'), 59 ('Ivory Chalice'), 17 ('Maxine Merrill') and 69 ('Yellow Lantern'). Visual rating of roots was influenced by two-way interactions between cultivar and collection date and between cultivar and IBA but a three-way interaction among cultivar, collection date and IBA did not occur. Mean rating of 2.1 or higher was received by cuttings of 'Yellow Lantern' collected 5 to 11 weeks after budbreak (Table 2). Cuttings of 'Ivory Chalice' collected 7 and 9 weeks after budbreak also received a mean rating of 2.1 or higher. Mean rating was ≤ 1.6 among cuttings of 'Butterflies', 'Golden Sun', 'Hot Flash', and 'Maxine Merrill' regardless of collection date (Table 2).

Application of 16,000 or 30,000 ppm IBA improved visual rating among cuttings of 'Ivory Chalice' and 'Yellow Lantern' (Table 3). Although callus was observed, 'Butterflies,' 'Golden Sun,' 'Hot Flash,' and 'Maxine Merrill' were not influenced by IBA and visual rating was ≤ 1.4 (Table 3).

High rooting percentages among 'Ivory Chalice' and 'Yellow Lantern' cuttings indicate they are promising choices for growers interested in cutting propagation of yellow-flowered cultivars of *Magnolia*. Their rooting is maximized by collection of terminal cuttings within 5 to 11 weeks after budbreak and treatment with 16,000 or 30,000 ppm IBA in talc.

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Table 1. Date of budbreak in 2004 and dates on which cuttings were collected among six yellow-flowered cultivars of *Magnolia*.

Cultivar	Date of budbreak	Collection date (weeks after budbreak)
'Butterflies' (USPP #7456)	March 15	April 20 (5) May 4 (7) May 18 (9) June 1 (11)
'Golden Sun'	March 15	April 20 (5) May 4 (7) May 18 (9) June 1 (11)
'Hot Flash'	March 15	April 20 (5) May 4 (7) May 18 (9) June 1 (11)
'Ivory Chalice'	March 8	April 13 (5) April 27 (7) May 11 (9) May 25 (11)
'Maxine Merrill'	March 15	April 20 (5) May 4 (7) May 18 (9) June 1 (11)
'Yellow Lantern'	March 8	April 13 (5) April 27 (7) May 11 (9) May 25 (11)

Table 2. Collection date (5, 7, 9 or 11 weeks after budbreak) influenced visual rating of cuttings from six yellow-flowered *Magnolia* cultivars when evaluated at ten weeks after collection date and rooting treatment. Because a three-way interaction was not observed between cultivar, weeks after budbreak and IBA, data for the collection dates were pooled over the four IBA levels. Means (n=32) with the same letter are similar as determined by Fisher's LSD (_=0.05).

Cultivar	Visual rating (0 – 5)[z]			
	Collection date (weeks after budbreak)			
	5	7	9	11
'Butterflies'	1.0 bc	1.0 bc	0.7 bc	0.9 bc
'Golden Sun'	1.5 b	1.3 b	1.0 bc	1.0 bc
'Hot Flash'	0.8 bc	0.8 bc	0.8 bc	0.9 bc
'Ivory Chalice'	1.4 b	2.5 a	2.1 a	1.2 b
'Maxine Merrill'	1.6 ab	1.0 bc	1.1 b	1.0 bc
'Yellow Lantern'	2.5 a	2.3a	2.3 a	2.1 a

[z]0 = neither callus nor roots were observed; 1 = presence of callus but roots absent; 2 = one or two roots ≥ 0.5 cm in length; 3 = few, relatively short roots; 4 = several, developed roots; and 5 = many, well-developed roots.

Table 3. IBA (0; 8,000; 16,000; and 30,000 ppm) influenced visual rating of cuttings from six yellow-flowered *Magnolia* cultivars when evaluated at ten weeks after collection date and rooting treatment. Because a three-way interaction was not observed between cultivar, weeks after budbreak and IBA, data for IBA were pooled over the four collection dates (5, 7, 9 or 11 weeks after budbreak). Means (n=32) with the same letter are similar as determined by Fisher's LSD (_=0.05).

Cultivar	Visual rating (0 – 5)[z]			
	IBA (ppm)			
	0	8,000	16,000	30,000
'Butterflies'	1.0 cd	0.9 cd	0.9 cd	0.9 cd
'Golden Sun'	1.0 cd	1.1 c	1.3 c	1.4 c
'Hot Flash'	0.8 cd	0.7 cd	0.9 cd	1.0 cd
'Ivory Chalice'	1.1 c	1.6 c	2.2 b	2.4 b
'Maxine Merrill'	0.9 cd	1.2 c	1.1 c	1.4 c
'Yellow Lantern'	1.4 c	1.9 bc	2.6 b	3.2 a

[z]0 = neither callus nor roots were observed; 1 = presence of callus but roots absent; 2 = one or two roots ≥ 0.5 cm in length; 3 = few, relatively short roots; 4 = several, developed roots; and 5 = many, well-developed roots.

Seed Germination Characteristics of Beach Vitex: An Invasive Coastal Plant

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Index Words: *Vitex rotundifolia*, seed viability, landscape plant

Significance to Industry: It is important for the industry to help solve problems with introduced landscape plants that become invasive. Vitex rotundiflora (beach Vitex) was introduced into the Carolinas in the mid -1980's from Korea as a salt-tolerant woody ground cover that grows well on dunes and has attractive flowers and foliage. It was planted in coastal areas of the Carolinas and has become invasive on primary and secondary dunes. The extensive growth of beach Vitex excludes native beach grasses and causes nesting problems to sea turtles. Beach Vitex spreads by long runners and also produces many seeds. This research indicates that beach Vitex seed is viable (1.2 viable seeds/capsule) and germinates better after receiving a cold stratification period of 5 or 10º C for 8 weeks or longer. However, 40% germination occurred at 15º C indicating potential movement to new areas and establishment of invasive populations along the coast.

Nature of the Work: Beach Vitex (*Vitex rotundifolia*) was introduced to the Carolinas in the 1980s as a landscape plant from Korea. This deciduous, low-growing vine or ground cover has beautiful foliage and attractive flowers and will thrive in the harsh beach environment even on the primary dune. It produces many seeds but the main method of propagation on the beach is through long runner branches that root very easily. It has now been planted in the coastal regions of North and South Carolina and is dominating the primary dune areas and excluding native species. (Gresham and Neal. 2004)

Sea turtle enthusiasts believe that it prevents egg-laying activities due to its thick growth, which does not allow turtles to navigate to an acceptable egg laying location. Preliminary research indicates that it does not trap as much sand as Southern sea-oats and may increase the hydrophobicity of sand. Beach Vitex may produce as many as 6,000 viable seed per meter (Gresham and Neal, 2004). It spreads very rapidly. In one location near Litchfield Beach, South Carolina, nine (one gallon) plants covered1700 sq. ft of primary dune in seven years.

Since this plant is problematic on the coast and is primarily limited to parts of the Carolina Coast, a task force involving multiple agencies and volunteers was created to detect and eliminate it from the South Carolina coast before it spreads further and develops into a significant invasive pest problem. There is very little known about the seed germination characteristics of beach Vitex. It produces many seeds but seedlings do not appear to be the primary means of spread. Viable seed, seed weight, and germination characteristics were investigated for beach Vitex from 2003 and 2004 seed lots.

Two lots of beach Vitex capsules were hand collected in December 2003 and 2004 near Pawley's Island, South Carolina. They were air-dried and stored in plastic bags at room temperature (25º C) for the remainder of the time. Four lots of 100 capsules from each year were measured with a digital caliper and weighed. Capsules were dissected in half and placed in 25 mls of 1% tetrazolium chloride in the dark over night at 20º C (ISTA, 1985). Seed number and viability were determined. Each capsule could contain four seeds. Seeds were counted and those that were stained pink were considered viable.

Preliminary studies indicated that a cold stratification period was required for germination and that eight weeks at 5º C was effective to obtain germination. Additional temperatures were evaluated to determine the potential for natural germination in South Carolina coastal areas. Capsules from the 2004 seed lot were soaked for two days in water at room temperature and then placed in flats using germination mix. The seeded flats were moistened and placed in the cooler at 5, 10, or 15º C for 12 weeks. The length of stratification period was also evaluated. Seeded flats were held in a cooler at 5º C for 4, 8, and 12 weeks. After the cold treatments, flats were placed in greenhouse mist chamber at 30º C for 4 weeks and emerged seedlings were counted. Seeded flats were replicated 8 times for a total of 60 viable seed. Analysis of variance was determined and means separated using LSD at P = 0.05.

Results and Discussion: There were no differences between 2003 and 2004 seed lots for capsule diameter, capsule weight, seed number per capsule, and viable seed per capsule (Table 1). It is interesting to note that our results show only an average of 1.25 viable seed per capsule out of a possibility of 4 seeds per capsule. This corresponds closely with visual observations in the germination studies where most capsules had one seedling emerging from each capsule.

Germination occurred at each length of stratification at 5º C. The lowest percent germination was observed at the shortest stratification period of 4 weeks. Germination was higher for the 5 and 10º C compared to 15º C. Some germination at 15º C indicates the potential for seedlings to emerge on the beach and become established. Higher temperatures will be evaluated to determine possible emergence and the increased risk of establishing new plantings of beach Vitex from seed. In each of the studies, unstratified seed were included as controls and no germination was observed.

These results indicate the beach Vitex produces viable seed that germinates best with a cold stratification period as short as 4 weeks. Best germination occurred after stratification of 5 and 10º C for 8 weeks or longer. Since germination did occur at 15 C, there is potential for beach Vitex seedling emergence and establishment in the coastal area of South Carolina.

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Table 1. Capsule and seed characteristics of 2003 and 2004 beach Vitex seed lots.

Seed Characteristics	2003 Lot	2004 Lot
Capsule diameter (mm)	6.38	6.53
Capsule wt. (mg)	48	50
Seed # /Capsule	1.3	1.3
Viable seed/capsule	1.2	1.3

There were no statistical differences detected between years at $P = 0.05$ according to ANOVA.

Table 2. Beach Vitex seed germination by weeks of stratification at 5 C (2004 seed).

Stratification	% Germination	# Seed
0 weeks	0 a	0 a
4 weeks	40 b	24 b
8 weeks	60 c	36 c
12 weeks	70.6 c	42 c

Means followed by the same letter are not different according to LSD at $P = 0.05$.

Table 3. Seed germination after 5, 10, and 15 C stratification temperatures for 12 weeks (2004 lot)

Temperature C	% Germination	Number
5	73 b	44 b
10	63 b	38 b
15	23 a	14 a

Means followed by the same letter are not different according to LSD at $P = 0.05$.