Propagation

Cecil Pounders
Section Editor and Moderator

Vegetative propagation of *Campsis grandiflora* 'Morning Calm' Trumpet Vine by Root Cuttings

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Index Words: J.C. Raulston Arboretum, plant production, non-native plants.

Significance to Industry: Nurserymen choosing to incorporate *Campsis* 'Morning Calm' trumpet vine into production can propagate the plant utilizing root cuttings. Two inch long sections ranging in diameters from 0.25 in to 0.57 in (6.4 mm to 14.6 mm) rooted successfully and produced vigorous plants. Some plants produced flowers on the new growth after propagation.

Nature of Work: *Campsis grandiflora* 'Morning Calm' Chinese trumpet vine grows 4.6 m to 7.6 m (15 to 25 ft) tall and has a 1.8 m to 2.7 m (6 to 9 ft) spread. The plant has 6 to 12 attractive, peach colored flowers with yellow throats that are borne on pendulous panicles. It is cold hardy in USDA zones 7 through 9 (1). 'Morning Calm' trumpet vine is a cultivar brought from Korea in September 1985 by the late J. C. Raulston who later named the plant in 1994. The name 'Morning Calm' was selected to honor Korea, the country of origin known as "the land of morning calm" (3).

Success is reported in rooting softwood stem cuttings of *Campsis sp.* (2), but variable success is reported when trying to root *Campsis* 'Morning Calm' from stem cuttings (3). Root cuttings have been a successful method of propagating various plants and nurseries continue to rely on this method of propagation. Because shoots often sprout from roots under and around *Campsis sp*. harvesting root cuttings might be a viable option for producing plants. Therefore, the objective of this study was to determine if the diameter of root cuttings affected success producing *Campsis* 'Morning Calm'.

Methods : Roots were removed from *Campsis* 'Morning Calm' growing on a trellis located at the Mountain Horticultural Crops Research and Extension Center in February 2007. Roots were cut into 5.1 cm (2 in) long pieces. The distal end of the root piece was cut at a 45 degree angle and root pieces were graded by caliper. Three grades were 6.4 mm (0.25 in), 8.0 mm (0.31 in), and 14.6 mm (0.57 in) in diameter. Root cuttings were inserted into a substrate of 2 peat : 3 perlite, which filled a 39.4 cm x 39.4 cm x 12.7 cm (15 in x 15 in x 5 in) plastic flat (Anderson Tool and Die, Co. Portland, Ore.). Moist peat moss was used to cover the root cuttings to a depth of 0.25 in (6.4 mm). Flats were placed

on a non-misted bench in a propagation greenhouse. Day temperatures were maintained at 22.2 C (72 F) and night temperatures at 14.4 C (58 F). Flats were hand-watered as needed and percent success, number of new roots, root length and stem length was recorded on May 1, 2007. Percent success was recorded as the appearance of new root and shoot growth.

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Results and Discussion: Root caliper of root cuttings did not affect percent success, number of roots, root length, or shoot caliper. Overall success was 84.9%. Each successful plantlet produced 3.5 roots with an average root length of 6.6 cm (2.6 in). Shoot length was affected by root caliper. Shoot length was 4.4 cm (1.75 in) on root cuttings with a caliper of 14.6 mm (0.57 in), whereas shoot length was 2.2 cm (0.86 in) on root cuttings with a caliper of 6.4 mm (0.25 in). Shoot length was 2.7 cm (1.1 in) on the intermediately sized root cuttings. The shoot caliper of all shoots was 1.8 mm (0.07 in), regardless of the root caliper or shoot length.

All three sizes of root calipers collected from *Campsis* 'Morning Calm' produced a rooted plantlet large enough for production. Lengths of root cuttings shorter or longer than 5.1 cm (2 in) have not been tested, nor have different substrates and collection times during the year. Potentially, root cuttings could be started directly in containers to reduce labor and transplanting. Root cuttings are a viable option to vegetatively propagate 'Morning Calm' trumpet vine without mist application or advanced technology.

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Ornamental Teaoil Camellia Cultivars and Their Hypocotyl Graft Propagation

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Index Words: *Camellia oleifera*, cultivar, hypocotyl grafting, teaoil, ornamental, propagation.

Significance to Industry: Many popular ornamental plants can be derived from other crop commodities, such as ornamental peaches derived from fruit peach breeding. Teaoil camellia (*Camellia oleifera* Abel) has been used for thousands of years in China for edible oil production and has potential for ornamental production. Together with many other outstanding ornamental camellias, it plays an important role in the field of ornamental horticulture, especially breeding for cold hardy camellia cultivars. The US National arboretum has released more than a dozen cold hardy ornamental camellia cultivars with *C. oleifera* in their parentage. In China, for the past 60 years, much breeding work had been conducted on teaoil to improve oil quality and yield which has also resulted in several cultivars that have very attractive ornamental features. This paper is to introduce new teaoil cultivars to our nursery industry with an associated hypocotyl grafting technique. The hypocotyl grafting technique may also have potential to improve propagation of other ornamental plants with similar seed characteristics.

Nature of Work: Teaoil camellia (*Camellia oleifera*) is native to China from 18°21' to 34°34' North latitude, and grows in acidic soils where January mean temperatures do not drop below 35.6F. It is a promising crop (edible oil and ornamentals) for US agricultural industries (Shanan and Ying, 1982). The U.S. National Arboretum (2007) used *C. oleifera* 'Lu Shan Snow' as a parent and produced more than a dozen ornamental camellia hybrids with improved cold tolerance (Ackerman, 1981; Ackerman and Egolf, 1981, 1991, 1992). During the long history of this crop's cultivation, many elite cultivars have been introduced. Work in Hunan Province alone has resulted in selection of 88 elite cultivars in recent years, and more than 100 superior clones are cultivated by the Hunan Oil Tea Growers (Chen and Wang, 2001). In the past three years, we have focused

our selection on its ornamental potential. Based on habit and fruit features, four cultivars from Hunan and Jiangxi plantations have been evaluated for use as ornamentals. Hypocotyl grafting, a popular camellia propagation technique in China, is also discussed.

Selected Cultivars: *Camellia oleifera* has been cultivated in China for thousands of years for its edible oil production. Although it is not common in ornamental gardens, the plant is one of the most cold hardy camellia species and usually hybridized with its close relatives, such as *C. sasanqua*. It is an evergreen shrub or small tress, which can reach 6 meters tall. Flowers are white to pink, usually bloom from October to January when few other plants are flowering. Fruits mature from October to December (Dirr, 1998). Flowering and fruiting overlap, which provides a very attractive winter display. Based on habit and fruit characteristics, four cultivars with better ornamental potential have been selected for introduction. These plants should grow well in USDA cold hardiness zones 6-9.

'Changlin': A selection, from open pollination in the Subtropical Forestry Research Center orchard, is a fastigiated shrub, to 1.5 × 3 m^2 in five years. The unique features are red fruits that have four distinguished "buttons" at apex. New growth flushes usually change from red to green.

'Hongqiu': Selected by Hunan Academy of Forestry for high yield of seeds. This plant has open canopy and is 2 × 2.5 m^2 in four years. It is different from other plants by its maroon fruits and long narrow leaves.

'Hunan Dome': Bred by Hunan Academy of Forestry from local high yield clones. The plant is rounded and the canopy is 2 × 2 m^2 from the ground. The distinguishing features are rounded habit with green new growth and capsules.

'Ninglin': This cultivar was selected from seedling populations in Jiangxi Province for better quality of edible oil production. Ornamental features include its columnar habit, usually one meter wide and three meters tall. The fruits are red with pubescences and the new growth is red.

Propagation: For clonal production, these cultivars can be propagated from cuttings (Ackerman, 2002). Studies on cutting propagation have focus on the hormonal responses and the season to collect cuttings. These plants can also be budded or grafted to other cultivars or common seedlings in early spring. In our production, hypocotyl grafting has been employed.

Hypocotyl Grafting: The uniqueness of hypocotyl grafting is to graft a mature hardwood scion (one-year-old node with a leaf) to a just-differentiated tender young stock (underground hypocotyl). The procedure can be described in six steps:

1. Seed germination (as rootstocks): Seeds are collected in October to December, and then stored in moist sand over winter with mean temperature around 5º. In late March or April (the mean temperature usually is higher than 15ºC), seeds germinate in the sand. Before hypocotyl reaches the sand surface (about 5-7 cm in length), the whole plant is removed from the sand bed.
2. Preparation of Rootstock: Both hypocotyl and radicle are trimmed to 4-7 cm long and split hypocotyl for grafting.
3. Preparation of Scion: One year old branches are collected from mature plants (usually 3-6 years old), then cut both sides of stem and make an one-node scion with an attached leaf.
4. Grafting: Gently insert the scion into the split rootstock.
5. Grafting Wrap: Prepare heavy aluminum foil strips (2-3 cm long and 0.3-0.6 cm wide), then gently wrapped around grafted unions;
6. Transplanting: Grafted seedlings are immediately transplanted into raised field beds, and then covered with plastic (plastic tunnel). The plastic tunnels should then be covered with shade cloth (30-60%) about one meter above the tunnel.

Hypocotyl grafting is a popular clonal propagation technique for teaoil camellia in China. The grafted unions completely join within 40 days. The survival rates are generally more than 95% in the past three years.

Availability: Hypocotyl grafted plants are available from Silviculture Programs at Central South University of Forestry and Technology or Economic Plant Programs of Hunan Academy of Forestry in Changsha, Hunan 41004, China. Cutting propagated plants will be available after year 2008 from the above institutions.

Acknowledgement: Financial support from Qinglong High Technology Co. (Yichun, Jiangxi, China), University of Maine (Orono, Maine, USA), and Central South University of Forestry and Technology (Changsha, Hunan, China) is appreciated.

Supporting Online Material:

http://www.umaine.edu/maineplants/PubDZ/SNA07Teaoil.pdf

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Figure 1. *Camellia oleifera* 'Changlin'

Figure 2. *Camellia oleifera* 'Hongqiu'

Figure 3. *Camellia oleifera* ‘Hunan Dome’

Figure 4. *Camellia oleifera* ‘Ninglin’

Micropropagation of an Ornamental Prickly-pear Cactus (*Opuntia lanigera* Salm-Dyck) and Effects of GA_3 on Plant Growth after Transplantation.

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Index Words*: In vitro* propagation, prickly-pear cactus, nopal, plant growth regulators.

Significance to Industry: The nursery industry in general and the fast growing prickly pear industry in particular, may be benefited from the reliable and efficient micropropagation protocol described here. The conditions to grow the plants through fertigation plus the application of GA_3 that induces changes in the appearance of the plant (phenotype) may be used in commercial exploitations to regenerate 12,500 plantlets on average after 10 months of culture. Propagules produce healthy plants with better ornamental characteristics and higher commercial value that can be used in landscaping for public, private and residential properties.

Nature of Work: We established the conditions to micropropagate the ornamental prickly pear cactus *Opuntia lanigera* Salm-Dick through axillary shoot development from isolated immature areoles and evaluated the effects of gibberellic acid (GA_3) on plant growth and development after transplantation and acclimatization. To start the process of micropropagation, healthy young cladodes about 5-6 cm in length were selected and excised from donor plants that were previously cultured under glasshouse conditions. After a cleaning process, pairs of young areoles were dissected and used as initial explants.

For the shoot proliferation stage different explant orientation (vertical and horizontal), type of cytokinin (BA [N6-Benzyladenine], K [6-furfurylaminopurine], DAP [6-(γ,γ-dimetylalilaminapurine)], Sigma-Aldrich), and concentrations (0, 1.25, 2,5, 5.0, 7.5 mg/L^{-1}) were evaluated in a 3 X 5 X 2 factorial experiment. Each treatment was represented by 25 explants (n=25). After 6 weeks of culture shoot number per explant and total length (mm) were determined. Media [Murashige and Skoog (1962) (2): 50, 100 %], and carbohydrate concentration (0.25, 0.5, 0.75, 1%) were studied in a 2 X 4 factorial experiment to optimize individual

shoot proliferation growth and elongation. Each treatment was represented by 25 explants (n=25) and total shoot length (mm) and fresh weight (mg) were determined after 42 days of culture.

All media for these experiments were prepared by using standard protocols, pH adjusted to 5.7, and autoclaved at 121°C for 20 min. The cultures were maintained in an incubation room calibrated to provide 200 $\mu mol/m^2/s^{-1}$ of photosynthetic photon flux density (PPFD) at plantlet level, a photoperiod of 16 h of light provided by cool white fluorescent light, and 25 $\pm$ 2°C of temperature. Adventitious root formation was induced on basal MS (1962) (2) medium supplemented with 5% sucrose and 0.7% agar.

Following micropropagation and plantlet acclimatization, the effects of GA_3 on plant growth were determined by spraying a series of increasing concentrations (0, 150, 300, 450 ppm), which were applied at night (between 8:00 and 9:00 PM), every 15 days for 6 times through spraying 15 mL of a liquid solution of each concentration directly to the cladode. The experiment was maintained in a glasshouse with maximum photosynthetic photon flux density (PPFD) of 1100 $\mu mol/m^{-2}/s^{-1}$ at plant level, and an average day/night temperature of 27/20 ± 2ºC. The plantlets were fertilized through an automated fertigation system programmed to irrigate during 60 s every 2h with a standard re-cycling nutrient solution (1) including N (150 mg/L^{-l}), P (40 m g/L^{-l}), K (225 mg/L^{-l}), Ca (210 mg/L^{-l}), Mg (40 mg/L^{-l}), Fe (12 mg/L^{-l}), Mn (2 mg/L^{-l}), B (0.6 mg/L^{-l}), Cu (0.1 mg/L^{-l}), Zn (0.2 mg/L^{-l}), Mo (0.05 mg/L^{-l}), which was daily adjusted to an electrical conductivity of 2 millisiemens (mS) [10% $Ca(NO_3)_2$ and KNO_3 solutions (1:1 v/v)], and pH of 5.8 [1N HNO_3 or $OHNH_4$ solutions]. Fresh nutrient solution was prepared from stocks and changed every 15 days. Each treatment was represented by 18 plants (n=18). Total shoot (cladode) length (cm) was measured every month following the first application of GA_3 for 310 days. Relative growth rate (RGR) of shoots was calculated by using the formula: RGR= $\ln TSL_2 - \ln TSL_1/t_2\text{-}t_1$, where ln= natural logarithm, TSL_2= total shoot lenght at final measurement, TSL_1= total shoot length at initial measurement, $t_2\text{-}t_1$= days between measurements. By the time of harvesting the experiment, the plantlets were evaluated for shoot (Cl-FW), root (R-FW), and total plant fresh (P-FW) and dry mass (Cl-DW, R-DW, and P-DW, respectively) (g), total shoot and spine-hair length (cm), and root to shoot ratio. Treatment effects in all experiments were determined by using analysis of variance (ANOVA) and Tukey (α= 0.05%) for mean separation (3).

Results and Discussion: The greatest propagation ratio (shoot proliferation) was obtained when explants were cultured in vertical orientation (4.98 shoots per explant) as compared to horizontal position (3.69 shoots per explant). The addition of BA to the media resulted in increased shoot number per explant (8) in comparison to K and DA, which produced only 2 shoots in average. However, after 42 days of culture, significantly higher shoot length was obtained with DAP (14 mm) compared to K and BA (4 mm, respectively).

After the shoot proliferation stage, an elongation subculture was performed prior rooting in which shoot growth was enhanced when crowns of shoots were cultured in 50 % of basal salt formulation of MS (1962) (2). The addition of low concentrations of sucrose to this medium during subcultures produced increased shoot length and total shoot fresh weight after 45 days of culture (25%= 19.94 mm and 0.32 g, 50%= 16.25 mm and 0.26 g, respectively) compared to data obtained from high concentrations (75%= 9.53 mm and 0.1310 g, 100%= 5.88 mm and 0.0921 g, respectively).

Exogenous application of GA_3 after plantlet acclimatization under glasshouse conditions increased spine-hair (developed from areoles in young plants) length as part of short-term effects. However, significantly higher values were obtained in plantlets treated with 300 ppm of GA_3 when compared with the rest of the treatments. At the end of the study, the most important long-term effect produced by GA_3 was the suppression of total shoot growth (Table 1).

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Table 1. Effects of exogenous GA_3 on plant (PL), cladode (CL), root (R) fresh (FW), dry (DW) weight, and spines length of *Opuntia lanigera* Salm-Dick, 310 days after the treatment.

GA_3 (ppm)	PL-FW (g)	CL-FW (g)	R-FW (g)	PL-DW (g)	CL-DW (g)	R-DW (g)	Spines Length (cm)
0	1058.77 a	961.62 a	97.15 a	166.93 a	152.3 a	14.63 a	0.87 d
150	703.03 b	649.35 b	53.68 b	119.20 b	108.17 b	11.03 b	1.40 c
300	531.40 b	491.10 b	40.30 b	95.42 c	83.78 c	9.63 b	3.33 a
450	419.38 b	382.62 b	36.77 b	95.83 bc	86.32 bc	9.52 b	2.63 b
MSD	302.60	281.10	30.30	23.48	22.68	1.88	0.41

Means with different letter are significantly different according to Tukey test (α= 0.05). n= 18.

Propagation of *Viburnum rufidulum* by stem cuttings

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Index Words: adventitious rooting, indolebutyric acid, plant propagation, rusty blackhaw, southern blackhaw

Significance to industry: Southern blackhaw Viburnum has potential to be a valuable nursery and landscape plant. Currently, propagation difficulties limit its availability and use. This report documents successful clonal propagation by stem cuttings using a quick dip of K-IBA at 0, 3000 (0.3%), 6000 (0.6%), or 9000 ppm (0.9%). Talc formulation of IBA were unsuccessful.

Nature of work: *Viburnum rufidulum* (southern blackhaw) is a large shrub or small tree with attractive spring flowers, lustrous green leaves, showy fruit, and beautiful fall color. The species is native to coastal North Carolina, south to the Florida panhandle, and west to central Texas, Oklahoma, Missouri, and southeastern Kansas (3). Plants occur on a range of soils from dry rocky hillsides to rich moist valleys. Once established, plants are remarkably drought tolerant and have few insect or disease problems. However, wide scale nursery production and landscape usage is minimal due in part, to propagation difficulties. The literature suggests stem cuttings root with ease when collected throughout summer and treated with 3000 (0.3%) to 8000 (0.8%) ppm indolebutyric acid (IBA) in talc or as a quick dip and placed under intermittent mist (1). Others reported stem cuttings treated with a quick dip of IBA at 5000 (0.5%), 10000 (1.0%), 15000 (1.5%), or 20000 (2.0%) ppm or 3000 (0.3%) ppm IBA in a talc formulation rooted 100% after 5 months in a fog chamber (2). These results have not been realized by growers. The ability to reproduce plants by rooting stem cuttings would allow growers to add southern blackhaw to their inventory. Additionally, successful clonal propagation would facilitate future cultivar development from this species, of which there is currently very few selections.

Stem cuttings, approximately 20 cm (8 in) in length were collected from three specimens on 25 Aug. 2005 (semi-hardwood), 14 June 2006 (softwood), and 15 Dec. 2006 (hardwood). Cuttings from all three stock plants were combined and processed randomly to avoid confounding treatment responses with the influence of stock plant genotype. From the initial cutting material, terminal cuttings 15.25 cm (6 in) in length were prepared and leaves were removed from the lower half of the cuttings prior to auxin treatment.

Following preparation, the basal 1 cm (0.4 in) of all cuttings was treated for 3 sec with 0, 3000 (0.3%), 6000 (0.6%), or 9000 ppm (0.9%) K-IBA dissolved in distilled water. Cuttings were air dried for 10 min before inserting the basal 4 cm (1.6 in) into flats 9 cm (3.5 in) deep containing a medium of 3 perlite : 1 peat (v/v). Bottom heat was maintained at 21 ± 1 C (70 ± 2 F) for hardwood cuttings only. Additionally, the basal 1 cm (0.4) of another group of cuttings was moistened with distilled water prior to treating with 1000 (0.1%), 3000 (0.3%), or 8000 ppm (0.8%) talc formulated IBA. Intermittent mist operated daily 6 sec every 8 min from sunrise to sunset for the softwood and semi-hardwood cuttings. Hardwood cuttings were misted once daily by hand. For each growth stage the experimental design was a randomized complete block with seven blocks, and five cuttings per treatment per block. Data were collected after 8 weeks for softwood cuttings, 11 weeks for semi-hardwood cuttings, and 10 weeks for hardwood cuttings. Data included percent rooting, number of roots ≥ 1 mm (0.04 in) in length, and root length of rooted cuttings. Data were subjected to analysis of variance and regression analysis.

Results and Discussion: A talc formulation of IBA was generally unsuccessful. Overall rooting was poor or nonexistent for softwood (24%), semi-hardwood (4%), and hardwood (0%) cuttings. This was unexpected given previous reports that cuttings root readily when treated in such a manner (1).

A quick dip of K-IBA improved rooting at all growth stages (Table 1). Maximum rooting of softwood cuttings (87%) occurred with 6000 ppm (0.6%), maximum rooting of hardwood cuttings (100%) occurred with 9000 ppm (0.9%), and maximum rooting (49%) of semi-hardwood cuttings occurred at 3000 ppm (0.3%) K-IBA. Semi-hardwood and hardwood cuttings did not root without K-IBA application, however, 3000 ppm (0.3%) improved rooting to 49% and 75%, respectively. Softwood cuttings did root without K-IBA (29%), however, rooting was doubled (60%) when 3000 ppm (0.3%) K-IBA was used. Increasing K-IBA concentration increased rooting of softwood and hardwood cuttings, however, rooting of semi-hardwood cuttings did not improve. These results were slightly different from previous reports where 100% rooting was achieved regardless IBA concentration (2). Perhaps most unexpected was the high rooting percentage of the hardwood cuttings. This procedure is promising as there would be little concern of overwintering difficulties with hardwood cuttings.

The number of roots per rooted cutting was affected by K-IBA only at the softwood growth stage. Root number per rooted cutting at 0, 3000 (0.3%), 6000 (0.6%), or 9000 ppm (0.9%) K-IBA was 2.6, 5.8, 7.9, and 8.4, respectively. Root number of the semi-hardwood (5.5) and hardwood (4.4) cuttings was unaffected by auxin treatment. Average root length was not influenced by K-IBA application and averaged 13.8 mm (0.5 in), 19.0 mm (0.7 in), and 21.4 mm (0.8 in) for softwood, semi-hardwood, and hardwood cuttings, respectively.

The results discussed herein demonstrate that stem cuttings of *V. rufidulum* can be rooted in commercially acceptable numbers using a quick dip of K-IBA. Commonly used talc formulations of IBA should not be used, as overall rooting was poor with this product. Liquid formulations of K-IBA from 6000 ppm (0.6%) to 9000 ppm (0.9%) were sufficient to root stem cuttings of *V. rufidulum* in only 8 to 10 weeks.

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Table 1. Effect of K-IBA concentration on percent rooting of stem cuttings of *Viburnum rufidulum*.

K-IBA concn. (ppm)	Softwood	Semi-hardwood	Hardwood
0	29[z]	0	0
3,000	60	49	75
6,000	87	31	90
9,000	77	40	100
Linear	**[y]	*	**
Quadratic	*	NS	**

[z]N=35; [y]NS = not significant; * = significant at $P \leq 0.05$; ** = significant at $P \leq 0.01$.

Winter Cutting Propagation of Dwarf Burford Holly

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Index Words: Adventitious Rooting, *Ilex*, Root-promoting Compounds

Significance to the Industry: Dwarf Burford holly (*Ilex cornuta* 'Dwarf Burford') is a common nursery crop and a widely used landscape plant in warmer regions of the United States. Plants can be propagated readily by stem cuttings. Use of an auxin treatment has historically been recommended for rooting cuttings. Experimental results indicate semi-hardwood, terminal stem cuttings of dwarf Burford holly can be successfully rooted during winter months without the use of an auxin treatment, permitting elimination of one step in the propagation process. Use of auxin at a moderate rate can sometimes result in larger root systems by the end of the rooting period; however, a high concentration of auxin can adversely affect rooting of cuttings.

Nature of Work: Dwarf Burford holly is a significant crop in ornamental nursery production (1). Plants are readily maintained in the landscape and suitable for small landscapes in USDA hardiness zones 7 to 9 (4). Although plants are self-fertile and produce some bright red berries in fall and winter (6), they are grown primarily for their form and foliage (1). Treatment of cuttings with auxin is typically recommended to promote rooting (1, 5). Cuttings of other hollies, including dwarf yaupon holly (*Ilex vomitoria* 'Nana') and 'Nigra' inkberry (*Ilex glabra* 'Nigra'), can be successfully propagated from winter cuttings without use of an auxin treatment (2, 3).

The objective of the present study was to compare rooting of winter stem cuttings of dwarf Burford holly propagated both with and without use of a basal quick-dip in a solution of indole-3-butyric acid (IBA) + 1-naphthaleneacetic acid (NAA). Winter is typically a slow period for many nursery activities. One step in the propagation process could be eliminated if an auxin treatment is not necessary.

Cutting propagation material of dwarf Burford holly was collected from mature landscape plants growing on the campus of Auburn University, Auburn, AL for three experiments. Terminal, semi-hardwood, 3-inch long stem cuttings were prepared using the previous season's growth (firm but green stems), with the lowest leaf removed from the base of each cutting.

Auxin solutions were prepared by diluting Dip ‘N Grow (IBA + NAA; Dip 'N Grow, Inc., Clackamas, OR) with deionized water. In all experiments, cuttings received no auxin treatment or a 1-second basal quick-dip to a depth of 0.5 inch in a solution of 2500 ppm IBA + 1250 ppm NAA. In Expt. 1, cuttings in a third treatment received a 1-second basal quick-dip to a depth of 0.5 inch in a solution of 5000 ppm IBA + 2500 ppm NAA. Cuttings were inserted to a depth of 0.5 inch into individual pots containing Fafard 3B mix (a blend of peat, perlite, vermiculite, and pine bark; Conrad Fafard, Inc., Agawam, MA) as the rooting substrate.

Expt. 1 was initiated on Dec. 29, Expt. 2 on Feb. 17, and Expt. 3 on Feb. 23. Cuttings were placed inside a high-humidity polyethylene-covered enclosure inside a greenhouse. Overhead mist was supplied once daily for 10 seconds at noon to maintain a relative humidity of 95% to 100%. Daily maximum/minimum temperature was 81 ± 10F/64 ± 5F.

A completely randomized design was utilized in all experiments with 30 cuttings per treatment in each experiment. Number of rooted cuttings, number of primary roots emerging from the stem of each rooted cutting, and total length of primary roots on each rooted cutting were determined after a rooting period of 145 days in each experiment. In Expt. 1, significance of an increase or decrease in response variable values with increasing auxin concentration was evaluated with Wald Chi-square statistics and t statistics (using the LOGISTIC, GENMOD, and GLM procedures of SAS (SAS Version 9.1; SAS Institute Inc., Cary, NC). In Expt. 2 and Expt. 3, between-treatment differences were evaluated with Fisher’s Exact Test and permutation tests using the MULTTEST procedure of SAS.

Results and Discussion: Rooting percentage tended to decrease with increasing auxin concentration in Expt. 1, while rooting percentage of nontreated cuttings was similar to that of cuttings treated with 2500 ppm IBA + 1250 ppm NAA in Expt. 2 and Expt. 3 (Table 1). Number of roots per rooted cutting was similar among treatments in all three experiments. Total root length per rooted cutting was similar among treatments in Expt. 1 and Expt. 2, but was greater on cuttings treated with 2500 ppm IBA + 1250 ppm NAA compared with nontreated cuttings in Expt. 3. In general, rooting percentages, root number, and total root length appeared to be greater with cuttings taken on February 17 and 23 compared with cuttings taken on December 29; however, this would need to be confirmed by a multi-year test.

Rooting results support prior observation (5) that dwarf Burford holly may be successfully propagated by stem cuttings late into the winter. However, results also indicate cuttings will root well without use of an auxin treatment. While use of an auxin treatment may sometimes produce larger root systems compared with nontreated cuttings, use of higher auxin concentrations can have a negative effect on rooting percentage.

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Table 1. Root development responses of terminal, semi-hardwood cuttings of *Ilex cornuta* 'Dwarf Burford' treated with and without auxins [indole-3-butyric acid (IBA) + 1-naphthaleneacetic acid (NAA)] in three experiments initiated during winter[1]. Cuttings were rooted in Fafard 3B substrate[2] in a warm, high-humidity rooting environment inside a greenhouse with a rooting period of 145 days for each experiment.

IBA + NAA (ppm)	Rooted (%)	Roots (no.)	Total root length (mm)
	Expt. 1		
Nontreated	67	6.2	266
2500 + 1250	63	6.6	281
5000 + 2500	27	7.4	277
Significance[3]	0.003	0.421	0.883
	Expt. 2		
Nontreated	93	7.9	444
2500 + 1250	93	8.6	513
Significance[4]	1.000	0.510	0.233
	Expt. 3		
Nontreated	90	9.1	419
2500 + 1250	83	10.4	624
Significance[4]	0.707	0.236	0.001

[1]Expt. 1: Dec. 29, Expt. 2: Feb. 17; Expt. 3: Feb. 23.
[2]A blend of peat, perlite, vermiculite, and pine bark.
[3]*P*-values for the significance of an increase or decrease in response variable values with increasing auxin concentration were obtained using Wald Chi-square statistics (for percent rooted and number of roots) and using a t statistic (for total root length).
[4]*P*-values for the differences between means in Expt. 2 and Expt. 3 were obtained using Fisher's Exact Test (for percent rooted) and permutation tests (for number of roots and total root length).

Using Hot Water Immersion to Control Nursery Pests

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Index Words: Hot water, immersion, ornamental plants, propagation, cuttings

Significance to Industry

Many nursery plant propagators are eager to adopt cost-effective and efficient methods of non-chemical control of pests. Concern over workers' prolonged exposure to chemicals has prompted many nursery owners to look for alternative methods to deal with insect and mite control that places less reliance on pesticides. Greater regulation on the use of chemical pesticides has created an opportunity to explore other methods of dealing with pests. Nursery managers propagate many species of plant material by taking cuttings from stock plants that can have a small infestation of insects or mites that are difficult to detect. We built and tested a portable hot water immersion system that is economical and relatively easy to construct and operate by nursery plant propagators. The system involves an instantaneous water heater that rapidly heats water to the proper temperature. The water is re-circulated around plant cuttings.

Nature of Work

One objective of this trial was to establish threshold temperatures at which temperate-zone plant material can be treated safely using hot water without damaging the plant material. A second objective was to establish whether these hot water treatment temperatures and the length of treatment also kill pests. The method of using hot water treatments to control pests is relatively simple and effective. Most pests of ornamental plants can survive at high temperatures, but there is a narrow temperature window at which insect pests die and plant material is tolerant. Several species of insects including aphids, scale, and mealybug have been effectively killed with hot water treatments of 120 °F for up to 15 minutes (1, 2). We tested multiple temperatures and treatment times on woody and herbaceous plant material. Five replications of whole plant cuttings were submersed in water and held at a constant temperature for a set amount of time with the water being re-circulated around the plant cuttings. The treated cuttings were cooled for 60 to 120 seconds and stuck using normal propagation methods.

Results and Discussion

Cuttings were observed over a six- to eight-week period. We noted if the treatments caused scorching of the foliage, dieback of the cutting or a lack of rooting. If any damage was recorded at temperature or time interval, it was determined to be unacceptable. The threshold that injury is incurred on several species of plants in our trial appears to be 120 °F. We found that 120 °F at 10- to 20-minute treatment times appears to be safe on azalea, ivy (*Hedera* sp.), boxwood (*Buxus* sp.), Leyland cypress (× *Cupressocyparis leylandii*) and 'Green Giant' arborvitae (*Thuja* 'Green Giant'). Boxwood cuttings were taken from plants infested with boxwood mite (*Eurytetranychus buxi*). Twenty 6-inch branch tips, taken randomly, were examined and the number of eggs recorded to establish a precount average number of eggs. The cuttings were treated at 120 °F for 15 minutes. Plants treated at 120 °F for 15 minutes gave 100 percent control of boxwood mite. Plants treated at 115 °F for 15 minutes only had a little more than 60 percent control. Twenty *Liriope* plants infested with fern scale (*Pinnaspis aspidistrae*)were examined and the amount of third-instar females present was recorded. This established an average number of scale per plant for the pretreatment count. We found that 115° was not sufficient to kill the scale, but 120 °F for 15 minutes gave 99 percent control. A precount was taken on 20 plants of container-grown *Miscanthus* infested with Miscanthus mealybug (*Miscanthicoccus miscanthi*) to establish an average number of overwintering mealybugs per plant. Whole plants were treated at 120 °F for 15 minutes and 125 °F for 15 minutes. Plants were examined a month later and the number of mealybugs counted. The mealybug hides between the leaf sheaths, and because this sampling method was destructive, only one data set could be taken. We found that treatment at 120 °F for 15 minutes gave a more than 99 percent level of control of Miscanthus mealybug. Treatment at 125° F also worked with no damage to the plants; however, a grower only needs to reach 120 °F to control this pest. We found that New Guinea impatiens cuttings are very tolerant of 120 °F treatments for up to 20 minutes. One pest that damages 'New Guinea' impatiens is the cyclamen mite (*Phytonemus pallidus*). Researchers have reported that treatment with hot water at 110 ° F for 15 to 30 minutes kills cyclamen mites (3). We conducted trials on several herb species and found tarragon (*Artemisia dracunulus*) could not withstand temperatures above 110 °F, sage (*Salvia*) only tolerated temperatures up to 112 ° F, and rosemary (*Rosmarinus officinalis*) could take 120 °F , but for just 10 minutes. Growers are not likely to kill many pests at this lower temperature and shorter treatment interval. These findings are unfortunate because there only are a limited number of labeled chemical options for growers to use on herb crops.

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Table 1. Highest temperature tolerated by plants

Plants Tested	Highest temperature plants tolerated (°F)	Greatest length of time plants tolerated (min)
× *Cupressocyparis leylandii*	120	15
Artemisia dracunulus	110	0
Azalea 'Rosebud'	120	10
Buxus sempervirens 'Rotundifolia'	120	15
Cotoneaster dammeri 'Coral Beauty'	120	10
Hedera colchica	120	15
Hedera helix 'Marginata of Hibbard'	120	20
Hedera helix 'Wingertsberg'	120	15
Impatiens 'New Guinea'	120	20
Pieris japonica	120	20
Rosmarinus officinalis	115	10
Salvia	112	20
Thuja 'Green Giant'	120	10

Table 2. Hot water treatment kills the following pests.

Insect treated with hot water	Temperature (°F)	Time to obtain >99% mortality
Boxwood mites	120	15 minutes
Miscanthus mealybug	120	15 minutes
Fern scale	120	15 minutes

Propagation of Selected Clones of Eastern Redbud (*Cercis canadensis*) by Stem Cuttings

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Index Words: Adventitious Rooting, Auxin, K-Indolebutyric Acid, Leguminosae

Significance to Industry: Cultivars of eastern redbud (*Cercis canadensis* L.) sold in the nursery trade typically are propagated by budding or micropropagation. This study showed that particular clones when propagated by stem cuttings root in high percentages. Propagation of some cultivars by stem cuttings may be an alternative to more expensive methods of propagation such as budding or micropropagation.

Nature of Work: Landscapers covet small flowering trees such as eastern redbud, and several cultivars are available, each bringing interest to the garden (5). Stem cuttings of redbuds are reportedly difficult to root (2, 5), and nurserymen commonly use chip-budding, t-budding, or tissue culture to propagate desirable clones. These techniques require significant skill and time and are a limitation to wider utilization of redbuds in the landscape. Additionally, trees propagated by budding may be prone to disease and can develop problems at the bud union (Dennis Werner, NC State Univ., personal communication). Many species propagated via tissue culture tend to lack vigor, and there are anecdotal reports of this occurring in redbuds. Thus, developing protocols for rooting stem cuttings of redbud would offer a simpler means of propagation and may yield more vigorous, problem free plants.

Previous research on propagating eastern redbud by stem cuttings has yielded mixed results. Tipton (6) working with *C. canadensis* var. *mexicana* (Rose) M. Hopkins (Mexican redbud) reported predicted maximum rooting response was 88% for cuttings taken 4 weeks after budbreak and treated with 21,000 mg•liter^{-1} (ppm) K-IBA [potassium salt (K salt) of indolebutyric acid (IBA)]. Similarly, Dillion and Klingaman (1) found high rooting percentage (94%) of a clone of *C. canadensis* from cuttings taken 3 weeks after growth began and treated with 20,000 mg•liter^{-1} (ppm) IBA. In addition, Pooler and Dix (4) reported 56% rooting of semi-hardwood cuttings of eastern redbud taken from plants in the juvenile growth phase. In contrast, Murphy (3) attempted to propagate *C. canadensis* 'Forest Pansy' by hardwood cuttings and was unable to root a single cutting. Other anecdotal reports of attempts to root softwood and semi-hardwood cuttings of 'Forest Pansy' support the findings of Murphy (3) and others (Dennis Werner, NC State Univ.,

personal communication; Wooldridge et al., unpublished data). The aforementioned studies suggest genotype plays a large role in the rootability of *C. canadensis*. Therefore, two studies were conducted to investigate the effect of auxin treatment on rooting stem cuttings of four related clones of *C. canadensis*.

The experiments were both 4 by 4 factorials in a randomized complete block design with four replications and six cuttings per replication. The main factors were four clones: 'Flame', dwarf white (an unreleased selection of the JC Raulston Arboretum), and two selections (99-6-1 and 99-6-2) of an F_1 generation from a cross of 'Flame' and dwarf white; and four rates of K-IBA [0, 5000, 10,000, or 15,000 mg•liter^{-1} (ppm)]. For each clone, cuttings were taken from a single tree in the adult growth phase. The trees of 'Flame' and dwarf white were located at the JC Raulston Arboretum, Raleigh, NC, while trees of the F_1 selections were located at the Sandhills Research Station, Jackson Springs, NC. In the first study, softwood cuttings were taken in May, and in the second study semi-hardwood cuttings were taken in July. For all four clones, the leaves of the softwood cuttings were not fully expanded and the stems were green. When the softwood cuttings were flexed, the stem broke without a snapping sound and remained attached. For all the semi-hardwood cuttings, the leaves were fully expanded and stems were a medium brown color. When pressure was applied, the stem broke with a snapping sound, but did not separate.

During preparation of the cuttings at both stages of growth, terminal succulent growth was removed to the first or second distal node. The cuttings were then trimmed at the base resulting in a final length of 15 cm (6 in) and leaves were removed from the basal 5 cm (2 in). Leaves larger than 10 cm (4 in) in width were cut in half perpendicular to the midrib and the basal 2 cm (0.8 in) of each cutting was dipped for 2 sec in a solution of K-IBA at 0, 5000, 10,000, or 15,000 mg•liter^{-1} (ppm). After 15 min of air drying of the IBA solutions, each cutting was set in a plastic Anderson Deep Tree Band (Anderson Tool and Die, Portland, OR) [6 x 6 x 12 cm (2.4 x 2.4 x 5 in)] containing 1 peat : 1perlite (by vol.) and placed under intermittent mist in a greenhouse. The mist operated from 7 am to 7 pm for 4 sec every 10 min in the softwood study and 4 sec every 8 min in the semi-hardwood study. The greenhouse was maintained at days/nights of 21/16° C (70/60° F). After 8 weeks the study was terminated and cuttings were evaluated for rooting. A cutting having one primary root ≥ 1 mm (0.04 in) in length was classified as having rooted. Rooting percentages were calculated and these data were subjected to analysis of variance (ANOVA) and regression analysis, where appropriate. When regression analysis was significant, simple linear and polynomial curves were fitted to data. The maximum of the polynomial curve was calculated as a first order derivative of the independent variable where the dependent variable equaled zero.

Results and Discussion: For softwood cuttings taken in May, rooting percentage was significantly affected by clone and K-IBA treatment but not the

clone by K-IBA interaction (Table 1). The response ranged from 0% rooting for nontreated stem cuttings of 99-6-1 to 75% for cuttings of 99-6-2 treated with K-IBA at 15,000 mg•liter^{-1} (ppm) (Fig. 1). For this treatment, the cuttings of 99-6-2 that rooted had a mean of 6.3 primary roots with a mean total primary root length of 29 cm (11 in) (data not presented).

For semi-hardwood cuttings taken in July, rooting percentage was again affected by clone and K-IBA treatment. In addition, there was a significant clone by K-IBA rate interaction (Table 1). Dwarf white did not root regardless of IBA rate, whereas 83% rooting was noted for stem cuttings of 'Flame' treated with 10,000 mg•liter^{-1} (ppm). Cuttings of 'Flame' treated with K-IBA at 10,000 mg•liter^{-1} (ppm) had a mean of 14.9 primary roots with a mean total root length of 76 cm (30 in) (data not presented). The rooting response of semi-hardwood cuttings of 'Flame' treatment to K-IBA was quadratic (P = 0.002, R^2 = 0.74) with the maximum rooting percentage predicted at 10,241 mg•liter^{-1} (ppm). The quadratic response was not seen for the other clones. Similar to dwarf white, low rooting percentages were observed for 99-6-1 and 99-6-2 regardless of auxin treatment (Fig. 2).

Although these studies were not designed to test the influence of growth stage on rooting, dwarf white, 99-6-1, and 99-6-2 rooted in higher percentages in the softwood study, while 'Flame' had a higher rooting percentage in the semi-hardwood stage. This was surprising as previous research indicated stem cuttings of eastern redbud generally root best from cuttings taken soon after budbreak (6). Data herein indicate clones of *C. canadensis* respond differently regarding adventitious rooting and propagation by stem cuttings may be a means to propagate particular genotypes.

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Table 1. ANOVAs for rooting percentage of softwood and semi-hardwood cuttings of *C. canadensis* as influenced by cultivar and K-IBA treatment.

Treatment	Softwood cuttings	Semi-hardwood cuttings
Cultivar	0.0101	< 0.0001
K-IBA rate	< 0.0001	< 0.0001
Cultivar x K-IBA rate	0.3629	0.0001

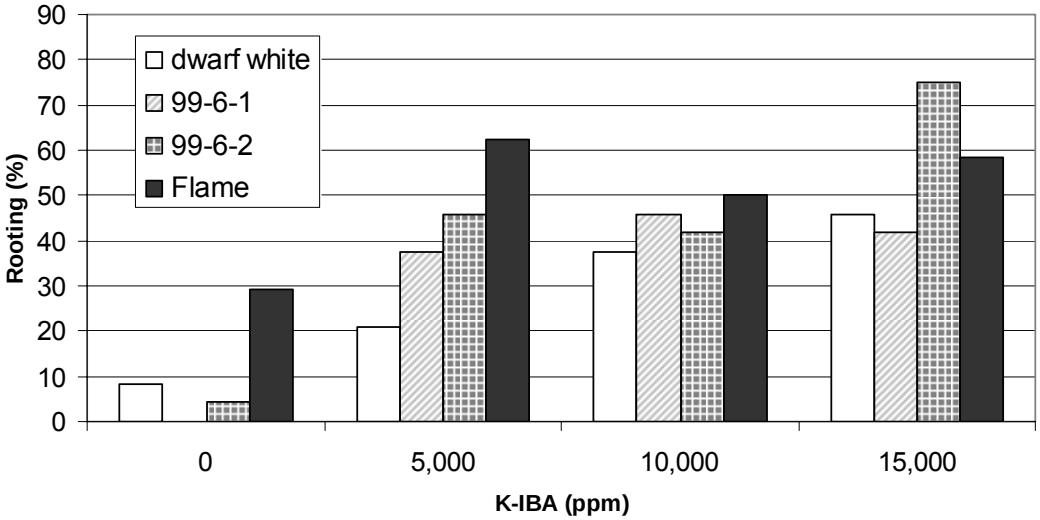

Fig. 1. Influence of K-IBA treatment on rooting softwood cuttings of selected clones of eastern redbud.

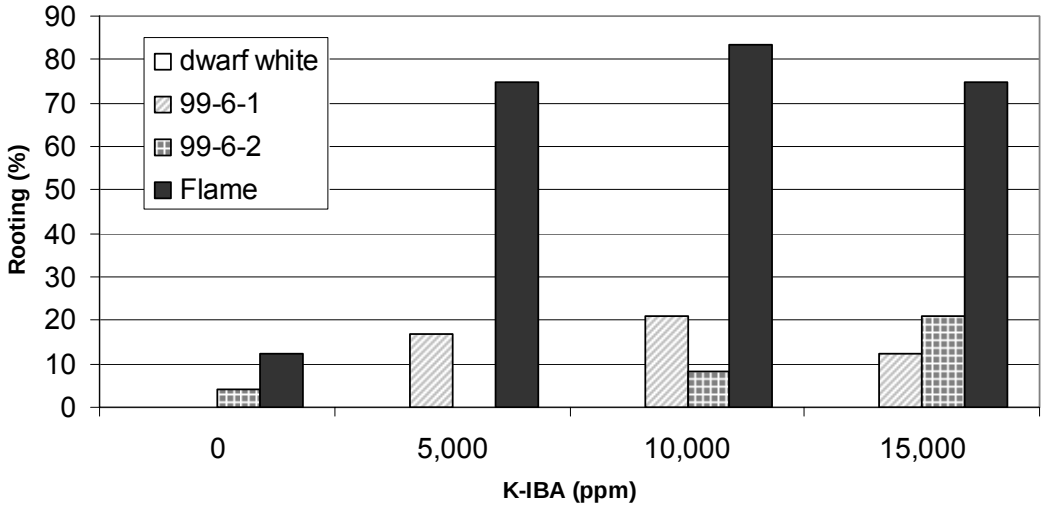

Fig. 2. Influence of K-IBA treatment on rooting semi-hardwood cuttings of selected clones of eastern redbud.

Influence of Light and Temperature on Germination of Seeds of Pinkshell Azalea (*Rhododendron vaseyi*) Collected from Two Locations in Western North Carolina

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Index Words: Sexual Propagation, Native Plants, Ericaceae, Rare Species

Significance to Industry: Quantitative data are presented concerning the influence of light and temperatures of 25C (77F) and 30/20C (86/68F) on germination of seeds of pinkshell azalea (*Rhododendron vaseyi* A. Gray) collected from two locations in western North Carolina. Regardless of temperature, light was required for germination and continuous light (24 hr) produced the highest germination for both locations at 30/20C (86/68F). Generally, the environmental variables required for germination did not differ between the locations and maximum germination for both locations was approximately 50%. Because of their small size, apparent low viability, and obligate light requirement, seeds of *R. vaseyi* should simply be dusted in copious quantities on the surface of a germinating medium and germinated under continuous light.

Nature of Work: The deciduous pinkshell azalea is a rare ericaceous species endemic to only six counties in western North Carolina. May through June, plants produce attractive, pink to rarely white flowers in clusters of 3-15 flowers. Because natural habitats are diminishing for this plant due to home and road construction, protocols for seed germination were developed and presented previously for seeds collected from one location in northwestern North Carolina (5). However, germination from this location was low (50%) and one reason may be yearly variation in seed viability. Another reason may be variability between populations/localities. Therefore, the following research was conducted to study the influence of light and temperature on germination of seeds of *R. vaseyi* collected from two locations in western North Carolina.

On October 15, 2006, mature seed capsules (fruits) were collected from four native populations of open-pollinated plants of *R. vaseyi* in western North Carolina. The first two populations were collected on Pilot Mountain in Transylvania County, and along the ridgeline between Jackson and Transylvania counties. All capsules were pooled and collectively designated Location 1. The second two populations of seeds were collected at mileposts 300 and 305 of the Blue Ridge Parkway in Avery county. Capsules were pooled from these two

populations and designated Location 2. The distance between Locations 1 and 2 was 120 miles.

Following extraction from the capsules, seeds were placed in covered 9-cm (3.5 in) diameter glass petri dishes, each dish containing two pre-soaked germination blotters moistened with tap water. Half the dishes were designated for germination at 25C (77F) and the other half for germination at an 8/16 hr thermoperiod of 30/20C (86/68F). Temperatures within germination chambers varied within ± 0.5C (0.9F) of the set point. The chambers were equipped with cool-white fluorescent lamps that provided a photosynthetic photon flux (400-700 nm) of approximately 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (3.2 klx). Within each temperature regime, seeds were subjected daily to the following eight photoperiods: 0 (total darkness) ½, 1, 2, 4, 8, 12, or 24 hr. Daily photoperiod treatments were regulated by removal and placement of the petri dishes into black sateen cloth bags. Petri dishes for the 24 hr photoperiod treatment remained continuously unbagged in the chambers. Dishes for the 0 hr (total darkness) treatment remained bagged throughout the experiment and germination data were recorded under a green safelight. Germination blotters were kept moist with tap water throughout the experiment. Within a temperature regime, each photoperiod treatment was replicated four times with a replication consisting of a petri dish containing 100 seeds.

Germination counts were recorded every 3 days for 30 days and seeds showing signs of decay were removed from the dishes. A seed was considered germinated when radicle emergence was ≥ 1 mm (0.04 in) in length. Percent germination was calculated as a mean of four replications per treatment. Data were subjected to analysis of variance procedures (SAS v. 9.1, SAS Institute, Cary, NC).

Results and Discussion: Light was required for germination, which is similar to other species of *Rhododendron* L. (1, 2, 3, 4). Germination increased as a function of photoperiod for each location at both temperatures. Germination was highest at 30/20C (86/68F) for Location 1 (45%) and Location 2 (50%) in the 24 hr photoperiod (Fig. 1). Seeds from Location 2 germinated at higher percentages compared to seeds from Location 1 at both temperatures and all photoperiods. As indicated by a significant interaction between location, temperature, and photoperiod (ANOVA not presented), however, the magnitude of difference between locations was not consistent for each temperature at all photoperiods (Fig. 1). For example, at 30/20C (86/68F), the difference in germination between locations for 8 hr was 22% (37% minus 15%), whereas for 24 hr the difference was 5% (50% minus 45%). At 25C (77F), the difference between locations was 10% (13% minus 3%) and 5% (30% minus 25%) for photoperiods of 8 and 24 hr, respectively (77F) (Fig. 1).

The alternating temperature of 30/20C (86/68F) partly compensated for the light requirement at shorter photoperiods. The extent of compensation depended on the location and the photoperiod. The difference in germination percentages

between both temperatures for Location 1 at the 4 hr photoperiod was 1% (3% minus 2%) and for Location 2 was 10% (15% minus 5%) (Fig. 1). At the 8 hr photoperiod, the difference between germination temperatures was 12% for Location 1 (15% minus 3%) and 25% for Location 2 (38% minus 13%). Although seeds from Location 2 germinated at slightly higher percentages than seeds from Location 1 at 24 hr, the response of both locations to temperature and photoperiod was similar. The difference in germination may be due to more vigorous seed from location 2 (4).

Effects of light and temperature on seed germination of *R. vaseyi* were very similar to previous reports for seeds from Location 1 (5) and other *Rhododendron* sp. (1, 2, 3, 4). However, the overall germination percentages for *R. vaseyi* were much lower than other *Rhododendron* sp. It is possible seed viability of *R. vaseyi* may be inherently low and methods to determine viability prior to germination would enhance the response. It is also possible there are other barriers to germination, in addition to the light requirement, that require further investigation. Additionally, germination tests of other populations of *R. vaseyi* within North Carolina, as well as closely related species, might indicate if low seed viability is widespread in the species.

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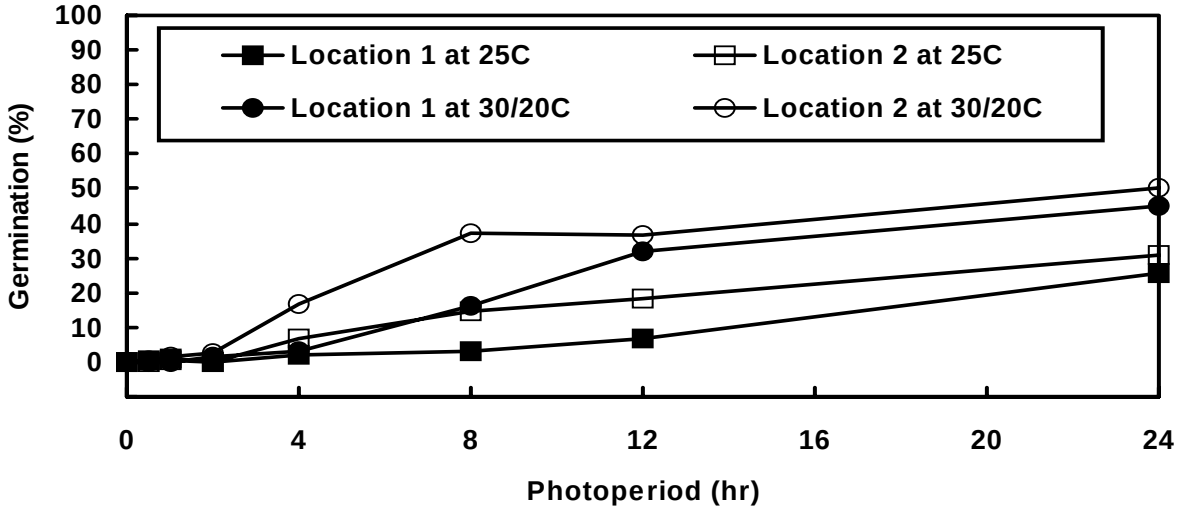

Fig. 1. Cumulative germination of seeds of *R. vaseyi* collected from two locations in western North Carolina and germinated at 25C (77F) or an 8/16 hr thermoperiod of 30/20 (86/68C) with daily photoperiods at each temperature of 0 (total darkness), ½, 1, 2, 4, 8, 12, or 24 hr. Symbols represents mean percentage germination of four petri dishes each containing 100 seeds.

Enhancing the Germination of Sunflowers (*Helianthus annuus* L.)

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Index Words: Asteraceae, native plants, seed density, seed germination

Significance to Industry: *Helianthus annuus* (annual sunflower) seeds do not germinate uniformly as the viability of achenes is influenced by many factors. This characteristic is a drawback for seed producers and growers desiring uniform stands of this Texas native. Enhanced germination of annual sunflowers allows greater utilization of this plant for wildlife management purposes. Finding a method of increasing germination uniformity without cracking the hull would be valuable as it would not compromise shelf-life of the seed.

Nature of the Work: There is great diversity within the annual sunflower species. Germination of the achenes can be influenced by environment, ripeness, density and size. Seed size is impacted by environmental conditions, plant density, cultivar differences and position on the head (Radford 1977). In cases where achene size affects germination, separating by size could lessen competitive suppression of the less viable achene size.

Seed density has been found to be a better indicator of germination (Krieg and Bartee 1975; Maranville and Clegg, 1977) than seed size. Smaller sunflower achenes are denser than larger achenes (Hernandez and Oriolo, 1982).

Methods: Four sequential experiments were conducted using annual sunflower seeds collected in Breckenridge, Texas. Viability of the seed lot was determined using the Association of Official Seed Analysts (AOSA) excised embryo tests. All experiments were conducted as a completely randomized block design with 7 replicates. The excised embryo treatment was used as the control in each experiment. Achenes were considered germinated when the radicle was visible. A $p \leq 0.05$ was maintained as the level of significance throughout the study.

The first experiment conducted tested germination uniformity of whole achenes following the AOSA guidelines. Low germination rates led to the second experiment in which an overnight deionized water seed soak was used to soften the seed coat. AOSA germination uniformity testing followed. A 500 ppm Gibberellic Acid (GA_3) treatment was employed in the third experiment to improve germination uniformity. In the final experiment, the seed were separated into three density groups using a Dayton Blower to determine the impact of density on germination uniformity. The volume of seeds in each density group was measured using a volumetric flask.

Results and Discussion: The final germination percentage of the excised embryos was 57.1% (Table 1). This average was used as the control. In experiment one, whole achenes exhibited a reduced germination percentage of 2.9% which represents a 54.2% decrease in germination and the germination rate extended 3 days. In experiment two, there was a 49.1% increase in the germination of soaked whole achenes as compared to the excised embryos (Table 1). The germination rate soaked achenes also increased from 2 to 3 days. Whole achenes treated with Gibberellic acid (GA_3) had only 10.5% germination (Table 1). Germination rate with GA_3 also increased from 2 to 4 days. In the final experiment, seeds separated by density showed no difference in germination rate between density groups (Table 2). Germination percentages differed with an increase density (40%, 80%, and 100%, respectively). Seeds soaked for 18 hours exhibited radicle emergence of 0%, 8.0% and 16.0% respective to increasing size.

In conclusion, whole achenes, whole achenes allowed to soak and whole achenes treated with gibberellic acid did not significantly improve germination. Separating annual sunflower *seed* by density may be used as a method of improving germination uniformity.

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Table 1. Germination rate and percent for whole achenes. Soaked achenes, and achenes treated with GA_3.

Test	Rate (days)	Percent
Viability (Excised Embryo)	2	57.1%
Whole Achene (Control)	5	2.9%
Soaked Achenes	3	8%
GA_3 Treated Achenes	4	10.5%
N =7		

Table 2. Germination rate and percent for density separated achenes.

Test	Rate (days)			Percent		
	Low	Medium	High	Low	Medium	High
Excised achenes (viability)	2	2	2	40.0%	80.0%	100.0%
Whole achene	10	5	5	0.5%	5.0%	3.5%
Whole achene soak for 18 hours	0	3	3	0.0%	8.0%	16.0%
N =7						