Propagation

Patricia R. Knight
Section Editor and Moderator

Putting the Speed Back in Quick-dip Auxin Application

Richard C. Beeson, Jr.
University of Florida, Mid-Florida Research and Education Center
Apopka, FL 32703
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Nature of Work: The Quick-dip method of applying rooting compounds to cuttings usually consists of dipping the basal end of a cutting into a liquid solution of the auxin or auxins for 2 to 4 seconds before inserting the cutting into the propagation substrate. Quick-dip concentrations usually range from 1500 to 7500 ppm auxin. While 2 to 4 seconds is quick compared to the 12 to 18 hours needed for the alternative dilute soak method, when thousands of cuttings are to be propagated it becomes a slow and laborious process. This is especially true compared to the rapid coating of the cut end of a cutting with talc-based auxin preparations. However, many species of woody plants root better when treated with the liquid solutions of auxins than with the powdered preparations.

In the mid-1990s, Cell-U-Wett (Griffin Corp, Valdosta, GA) a spray adjuvant, was quietly promoted as a dilutant for liquid solutions of auxins. Some was obtained and tried. While the rooting percentages were satisfactory in non-replicated use, more impressive were its other advantages and anecdotal reports of its use. An experiment was set up to formally evaluate Cell-U-Wett in comparison to water as the dilutant in Quick-dip solutions with Dip & Gro (Astoria-Pacific, Inc. Clackamas, OR).

In early November 1998, Quick-dip solutions consisting of 1875 (1:7), 3750 (1:3) and 5000 (1:2; Dip & Gro:dilutant, respectively) ppm auxin from Dip & Gro (10,000 ppm IBA + 5000 ppm NAA) were made using either water or an aqueous solution of Cell-U-Wett. Cell-U-Wett was received as a powder and first dissolved in water (2 Tbsp (12 g) per liter of water). On November 4 and 5, uniform cuttings 2 to 4 inches in length (depending on species) were removed from healthy mother plants. Leaves were stripped from the bottom two-thirds of each cutting, and the cut ends were treated with an auxin solution and stuck in a moist peat-perlite rooting substrate. Cuttings treated with water-diluted solutions (WATER) were held for 3 to 4 seconds at a depth of 3/8 to 1/2 inch before sticking. Cuttings treated with Cell-U-Wett-diluted solutions(CELL) were dipped in and out of a solution of a similar depth as quickly as

possible before sticking. Dipping solutions were replenished as needed from stock solutions. Thirty-two cuttings per species for each solution were placed in peat cups (Jiffystrips, Jiffy Products Ltd., Shippagan, Canada) in plastic trays on a heated rooting bed with intermittent mist. Minimum soil temperature was maintained at 70F. Species utilized were *Ligustrum japonicum* (common ligustrum), *Photinia x fraseri* (Red-tip photinia), *Loropetalum chinense* (loropetalum), *Pittosporum tobira* 'Variegata' (Variegated pittosporum), and *Viburnum odoratissimum* (sweet viburnum). From mid-January 1999 until early March, trays of cuttings were removed by species as they became rooted, the roots washed and root fresh and dry weights determined. Rooting percentage was also recorded. Root masses were analyzed as a 2 x 3 factorial within each species with mean separation by F-Protected LSD were appropriate.

Results and Discussion: There were no differences in the rooting percentages between dilutants or auxin concentrations within a species. For ligustrum and viburnum, 100% of the cuttings rooted. All pittosporum treatments achieved 100% rooting except for the 5000 ppm CELL treatment (97%), in which 1 of the 32 cuttings failed to root. Rooting percentage was more variable with loropetalum, where the two lowest WATER concentrations and the two highest CELL concentrations were 100%. One cutting did not root with CELL at 1875 ppm and 3 did not root with WATER at 5000 ppm. Rooting percentages were poorest with photinia (91, 100 and 84% - WATER; 87, 97 and 91% - CELL at 1875, 3750 and 5000 ppm respectively), but still little difference was observed between dilutants.

There were no differences in root fresh weight as functions of auxin concentration or dilutant for viburnum or ligustrum. For loropetalum, the largest root fresh weights were obtained at the 3750 ppm concentration with either dilutant (Table 1). For photinia, the largest root fresh weights were obtained at the 3750 ppm concentration with either dilutant or with Cell-U-Wett at 5000 ppm. For pittosporum, the largest fresh weights were obtained with WATER at 1875 ppm (Table 1), although there were no differences in fresh weights among the other treatments.

For root dry mass, there were no differences among treatments for pittosporum and again for viburnum. As with fresh weights, the largest root dry mass was obtained with either dilutant at 3750 ppm auxin for loropetalum and photinia and Cell-U-Wett at 5000 ppm for photinia (Table 2). For ligustrum, there were no differences in root dry mass between dilutants at the two highest concentrations (Table 2).

Significance to Industry: Overall, the best rooting in terms of percentage and mass of roots generated occurred with the 3750 ppm concentration of Dip & Gro. The liquid used to dilute the Dip & Gro made no difference. However, in terms of practicality, using Cell-U-Wett as the dilutant more than doubled the number of cuttings which could be stuck in a given time frame. The speed at which cuttings can be stuck with Cell-U-Wett-diluted Dip & Gro is identical to that of powdered rooting compounds. The Cell-U-Wett solution has a texture similar to thin honey and thus clings to the stem, providing extended coverage of the basal portion of the cutting not provided by powders.

Another advantage of using Cell-U-Wett as the dilutant is that the alcohol-based auxins remain in solution and active for at least several weeks without refrigeration. With water-diluted Dip & Gro, the auxins bind and settle out of solution as the alcohol evaporates. This occurs quickly in Southern greenhouses in the summer, and can occur in sealed stock solutions overnight if there is a large air volume, resulting is wasted material and daily mixing of solutions. Over 5 years, none of these problems have been experienced with Cell-U-Wett solutions.

There are anecdotal reports of improved rooting percentages for some low-rooting species. However, Cell-U-Wett is not the “golden bullet”. Use of Cell-U-Wett won’t induce rooting of non-rooting species or cultivars. There are also several accounts from commercial propagators of more rapid root generation using Cell-U-Wett. This was not evaluated in this experiment.

Overall, rooting results using CELL treatments were identical to those achieved with fresh-made WATER treatments. The practical advantages of Cell-U-Wett alone should made water obsolete as the dilutant for alcohol-based auxin solutions.

Table 1. Fresh weight (g) of roots of three landscape plant species propagated with three concentrations of auxin prepared with Cell-U-Wett or water. Means with different letters are significantly different (P = 0.05 level) within species.

Auxin concentration	Dilutant	Loropetalum	Photinia	Pittosporum
1875 ppm	Water	.2802 c	.3407 b	1.655 a
	Cell-U-Wett	.3042 c	.2788 b	1.343 b
3750 ppm	Water	.4507 a	.6617 a	1.198 b
	Cell-U-Wett	.4301 ab	.7304 a	1.191 b
5000 ppm	Water	.3283 bc	.3791 b	1.137 b
	Cell-U-Wett	.3603 bc	.8275 a	1.113 b

Table 2. Dry weight (mg) of roots of three landscape plant species propagated with three concentrations of auxin prepared with Cell-U-Wett or water. Means with different letters are significantly different (P = 0.05 level) within species.

Auxin concentration	Dilutant	Loropetalum	Photinia	Ligustrum
1975 ppm	Water	60.25 c	61.48 b	194.79 bc
	Cell-U-Wett	62.53 c	61.19 b	187.79 c
3750 ppm	Water	131.8 a	132.99 a	230.02 a
	Cell-U-Wett	108.8 ab	147.02 a	221.63 ab
5000 ppm	Water	76.81 bc	70.90 b	232.49 a
	Cell-U-Wett	80.81 bc	158.29 a	212.40 abc

Influence of Commercial Auxin Formulations on Cuttings of Camellia Cultivars

Gene Blythe[1], Terry Denlay[2] , and Jeff L. Sibley[3]
[1]Monrovia Nursery Company, Azusa, CA 91702-1386
[2]Monrovia Nursery Company, Woodlake, CA 93286
[3]Department of Horticulture, Auburn University, AL 36849

Index Words: *Camellia japonica, Camellia sasanqua,* Auxins, Rooting Hormones, Cutting Propagation

Nature of Work: While most camellia cultivars and hybrids are produced commercially from cuttings (3) recommendations for appropriate auxin levels vary greatly (1,2,3). In this study cuttings of 20 cultivars of *Camellia* were obtained from container-grown production plants at Monrovia Nursery Company in Azusa, California. Semi-hardwood tip cuttings were prepared in early- to mid-May using firm, green wood following the spring flush of growth. Cuttings of *Camellia* hybrid'Freedom Bell' and *Camellia japonica* cultivars were prepared with a 2" stem, two mature leaves (with leaves cut in half), and a leafless node at the base. Cuttings of *Camellia sasanqua* cultivars were prepared with a 2.5" stem, three mature leaves, and a leafless node at the base. Knives were used to make all cuts. Cuttings received a quick basal dip into their respective auxin treatments and were stuck into 16" x 17" x 2 $^{1}/_{2}$" polypropylene cutting flats containing a 1:9 (v/v) peat/coarse perlite medium.

The three auxin treatments used in the trials consisted of 1. Dip 'N Grow® liquid (Astoria-Pacific, Clackamas, OR) diluted 1:9 (v/v) with a 50% methanol solution, providing 1000ppm IBA and 500ppm NAA; 2. Dip 'N Grow® liquid diluted 1:3 (v/v) with a 50% methanol solution, providing 2500ppm IBA and 1250ppm NAA; 3. Hormex No. 3 powder (Brooker Chemical, Chatsworth, CA), providing 3000ppm IBA. There were 200 cuttings per replicate (cutting flat) and 20 replicates per treatment per cultivar.

Cutting flats were placed in a randomized order on outdoor, concrete rooting beds in Azusa, California with 70°F bottom heat (supplied through June only) and 55% shade provided by overhead shade fabric. Cuttings received overhead mist with 1/4 E5 parasol nozzles (Spraying Systems, Wheaton, IQ provided with an 8-second duration and varying frequency (once every 6 to 60 minutes during daylight hours, depending on ambient temperature and wind, in order to keep cuttings slightly moist). Cuttings received fungicide sprays every two weeks. Overhead mist was discontinued after five months to acclimate the rooted cuttings. Rooting

percentages were determined after one additional month. Cuttings were considered rooted if the root systems were judged large enough to endure hand potting and subsequently provide high survivability and growth in liner pots.

Results and Discussion: Rooting percentages among the three treatments varied from one cultivar to another (Table 1). Cuttings of Camellia hybrid 'Freedom Bell' (a more challenging cultivar to root) produced the best results (52%) with Dip 'N Grow® 1: 9, compared to 40% for the other two treatments. Cuttings of C. Japonica 'Chandleri Elegans Variegated' and 'Elegans Splendor' rooted significantly better using Dip 'N Grow® 1: 3 and Hormex No. 3 (77% to 81 %) compared to Dip 'N Grow® 1: 9 (62% to 64%). Cuttings of 'Glen 40', and 'Silver Waves' produced better rooting percentages (76% to 79%) with Dip 'N Grow® 1: 3 than Hormex No. 3, but were similar to Dip 'N Grow® 1:9. Cuttings of C. *japonica* 'Debutante' and 'Spellbound' (two of the easier-toroot cultivars) rooted at 90% or higher for all three treatments, while 'Elizabeth Dowd Silver' rooted close to 80% for all three treatments. While some other treatments were similar, Hormex *No.* 3 produced the highest rooting percentages for cuttings of C. *japonica 'Nuccio's* Pearl', 'Pink Parade' and 'Magnoliaeflora' (84%, 95%, and 75%, respectively). Cuttings of C. *japonica* 'Nuccio's Jewel' and 'Shiro Chan' rooted better (at least 75% and 68%, respectively) with Dip'N Grow® 1:9 and Hormex No. 3 than with Dip 'N Grow® 1:3. Cuttings of the *C. sasanqua* cultivars (which are typically easier to root than *C. japonica* cultivars) produced rooting percentages that were quite close among the three treatments (generally 85% to 90%), with the exception of 'Hana-Jiman' for which Dip 'N Grow® 1:3 produced significantly better results (85%) than the other two treatments (75%).

Significance to the Industry: While general guidelines on the selection of commercial auxin formulations for cutting propagation of woody ornamentals are useful as a starting point, commercial propagators should not assume that that a single product or concentration is optimal for all cultivars within a genus, such as *Camellia.* With several *Camellia* species and so many cultivars in the trade, a clear-cut consensus as to the "best" auxin formulation and rate is not readily available. Also, the treatment that is optimal for the propagator at one nursery may not be optimal for the propagator at another nursery due to other factors that influence the rooting of cuttings, such as stock plant source, condition of cutting wood, time of year, cutting environment, etc. Some cultivars may respond equally well to a range of auxin treatments, allowing the propagator to select the product and concentration that is most economical, both in terms of product cost and efficiency of use. However, in

cases where cultivars exhibit varying responses to certain auxin treatments, propagators may wish to select the treatment that provides the best rooting response. In addition to rooting percentage, this optimal rooting response may also be determined by the size of the root systems, uniformity of root development, amount of callus, and other factors that may affect further development of the newly rooted plants. By conducting trials with the commercial auxin formulations available to them, commercial propagators may select the optimal treatments for their specific cultivars, growing conditions, and propagation environments.

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Table 1. Mean rooting percentages for cuttings of 20 *Camellia* cultivars treated with commercial auxin formulations.

Cultivar	Dip 'N Grow® 1:9	Dip 'N Grow® 1:3	Hormex No. 3
Camellia hybrid 'Freedom Bell'	52.4% a	39.7% b	39.5%b
Camellia japonica 'Chandleri Elegans Variegated'	62.0% b	76.8% a	81.2%a
Camellia japonica 'Colonel Fiery'	72.3% a	75.9% a	69.6% a
Camellia japonica 'Daikagura Variegated'	65.8% a	65.6% a	74.9% a
Camellia japonica 'Debutante'	89.6% a	89.9% a	93.6% a
Camellia japonica 'Elegans Splendor'	63.8% b	78.2% a	81.1% a
Camellia japonica 'Elizabeth Dowd Silver'	77.8% a	77.4% a	80.6% a
Camellia japonica 'Glen 40'	72.5% ab	77.9% a	66.6% b
Camellia japonica 'Magnoliaeflora'	60.5% b	69.9% ab	75.4% a
Camellia japonica 'Nuccio's Jewel'	78.9% a	64.6% b	74.7% a
Camellia japonica 'Nuccio's Pearl'	77.0% ab	73.8% b	83.5% a
Camellia japonica 'Pink Parade'	83.2% b	86.5% ab	95.2% a
Camellia japonica 'Shiro Chan'	68.5% a	57.4% b	67.5% a
Camellia japonica 'Silver Waves'	75.8% ab	79.3% a	68.3% b
Camellia japonica 'Spellbound'	95.4% a	93.8% a	95.3% a
Camellia sasanqua 'Bonanza'	91.4% a	88.8% a	88.7% a
Camellia sasanqua 'Hana-Jiman'	74.5% b	84.6% a	75.0% b
Camellia sasanqua 'Kanjiro'	90.9% a	92.0% a	92.5% a
Camellia sasanqua 'Shishi Gashira'	90.9% a	86.4% a	88.7% a
Camellia sasanqua 'Showa-No-Sakae'	90.8% a	83.6% b	87.8% ab

For each cultivar, means followed by the same letter are not significantly different at the 5% level.

Evaluation of Stem Cuttings During Cold Storage Utilizing Chlorophyll Fluorescence

Sarah E. Bruce and D. Bradley Rowe
Dept. of Horticulture, Michigan State University
East Lansing, MI 48824

Index words: Chlorophyll fluorescence, propagation, cold storage, *Taxus* sp.

Nature of Work: Traditionally, stem cuttings of *Taxus* sp. are collected between November and February, after natural exposure to cold temperatures (5). Today, most cuttings are taken during September or October, often before the first frost, stored at 2 - 5°C (35 - 41°F), and stuck a month or so later (4). This process enables propagators to adjust production schedules to coincide with a seasonal work force and hospitable weather conditions. It also ensures sufficient cold duration and consistency among cuttings because propagators can control the cold exposure cuttings receive. Since storage conditions are a major factor in cutting quality and subsequent rooting, a method of quantifying cutting health would be advantageous. A potential method is via chlorophyll fluorescence measurements.

Chlorophyll fluorescence is the small portion of light that is re-emitted from chlorophyll during photosynthesis. It is an estimation of photosynthetic efficiency and, in turn, provides an indirect measure of plant stress, potentially measuring detrimental plant stress levels before they are visible to the naked eye. Previous research includes detection/evaluation of such environmental stresses as cold (6), heat (3), and water (2), as well as nutrient deficiencies, irradiance levels, and air pollution. One study involved the use of chlorophyll fluorescence in conventional propagation by stem cuttings (1), but there are none pertaining to storage conditions of cuttings. Therefore, the objective of this study was to examine the effect of storage temperature, desiccation, and storage duration in four cultivars of *Taxus* x *media*, quantifying stress in relation to subsequent rooting using chlorophyll fluorescence measurements.

Seven hundred and twenty, 15 cm (5.9 in) cuttings were collected from each of four cultivars of *Taxus* x *media* (Brownii, Dark Green Spreader, Hicksii, and Wardii) on Oct. 14, from fields at Zelenka Nursery, Grand Haven, Mich. Cuttings were randomly divided into three desiccation treatments [sealed plastic bags (low desiccation), sealed plastic bags with holes punched in them (medium desiccation), and open plastic bags (high desiccation)] in combination with five temperature treatments [0, 2.5, 5, 10, and 20°C (32, 36, 41, 50, and 68°F)].

After 34 days in storage, half the cuttings were recut to 11.4 cm (4.5 in), treated with a 1.03% IBA, 0.66% NAA mixture (Woods Rooting Hormone, Earth Science Products Corp., Wilsonville, Ore.) at 2800 ppm (1:5 ratio), and placed in 100% perlite media within a greenhouse. The experimental layout consisted of a split-plot design with three splits (duration, cultivar, and desiccation) and four blocks. Sixty-five days after the original cutting collection (Dec. 18), the remainder of the cuttings were removed from storage and planted similarly to the first set. All cuttings were watered as needed until spring. At harvest, June 11-18, cuttings were gently uprooted and the roots were dried at 28°C (82°F) for 3 days and weighed.

Chlorophyll fluorescence (F_v/F_m) measurements were taken of storage material at cutting collection, 10 per cultivar, and then 5 per treatment at 24, 35, 50, and 65 day storage duration (Oct. 14, Nov. 7, Nov. 18, Dec. 3, and Dec. 18, respectively). Measurements were taken with a Morgan CF-1000 fluorometer (P.K. Morgan Instruments, Inc., Andover, MA) as described previously (1). Harvest and F_v/F_m data were subjected to analysis of variance (ANOVA) and LSD tests performed overall and at the individual cultivar level. Correlation calculations were done between harvest and F_v/F_m data (Proc anova, proc corr, SAS Institute Inc., Cary, NC).

Results and Discussion: At low desiccation, F_v/F_m decreased only in the 20°C (68°F) treatment, the decrease visible by day 35 (Fig. 1). At medium and high desiccations, however, a decrease in F_v/F_m in the 20°C (68°F) treatment can be detected by day 24, and a decrease in the 10°C (50°F) treatment by day 35 or 50. In general, 0, 2.5, and 5°C (32, 36, 41°F) showed almost no difference in F_v/F_m during storage. Wardii, as a cultivar, showed the most consistent F_v/F_m. Fluorescence correlated with temperature from day 24 in all cultivars (Table 1). Correlations also existed with rooting percentages in Dark Green Spreader (day 24 = 0.539, day 35 = 0.709), Hicksii (day 24 = 0.660, day 35 = 0.723), and at day 35 in Wardii (0.556). These correlations grew stronger with increased duration in storage.

Desiccation treatments reduced rooting percentages at higher temperatures [10, 20°C (50, 68°F)] in all cultivars, but caused minimal differences in root dry weights (data not presented). Low desiccation tended to decrease the negative effects of higher temperatures. Wardii and Hicksii showed some rooting in the 20°C (68 °F) treatments, but Brownii and Dark Green Spreader succumbed to fungal rot under these treatments. At lower temperatures (0 to 5°C) (32 to 41°F) desiccation treatments had little effect.

Storage temperature was negatively correlated with rooting percent in Brownii (-0.535), Dark Green Spreader (-0.842), and Hicksii (-0.766) (Table 1). In Hicksii, it was also correlated with root dry weight (-0.722). Rooting percentages for Dark Green Spreader at low and medium desiccation treatments were similar at all temperatures where rooting occurred. However, at high desiccation, 0 and 2.5°C (32 and 36°F) produced the highest rooting data. In Hicksii, increasing temperature decreased root dry weight while temperature had little effect on Wardii. Overall, rooting percentages decreased with increasing temperatures at medium and high desiccations (data not presented). The highest root numbers and dry weights were produced at 0°C (32°F).

Significance to Industry: Chlorophyll fluorescence measurements can detect damage caused by adverse temperature and desiccation effects in *Taxus* cutting material by 3 weeks into storage. Since temperature and desiccation closely affect rooting, chlorophyll fluorescence can serve as an indicator of rooting in extreme temperature and desiccation conditions. However, at low desiccation, chlorophyll fluorescence failed to consistently distinguish between 0, 2.5, 5, and 10°C (32, 36, 41, and 50°F) treatments, a range within which there are clear rooting differences. Factors causing decreases in rooting of *Taxus* as a result of these stresses do not affect plant photosynthetic efficiency in a comparative manner. Chlorophyll fluorescence values, as measured by F_v/F_m ratios, are not reliable predictors of rooting in *Taxus*.

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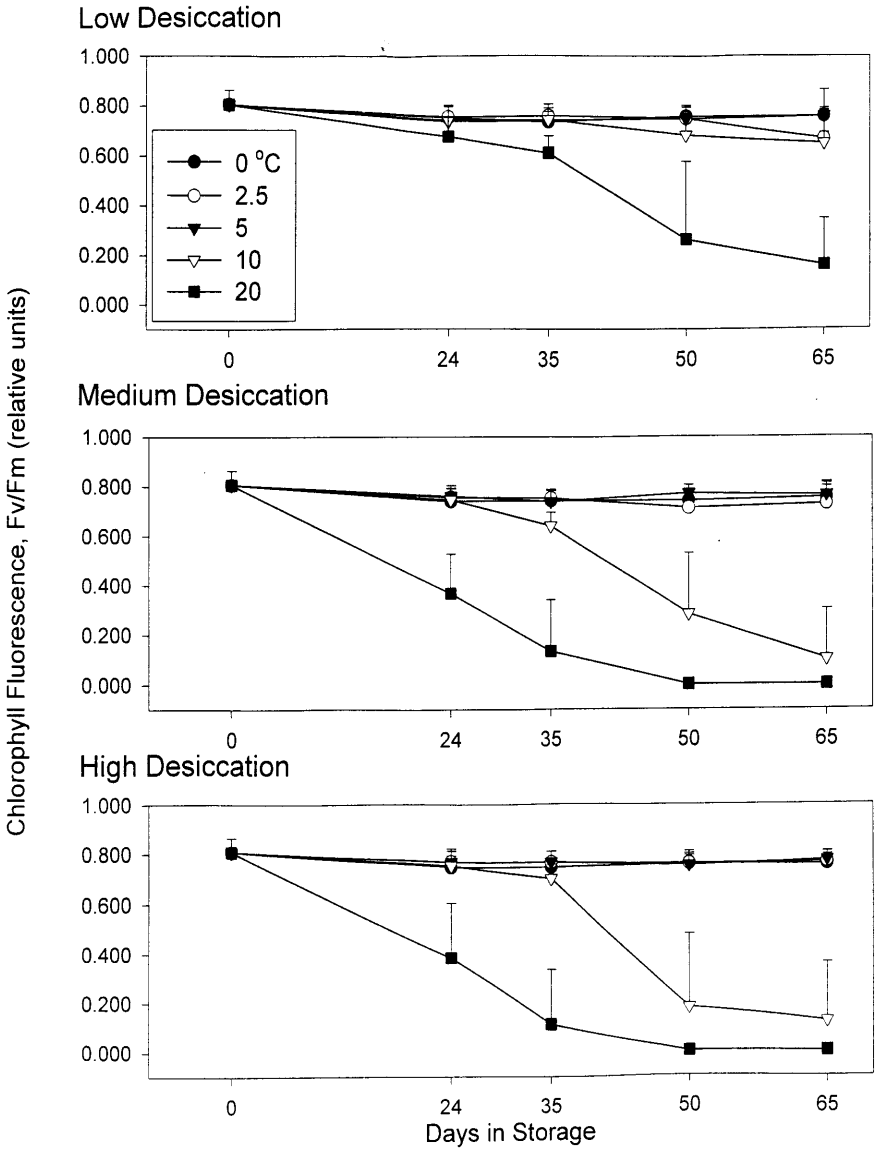
Table 1. Correlations of chlorophyll fluorescence (F_v/F_m) and storage temperature with rooting percentage of cuttings of *Taxus* (Storage duration = 34 days).

	Brownii	DGS	Hicksii	Wardii
F_v/F_m Day 24	0.286	0.539*	0.660**	0.502
F_v/F_m Day 35	0.361	0.709**	0.723**	0.556*
Temperature	-0.535*	-0.842**	-0.766**	-0.494

*, ** Significant at P <= 0.05 or 0.01, respectively
DGS = Dark Green Spreader

Fig.1. Effect of storage temperature and desiccation on chlorophyll fluorescence of cuttings of *Taxus*. The first point of each line represents a mean of 40 readings, all other points represent means of 80 readings. Vertical bars represent + SE.



Mycorrhizal Fungi Enhance Growth and Nutrient Uptake of Prickly-Pear Cactus (*Opuntia albicarpa* Scheinvar cv. 'Reyna') Plantlets After *ex vitro* Transplantation.

Andres Estrada-Luna and Fred T. Davies Jr.
Department of Horticultural Sciences. Texas A&M University, College Station, TX 77843-2133. USA

Nature of Work: We studied the effects of a Mexican arbuscular mycorrhizal (AMF) isolate [composed of *Glomus albidum, G. diaphanum,* and *G. claroides*] and low phosphate supply [0 and 11μg P ml^{-1}] on growth and nutrient uptake of micropropagated prickly-pear cactus (*Opuntia albicarpa* Scheinvar cv. Reyna) plantlets. After seven months of glasshouse culture, there was 100% survival of the micropropagated plantlets. Mycorrhizal colonization occurred rapidly within 5 days of inoculation, with the development of internal hyphae in root cortical cells. At the end of the study, high levels of colonization were observed (48 to 54%). Plantlets transferred to soil began to actively grow with no lag phase. However, plant growth rate was significantly affected by treatments. Poorest plantlet growth occurred with non-colonized plantlets that lacked P supply. In contrast, the combination of mycorrhizal colonization and supplementary P significantly increased shoot length, shoot and root DM and surface area of the plantlets. AMF enhanced nutrient uptake of P and Zn in the cladodes. We conclude that AMF can be used as a biotechnological tool that allows more efficient, low P input to enhance ex *vitro* transplantation of O. *albicarpa.*

Prickly-pear cactus [(PPC) *Opuntia* spp. Cactaceae] is native to the Western Hemisphere. PPC has been disseminated worldwide in arid and semiarid regions and is the most commercially important cacti (Barbera 1995). This plant species is commonly propagated via vegetative propagation of cladodes (Lazcano, et al., 1999). However, micropropagation offers more advantages with greater multiplication rates, higher quality with more uniform plant size (Escobar et al. 1986, Estrada-Luna 1988). Micropropagated PPC plantlets are hindered by slow growth rates during the first several months after *in vitro* production, particularly if establishment is during low temperatures and short-day conditions (Calderón 1995).

Colonization with arbuscular mycorrhiza (AMF) can be beneficial in enhancing overall plant growth in micropropagated, slower growing woody species (Fortuna et al. 1996, Azcón-Aguilar and Barea 1997). AMF benefits are important for plantlets that will be grown under field conditions in which abiotic and biotic constraints are common.

To our knowledge, no research has been done on AMF enhancement of micropropagated PPC plantlets. The objectives of the study were to assess root colonization by AMF and to evaluate the effects of a selected Mexican mycorrhiza isolate, which originated from a site utilized in commercial production of PPC. We also evaluated more efficient, low P inputs on growth and nutrient uptake of colonized PPC plantlets.

Results and Discussion: After micropropagation, all PPC plantlets survived *ex vitro* transplantation. However, AMF and P fertilization regime directly affected overall plant growth of the plantlets. Across all P levels, AMF significantly increased shoot length, total plant surface area, root, shoot and total plant DM compared to NonAMF plantlets.

Across all AMF levels, supplementary P significantly enhanced shoot length, total plant surface area, root, shoot and total plant DM. AMF and P level affected cladode tissue elemental concentration of the plantlets. In general, AMF had increased concentrations of cladode tissue P and Zn, but had lower K, Ca, Mg, Fe, Mn, and Cu than NonAMF plantlets. Supplementary P supply enhanced P and B uptake in the cladodes, however, lower concentrations of K, Ca, Mg, Fe, Mn, and Cu, and Zn occurred compared to treatments lacking supplementary P supply.

Mycorrhizal colonization occurred rapidly in PPC plantlets within five days after inoculation with the development of intraradical hyphae in the root cortical cells. Development of vesicles and endospores occurred within 10 days of inoculation. By the time the experiment was terminated (210 days), the NonAMF plantlets remained free of mycorrhiza, while extensive colonization was observed in AMF plantlets

Significance to Industry: The nursery industry stands to benefit from naturally occurring mycorrhizal symbionts that potentially help reduce fertility and irrigation needs and reduce pesticide usage during production. This is the first report of AMF enhancement of tissue culture produced PPC plantlets during acclimatization and *ex vitro* transplantation. We demonstrate that AMF can be used as a biotechnological tool that allows more rapid growth and more efficient low P usage to enhance *ex vitro* transplanting. The benefits of VAM symbiosis are of interest for a low input-sustainable agricultural system both at the nursery production stage and field production stage. In summary, AMF plantlets were extensively colonized. Low P supply stimulated mycorrhizal colonization. There is great potential in using selected AMF isolate for improving growth of slow-growing, micropropagated PPC plantlets during field establishment of commercial orchards. This has application to woody plant commercial nursery production systems in the US .

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Seed Germination of *Rhododendron chapmanii*: Influence of Light and Temperature

Luis O. Arocha, Frank A. Blazich, Stuart L. Warren, Mack Thetford, and James B. Berry
Dept. Of Horticultural Science, North Carolina State University, Raleigh, NC 27695-7609

Index Words: Sexual Propagation, Chapman's Rhododendron, Ericaceae, Native Plants, Rare and Endangered Species

Nature of Work: Seeds of *Rhododendron chapmanii* A. Gray (Chapman's rhododendron), a rare and endangered species, were germinated at 25^{0}C(77^{0}F) or 8/16 hr thermoperiods of 25/15^{0}C(77/59^{0}F) or 30/20^{0}C(86/68°F) with daily photoperiods of 0 (total darkness), 1/4, 1/2, 1, 2, 4, 8, 12 or 24 hr (1).

Results and Discussion: For all temperatures, no germination occurred during a 30-day period for seeds not subjected to light. At 25^{0}C(77^{0}F) germination was a function of photoperiod. The longer the photoperiod the greater the germination with 30-day germination $\geq$ 90% for photoperiods $\geq$ 8 hr. Alternating temperatures, particularly 25/15^{0}C(77/59^{0}F) enhanced germination when light was limiting. The highest germination (80%) by day 30 for seeds at 30/20^{0}C(86/68^{0}F) was realized with a 24-hr photoperiod, whereas 30-day germination $\geq$ 90% occurred at 25^0/15^{0}C (77/59^{0}F) and 25^{0}C(77^{0}F) for photoperiods of 8 and 12 hr and 8, 12, and 24 hr, respectively.

Significance to Industry: Seeds of *Rhododendron chapmanii* were relatively easy to germinate although light was required. Photoperiods that maximized germination varied depending on the temperature. Germination $\geq$ 90% occurred at 25/15^{0}C(77/59^{0}F) and 25^{0}C(77^{0}F) for particular photoperiods whereas 80% germination occurred at 30/20^{0}C(86/68^{0}F) for seeds subjected to constant light. When propagating this plant by seeds, seeds should be simply dusted on the surface of the germinating medium because of their small size [approximately 815,000 seeds per 28g (1 oz)] and light requirement.

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Vermicompost and Coir Enhance Germination of *Echinacea purpurea*

Sara Michelle Wills and Gary R. Bachman
Tennessee Technological University, School of Agriculture, Cookeville, TN 38505

Index Words: *Echinacea purpurea*, vermicompost, coir

Introduction: The horticulture industry is always looking for alternative soilless media components. Earthworm casts and coconut pith are two such materials. Earthworms casts or vermicompost (VC) have long been reported to have beneficial effects on plant growth. Charles Darwin conducted extensive research on VC and plant growth (2). Addition of vermicompost to soilless media is reported to improve water holding, nutrient content, and enhance seed germination and development through the action of plant hormone-like substances (3, 6). Growth increases up to a 40% have been reported for *Tagetes patula* and *Solanum lycospersicon* (1) while *Juniperus communis, Chamaecyparis lawsoniana, and Elaeagnus pungens* had up to 50% greater growth (4) with the addition of VC in the growing media.

Coconut pith or coir is the waste material left after the long fibers have been removed from coconut husks and is considered a viable substitute for peat. It is similar in appearance and physical properties such as water holding and drainage. Use of coir in growing media is reported to affect seed germination of woody plants. Germination percentages of *Magnolia, Rhamnus, and Callicarpa* were greater in 100% coir media versus coir media amended with perlite/vermiculite or a commercial peat-based media (5). The objective of this research was to evaluate the germination effects of vermicompost amended coir media on *Echinacea purpurea* 'Bravado' seed having varying viability.

Three sources of varying viability of *Echinacea purpurea* 'Bravado' seed were used for the experimental purposes. Seeds were field-collected in 1998 and 1999 and were stored in paper bags at room temperature. Additionally, commercial seed was purchased (Park Seed Company, Greenwood, SC) and was considered stored in optimal conditions. Field-collected seeds were cleaned using a Waring blender pulsed at low speed to loosen seeds from seed heads. Removed seeds were separated from the chafe using USA Standard Testing Sieve No. 7.

Seed viability from each seed source was determined using a "ragdoll" germination test. Paper towels were moistened and 25 seeds from each

source were placed on the towels and rolled. The “ragdolls” were put in plastic bags and placed in a 28C (82F) incubator. This was replicated four times. Germination was determined at 7, 11, and 18 days after treatment (DAT). After 18 DAT, the germination percentages were 79%, 67%, and 1% for the commercial, 1999 field-collected, and 1998 field-collected seed, respectively. This resulted in seed viability treatments being high, medium, and low.

The growing media was coir-coconut fibers (Crystal Company, St. Louis, MO) and vermiculite mixed at three different formulations: 100% coir, 90/10% coir/vermiculite, 70/30% coir/vermiculite. Half of each coir/vermiculite formulation was amended with vermicompost at 10% by volume. This resulted in six different media formulations. Seed from each seed source was sown into each media formulation using 128 plug trays.

Germination was checked daily beginning seven days after placing plug trays under mist system. Seeds were considered germinated when cotyledons had fully emerged from the media. Daily counts continued for twenty-four days after treatment initiation. All data were analyzed using Analysis of Variance (ANOVA) and significant data were further separated using Least Significant Difference (LSD), P=0.05.

Results and Discussion: Seed germination rates of the three seed sources were established across all treatments. The Park seed lot had the highest germination rates followed by the 99 field collected and the 98 field-collected. These germination results were corroborated by the ragdoll germination tests that were performed on the seed lots.

The addition of vermicompost had a significant effect on the seed germination of all three seed sources (Table 1). Twelve days after initiation, VC amendment increased germination for 1998 field-collected (100% coir), 1999 field-collected (100%coir, 90/10 coir/vermiculite) and commercial seed (100 coir, 90/10 and 70/30 coir/vermiculite). At the project conclusion, 24 DAT, germination was increased in 100% coir and 90/10 coir/vermiculite amended with VC for all three seed sources.

There were germination differences between the three media formulations. For the 1998 and 1999 field-collected seed, the 100% coir and 90% coir-10% vermiculite formulation had significantly greater germination than the 70% coir-30% vermiculite (Table 2). However, germination of the Park seed was significantly greater in 90% coir-10% vermiculite and 70% coir-30% vermiculite when compared to the 100% coir.

Significance to the Industry: Though the 1998 field collected seeds had extremely low germination, the addition of vermicompost or higher percentages of coir did promote greater germination. This is important when considering seeds that have low viability, whether from improper storage or natural causes. Germination percentages of other plant species seeds having inhibited viability may be increased with the addition of vermicompost and/or coir fiber to the growing media. More research needs to be conducted to further elucidate the effects of vermicompost and coir on seed germination.

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Table 1. Germination of *Echinacea purpurea* 'Bravado' seed of differing viability in three coir-based media formulations amended with vermicompost 12 and 24 days after treatment initiation.

Media Formulation	1998 Seed		1999 Seed		Commercial Seed	
	12 DAT	24 DAT	12 DAT	24 DAT	12 DAT	24 DAT
100% Coir	0 b[z]	0 b	41 b	64 b	25 c	56 b
100% Coir + VC	4 a	6 a	64 a	85 a	55 b	80 a
90%Coir/10%Vermiculite	0 b	1 b	39 b	67 b	41 b	65 b
90%Coir10%Vermiculite + VC	0 b	5 a	67 a	82 a	79 a	91 a
70%Coir/30%Vermiculite	0 b	2 b	32 b	62 b	29 c	77 ab
70%Coir/30Vermiculite + VC	1 b	2 b	24 b	59 b	67 a	87 a

[z]Mean separation within columns by least significant difference (LSD), P=0.05.

Table 2. Mean germination of *Echinacea purpurea* 'Bravado' seed of differing viability in three coir-based media formulations regardless of vermicompost amendment.

Media Formulation	Seed Viability Treatments		
	1998	1999	Commercial Seed
100% Coir	2.1 a[z]	56.7 a	46.9 b
90%Coir/10%PV	1.6 a	54.3 a	62.9 a
70%Coir/30%PV	0.9 b	39.5 b	60.4 a

[z]Mean separation within columns by least significant difference (LSD), P=0.05.

Design and Construction of a Recirculating Subirrigation System for Propagation.

James S. Owen, Jr., Brian K. Maynard, and William A. Johnson.
University of Rhode Island, Kingston, Rhode Island 02881

Index Words: Subirrigation, *Ilex glabra,* Rooting Environment

Nature of Work: Recirculating subirrigation is a hybrid of hydropropagation (Boland and Hanger, 1991) and subirrigation (Holt et al., 1998) that offers exceptional control of the rooting environment. Subirrigation is a recent innovation which has shown promising results for rooting several woody species (Aiello and Graves, 1998; Giroux et al., 1999). Cuttings are successfully propagated without mist by water provided through capillary action from a water reservoir maintained in the propagation tray. Subirrigation propagation can alleviate problems associated with mist, such as nutrient leaching, medium drainage, pathogen control, and inadequate environmental monitoring (Wells, 1965).

Recirculating subirrigation adds to subirrigation the advantages of hydropropagation, a nutrient film technique (NFT) type system, by giving the propagator control of exogenous auxin concentration, nutrients, temperature and aeration, each of which have been shown to affect root number and root length on various species (Wilkinson, 1993). Recirculating subirrigation requires little monitoring while allowing control of the rooting environment, and may increase rooting success of “hard-to-root” species, reduce costs, increase ease of compliance with OSHA/EPA regulations and allow chemical application with reduced employee exposure. In the future, recirculating subirrigation might be installed affordably into existing greenhouses by filling modified ebb-and-flow benches with perlite rooting medium.

Experimental recirculating subirrigation units were designed and constructed at the University of Rhode Island using 125 L (33 gal) Sterilite© totes (Sterilite Corp., Townsend, MA) as the water reservoirs and modified 26.4 L (7 gal) Rubbermaid® storage boxes (Rubbermaid Inc., Wooster, OH) as propagation trays (Fig. 1). Water heated by 100 watt Rena Cal Top Light Excel Aquarium Heaters™ (Aquarium Pharmaceuticals, Inc., Chalfont, PA) was recirculated using one 402 Powerhead™ Pump (Rolf C. Hagen Corp., Mansfield, MA) through $^1/_2$” polyethylene pipe. Perlite (Whittemore Co., Inc., Lawrence, MA), sifted through 8-mesh hardware screen was used as the rooting medium. In the center of the propagation tray a 44.5 cm (17.5 in) perforated length of schedule 80 PVC, covered with screen (1 mm mesh), was suspended in perlite to drain and control water table height.

Preliminary experiments were conducted in a greenhouse between February 22 and March 27, using a split-plot design. Three recirculating subirrigation experimental units were used to determine if *Ilex glabra* (inkberry holly) could be successfully propagated without any overhead irrigation during root initiation and root development. Three sets of eighteen inkberry holly cuttings were collected and treated with a basal quick dip of 0, 5, or 20 mM aqueous solutions of indole-3-butyric acid, potassium salt (K-IBA). Basal temperature was maintained at 22°C (72°F), 29°C (85°F) or 34°C (93°F). Water temperatures and the greenhouse environment were monitored throughout the trial using a 21X(L) Micrologger™ (Campbell Scientific Inc., Logan, UT) with thermocouples, relative humidity and quantum light sensors. After 34 days cuttings were harvested and rooting percent, root number, and root length data collected.

Results and Discussion: Within all three treatments the percentage rooting ranged from 50% to 100%. As stem base temperature increased cuttings treated with 5 mM or 20 mM K-IBA had more and longer roots, ranging from 32-76 roots per rooted cutting, and 16 to 24 mm length of longest root (Fig. 2). Inkberry holly cuttings rooted with only tap water and a stem base temperature of 22°C, rooted 100% and had an average of 38 roots with a longest root length of 9 mm. At higher temperatures 50% of cuttings treated with only tap water rooted, had the lowest average root number, 12 roots per rooted cutting with a longest root length of only 6 mm. Cuttings treated with 20 mM K-IBA with a stem base temperature of 34°C had an higher average root number, 76 roots per rooted cutting, that averaged 24 mm in length. Cuttings treated with either 5 or 20 mM K-IBA responded positively to increasing root zone temperatures, both rooting 100% with the largest average number of roots at 34°C.

In conclusion, recirculating subirrigation may be used to root semi-hardwood cuttings of *Ilex glabra* successfully within 34 days without any overhead irrigation. Rooting success varied with hormone treatments and basal temperatures. Root temperature had a strong affect on root development. Root number and length increased as root temperature and auxin concentration increased. Further studies are needed to better evaluate the interaction of basal temperature and auxin application in a range of woody taxa.

Significance to the Industry: Using recirculating subirrigation a propagator can avoid mist problems and achieve greater control of the rooting environment. In addition, propagators can safely apply chemicals (fungicides and growth regulators) while reducing exposure, enhancing environmental quality and reducing water use. This technique of

propagation could also improve knowledge of plant stress, nutrient, and temperature-auxin interactions on rooting success of cuttings. This could, in turn, minimize inputs used in propagation while maintaining or improving rooting percentages and cutting quality.

Rhode Island Agriculture Experiment Station Contribution No. 3807

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Figure 1 Design of recirculating subirrigation experimental unit.

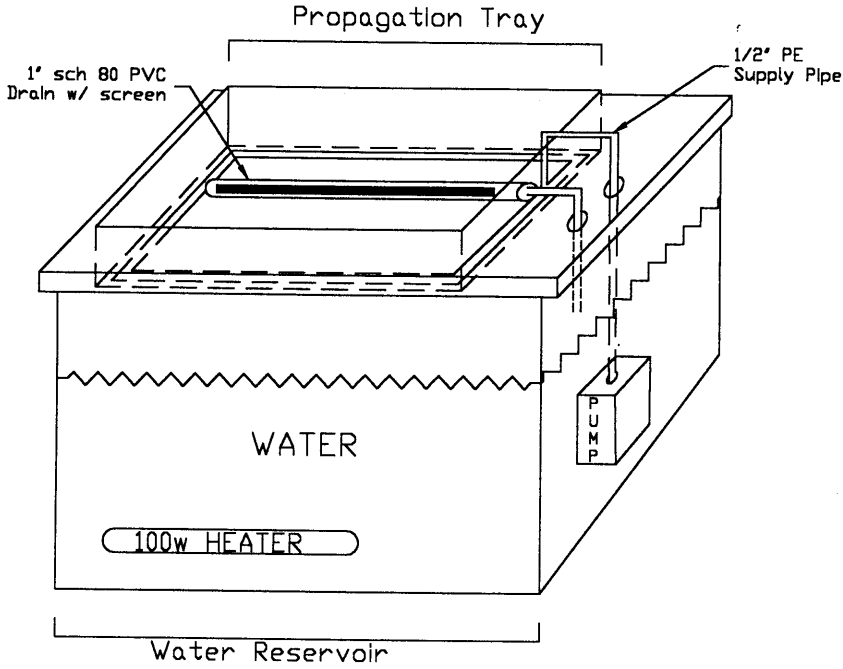
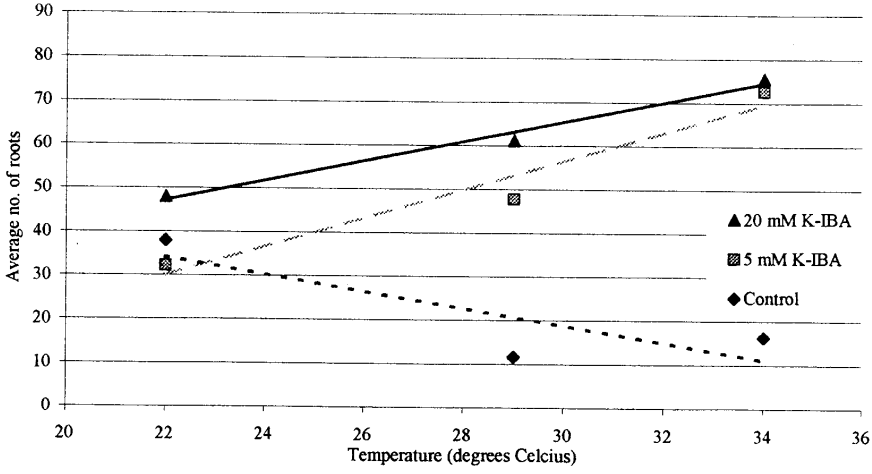


Figure 2 Rooting respose of *Ilex glabra* to three auxin concentrations and three temperatures while rooting for 34 days.



Effect of Timing and IBA on Rooting of Caddo Sugar Maple Stem Tip Cuttings

Clydette M Alsup and Janet C. Cole
Oklahoma State Univ., Dept. of Horticulture and Landscape Architecture, Stillwater, OK 74078-6027

Index Words: *Acer saccharum*, vegetative propagation, difficult-to-root ornamentals, IBA

Nature of Work: Sugar maples are an attractive landscape ornamental, prized for their strong wood, pleasing form and vivid autumn color. However, sugar maples grown in the Midwest are subject to extreme heat and high winds, which may result in leaf tatter and scorch (Conley et al., 1995). Caddo sugar maples, an ecotype native to the Red Rock Canyon in Caddo County, OK (Dent and Adams, 1983), are highly resistant to drought, leaf tatter and scorch (Pair, 1994).

Desirable selections of Caddo maples would be a valuable addition to the nursery industry; however, sugar maples are difficult to propagate. Seed propagation is common but sugar maples tend to be alternate bearing and seed coats are often empty (Dirr, 1998). Sexual propagation is further complicated because individual trees show variable traits such as fall foliage color. Vegetative propagation methods such as cuttings allow the cloning of trees with desirable characteristics, but often the desired characteristics are not evident until the trees are mature, and cuttings from mature trees are difficult to root. The objective of this study was to determine the optimum timing and rooting hormone concentration to produce rooted cuttings from mature Caddo sugar maples.

Cuttings were taken twice a month from May through October from three mature trees growing in Stillwater, OK. Cuttings consisted of the entire current season's growth, from the terminal bud to just below the basal node. The cuttings were immediately placed in tap water to prevent desiccation, then taken to a greenhouse and re-cut just below the lowest node. All cuttings were wounded by removing the lowest set of leaves and scraping bark from opposite sides of the lower 1 cm of the cutting. Four concentrations of indole-3-butyric acid (IBA) were used. The basal 1 cm of cuttings were dipped for 10 seconds in 0, 5,000, 10,000 or 15,000 ppm IBA. The IBA was dissolved in 50 ml of 70 percent isopropyl alcohol and tap water was used to bring the solution to 100 ml. Cuttings were stuck to a depth of about 5 cm in plastic rooting flats 16 cm wide x 24 cm long x 8 cm deep, in moist high-porosity medium, and kept in a polyethylene-covered greenhouse under natural photoperiod and

temperature ranging from about 60°F to 80°F. Flats were placed on raised benches equipped with nozzles placed 20 inches above the flats at 48-inch intervals. Shade cloth was placed over the greenhouse to prevent excessive heat during the warm season. Cuttings received mist irrigation between 0800 and 1800 HR daily. Mist cycles were adjusted as necessary to allow foliage to dry between misting (average 2 second duration every 2 minutes). Cuttings were evaluated for number and length of roots 12 weeks after sticking. Rooting was defined as the presence of at least one root on a cutting.

Results and Discussion: Root formation occurred only on cuttings taken early in the season; cuttings taken after June 1 did not root. Rooting percentage and estimated probability of rooting were greatest with cuttings taken in early May (Table 1). These cuttings were in the green softwood stem stage, when leaves had reached full size and were bright green, shoots had begun to stiffen slightly, and lenticels were pronounced. Similar visible characteristics led to maximum rooting of eastern sugar maples (Donnelly, 1977). The 5000 ppm IBA treatment resulted in optimum rooting for the green softwood cuttings taken on May 6 and 18, but cuttings taken on June 1, which had begun to harden and turn red at the base, responded best to 15000 ppm IBA. There was a significant interaction between date and IBA concentration on the estimated probability of rooting. On May 6, the probability of rooting was similar regardless of IBA concentration, but on May 18 and June 1 rooting improved as the IBA concentration increased.

The average number of roots per cutting increased as the IBA concentration increased (Table 2). Cuttings dipped in 0 ppm IBA had the shortest roots. Root length was greatest with the 5000 ppm IBA treatment, then decreased as the IBA concentration increased.

Significance to Industry: Caddo sugar maples possess outstanding ornamental and drought-resistant characteristics. Cutting propagation may allow commercial producers to clone desirable Caddo sugar maples. Optimum rooting was obtained with stem tip cuttings taken in the green softwood stage and treated with 5000 ppm IBA. Cuttings taken later in the season appear to require higher IBA concentrations to form roots.

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Table 1. Estimated and observed rooting percentage of Caddo sugar maple cuttings rooting on various dates when treated with four concentrations of IBA.

Date	IBA Treatment (ppm)	Rooting Percentage	
		Estimated[z]	Observed
May 6, 1999	0	31	14
	5000	33	54
	10000	34	38
	15000	36	28
May 18, 1999	0	9	2
	5000	13	30
	10000	19	18
	15000	26	18
June 1, 1999	0	2	2
	5000	4	2
	10000	9	8
	15000	19	22

[z]Estimated rooting percentage = 2.98 - 1.91D - 0.67I + 0.36DI, $P \leq 0.001$, where D = date and I = IBA, and date = 2, 3 or 4 for May 6, May 18 and June 1, respectively, and IBA = 1, 2, 3 or 4 for 0, 5000, 10000 and 15000 ppm, respectively.

Table 2. Number of roots and length of roots of Caddo sugar maple stem tip cuttings during three cutting dates in spring 1999 when treated with 0, 5000, 10000 and 15000 ppm IBA.

IBA Treatment (ppm)	Number of Roots	Length of Roots (cm)
0	0.07	0.36
5000	0.97	2.24
10000	0.85	1.87
15000	1.14	1.58
Linear	*	NS
Quadratic	NS	**
Cubic	NS	NS

NS denotes non-significant; * and ** denote significant at P=0.05 and P=0.01, respectively.

In Vitro Culture of *Gladiolus*

Willie Taylor, Sarabjit Bhatti, Deborah Long and Roger Sauve
Cooperative Agricultural Research Program, Tennessee State University, 3500 John A. Merritt Blvd., Nashville, TN 37209-1561

Index words: protoplasts, callus, regeneration, in vitro

Nature of work: The regeneration of plants from protoplasts offers the opportunity for the improvement of important agronomic and horticultural crops due to somaclonal variations. Commercially important floral bulbs have not yet been manipulated in culture to undergo plant regeneration from protoplasts. In *Gladiolus* micropropagation has occurred by the development of axillary buds into plantlets (Hussey, 1997). Regeneration of *Gladiolus* has been reported from floral explants (Ziv et al, 1970). The purpose of this preliminary study was to establish a new plant regeneration system from the protoplasts of *Gladiolus*. Tissue explants were obtained from the two *Gladiolus* cultivars, Jenny Lee and Peter Pear. Calli were initiated from either cormel slices or in vitro grown plantlets (Kamo et al, 1990) and maintained in vitro for 4 to 6 months in a liquid medium. Callus tissue was maintained on MS medium supplemented with 0.5 mg/l BA, 2 mg/l dicamba, 3% sucrose and 1% agar. Calli were transferred weekly and kept on a gyratory shaker at 120 rpm in the dark. For the isolation of protoplasts, callus tissue was placed in 60x15 mm plastic petri-dishes containing 5 ml of two different maceration media. The composition of these maceration media is shown in Table 1. The maceration media were adjusted to pH 5.7 and sterilized through a Millipore filter (Millex-HA, 0.45 um) before use. The calli were incubated for 16 hours on a reciprocal shaker at 25 r/min at 25^{0}C. The protoplasts were isolated by filtering through a single layer of Miracloth.(Calbiochem, U.S.A.) The filtering process was followed by 3 centrifugations at 100 g for 5 min in a 1/2 MS and 10% sorbitol solution. The protoplasts were suspended at final densities of 1x10^{5} and 5x10^{5} cells ml^{-1} of MS medium containing 0.3M sucrose, 0.3M sorbitol and 2 mg/l dicamba. All suspensions were cultured in this liquid medium in 60x15 mm plastic petri-dishes (Nanc, Denmark) and sealed with parafilm. After 21 days of incubation, a dilution solution (MS, 2 mg/l dicamba, and 10% sucrose) was added to the cultures to decrease osmotic pressure. The plates were incubated at room temperature in the dark. The cell division was recorded at 2, 7, and 14 days after incubation.

Results & Discussion: The quality of the source calli directly affected the yield and viability of protoplasts. It was already determined in previous studies that *Gladiolus* calli thrive in a liquid medium similar to

the one used in this study. The optimum concentration of enzymes used in the protoplast isolation medium has been determined. About 10^6 protoplasts were obtained from 1 gm of callus. It was observed that more frequent sub-culturing increased the yield of protoplasts for both the liquid and solid medium. The maceration medium II yielded viable protoplasts that divided and formed colonies. The protoplasts were viable up until 21 days as shown by the increasing rate of division (Table 2). Protoplast division rate was determined by dividing the number of protoplasts undergoing division by the original number of cultured protoplasts. Maceration medium I had very low protoplast yield because of incomplete maceration of cell walls, perhaps due to a low percentage of cellulase and the bursting of isolated protoplasts. The colonies that formed as a result of the dividing protoplasts will be used to regenerate plants.

Significance to Industry: Many of the recent advances in biotechnology for the improvement of plants have not been used with ornamentals. This technology has been used primarily for the development of new food and fiber plants. This study was conducted to develop a regeneration system for gladioli. The ability to isolate protoplasts and to regenerate plants from protoplasts is important for future experiments in genetic engineering.

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Table 1: Composition of the maceration media

	Medium I	Medium II
Cellulase	0.5%	1.0%
Macerozyme	0.5%	0.5%
Pectinase	0.1%	0.1%
Driselase	0.2%	0.1%

Table 2: Protoplast Division Rate

	Jenny Lee	Peter Pear
Day 2	15%	13%
Day 7	21%	22%
Day 14	33%	34%
Day 21	32%	34%

In Vitro Propagation of Japanese Flowering Cherry

A. Naseer Aziz, Roger J. Sauve and Jing-Tian Ling
Cooperative Agricultural Research Program, Tennessee State Univ.
3500 J. A. Merritt Blvd., Nashville, TN 37209-1561

Index Words: Tissue Culture, In vitro Germination, Prunus serrula, Paperbark Cherry, Flowering Cherry

Nature of Work: The flowering group of cherries is comprised of some 200 species in the genus *Prunus*. All have pink or white flowers with a delicate sweet scent. A garden with assorted species would begin to bloom with the onset of spring from mid to late April. Clark (1963) describes the beauty of inflorescence, foliage and bark of the flowering cherries depicting their garden ornaments for all seasons. Like other plants, flowering cherries are also prone to various diseases. Bacterial canker is a sporadic but economically important disease of several *Prunus* spp. (Liang *et al.*, 1994). Genetic improvement in current nursery stock is required to develop disease resistant varieties. Traditional breeding methods are long and difficult since they include controlled cross-pollination, seed isolation, germination, selection and vegetative propagation of superior stocks. Biotechniques for direct or mediated gene transfers and recovery of improved genotypes of elite cultivars can shorten the conventional breeding process (Miguel *et al.*, 1996). Genetic transformation depends on plant tissue culture techniques; therefore development of an in vitro shoot regeneration system greatly facilitates such process. This report attempts to establish an in vitro regeneration system for *Prunus serrula* commonly known as paperbark cherry.

For germination, surface sterilized and de-shelled *P. serrula* seeds were placed in 100, 250, and 500 mg/l each of GA3 (gibberellic acid), BA (benzyladenine), and their 1:1 combination, for 16 hours. Each treatment contained 30 seeds. The seeds were then placed on agar (1%) only media at 25°C and 18-hr photo-period (20-22 μmole/m^2/s). Shoot tips of the germinated seedlings were used to initiate in vitro cultures. Modified (Eliasson *et al.*, 1994) Murashige and Skoog (Murashige and Skoog, 1962) semi-solid (1% agar) media with varying concentrations of IBA (3-indole butyric acid) BA (benzyladenine), CPFPU (N<2-chloro-4 pyridyl>N'-<3,5-difluorophenyl>urea), 2IP (N6-<2-isopentyl>adenine), Kinetin and TDZ (thiadiazuron) were used to maintain the callus cultures (Table 1). Cultures were incubated at 31 °C (day) and 24 °C (night) with 16-hr photoperiod (20 (mole/m^2/s).

Results and Discussion: De-shelled *Prunus serrula* seeds started in vitro germination after 30 days of incubation. Germination resulted after only two growth regulator treatments, i.e., 500 mg/l GA + 500 mg/l BA

and 250 mg/l GA + 250 mg/l BA. Higher levels of growth regulators (500 mg/l GA + 500 mg/l BA) resulted in the germination of 14 seeds out of 30 (47% germination), whereas '250 mg/l GA + 250 mg/l BA' resulted in 13% (4 out 0f 30 seeds) germination. Shoot regeneration from callus cultures was obtained with about 30 days of incubation. Exogenous auxin levels were kept constant by providing 0.05 mg/l IBA in all media, while different concentrations of the cytokinins were used (Table 1). Higher shoot regeneration was obtained with 5 mg/l of BA and TDZ than their other levels (1.0 and 10 mg/l), while 1.0 mg/l of CPFPU, 2IP and Kinetin were found to promote good shoot regeneration (Table 1).

Significance to Industry: A disease free stock of the nursery plants can efficiently be propagated via in vitro techniques. Difficult-to-propagate nursery crops can be grown via in vitro germination techniques to develop pathogen-free plants. This work has established an in vitro seed germination and shoot regeneration protocol for *Prunus serrula.* The procedures described herein would enable nursery growers to maintain disease free paperbark cherry (*P. serrula*) stocks year-round. The results presented in this report would also facilitate genetic transformation efforts to produce disease resistant flowering cherries.

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Table 1. Percent shoot regeneration per number of *P. serrula* calli cultured on MS media containing varying concentrations of different growth regulators.

Growth regulators in semi-solid MS media	No. of calli cultured	Shoots formed (%)
0.05 mg/l IBA+ 1.0 mg/l BA	4	3 (75%)
0.05 mg/l IBA+ 5.0 mg/l BA	19	15 (79%)
0.05 mg/l IBA+ 10 mg/l BA	13	8 (61%)
0.05 mg/l IBA+ 1.0 mg/l CPFPU	8	7 (87%)
0.05 mg/l IBA+ 5.0 mg/l CPFPU	68	46 (68%)
0.05 mg/l IBA+ 10 mg/l CPFPU	9	7 (78%)
0.05 mg/l IBA+ 1.0 mg/l 2IP	30	22 (73%)
0.05 mg/l IBA+ 1.0 mg/l Kinetin	4	3 (75%)
0.05 mg/l IBA+ 5.0 mg/l TDZ	56	32 (57%)
0.05 mg/l IBA+ 10 mg/l TDZ	19	10 (53%)

Morphology and Predation of Redbud Seeds

W. E. Klingeman and M. S. Carrington
University of Tennessee, Department of Ornamental Horticulture and Landscape Design, P.O. Box 1071, Knoxville, TN 37901-1071

Index Words: *Gibbobruchus mimus*, *Cercis* spp., pest resistance, Bruchidae, Eulophidae, Eupelmidae, seed parasitism

Nature of Work: Eastern Redbuds (*Cercis canadensis* L.) are small- to medium-sized (20' to 35'), deciduous, native trees that present a profusion of rosy-pink to purplish blossoms along bare stems and trunks during March-April. Numerous cultivars of *C. canadensis* are available with attractive forms featuring white or pink flowers, leaf spot-resistance, or leathery leaves. Cultivars of the Chinese redbud, (*C. chinensis* Bunge), are also commercially available (3). Redbud trees produce abundant numbers of seeds, which are easily collected, but trees are notoriously difficult to propagate by stem or root cuttings (1, 3, 10).

In general, established redbuds are low maintenance trees. In late summer and fall, leaf spot diseases become common though seldom warrant controls. Stem and bole cankers often cause the decline and death of trees under stress (3). Redbuds are also subject to few arthropod pests. Problematic to U. S. producers of ornamental redbuds, however, are the Groundnut Seed Beetle, *Caryedon serratus* (Ol.) in the western U. S., and the Redbud Seed Beetle, *Gibbobruchus mimus* (Say), which occurs throughout the range of native *Cercis* sp. (7, 8, 9).

G. mimus is a 1.5 to 2 mm small, checkered brown, spherical beetle that lays its eggs individually or clustered on immature green pods of *Cercis* sp. trees. Behaviorally secretive, Redbud Seed Beetles are not readily observed on redbuds except during flowering. Yet this beetle is abundant throughout the eastern U.S., is active yearlong, and can infest large numbers of seeds (7, 9).

To better understand *G. mimus*' host range and preferences, fully developed seedpods of Eastern Redbud (*Cercis canadensis*), 4 *C. canadensis* cultivars, and 'Avondale' Chinese Redbud (*C. chinensis* 'Avondale') were collected from each of 6 trees in November 1999. Trees were part of a 10-yr old, massed, roadside planting located near the University of Tennessee in Knox County, TN. Seedpods were stored in a walk-in cooler maintained at 34(1°C until measurements could be taken. Measurements included pod length and width, seed number, number of pre-existing emergence holes, and seed size. Seeds were graded into size

classes using U. S. Standard Test Sieves such that Size 7 = ≥ 2.80 mm; Size 8 = 2.36 to 2.79 mm; Size 10 = 2.00 to 2.35 mm; and Size 12 = ≤ 1.99 mm diameter. Once sized, seeds were float-tested to ascertain seed soundness. Floating seeds were agitated to break the surface tension of the water. The viability of floating and sinking seeds were determined by excising up to 200 seeds per size class. Seeds were classed as either infested, dead or empty, having an emergence hole, or viable. Seeds were considered viable if they contained complete, yellow cotyledons and a visible endosperm.

Results and Discussion: Seedpod morphology among the selected species and cultivars of ornamental redbuds was varied and not surprisingly, seed numbers were increased in larger pods (Fig. 1). *Cercis canadensis* 'Forest Pansy' had the longest seedpods, which were comparable in length to *C. canadensis* (*sensu stricto*). The smallest seedpods were attributed to *C. canadensis* 'Rubye Atkinson' and *C. chinensis* 'Avondale' (Fig. 1). In contrast to length variability, pod widths measured across the widest part of the seedpod remained relatively consistent, regardless of tree type (Fig. 1). Mean seed numbers among 100 seedpods per tree also varied between cultivars (Fig. 2). *Cercis canadensis* 'Oklahoma' had more than 400 seeds per 100 pods with nearly all seeds larger than 2.8 mm. *C. canadensis* 'Alba', *C. canadensis* (*s.s.*), and *C. canadensis* 'Forest Pansy' also had over 300 seeds per 100 pods. However, seeds of *C. canadensis* 'Forest Pansy' were dark brown to black, had a wrinkled seed coat, and had no seeds larger than 2.79 mm (Fig. 2).

Successful germination of redbud seeds requires that eco- and endodormancy both be overcome (6). Scarification allowing seed imbibition must be followed by several months of chilling stratification (4, 5, 6, 10). Due to constraints of time, germination trials were not conducted. Seed viability was attributed if seeds possessed fully formed, yellow cotyledons and a visible endosperm. Excisions of seeds (n = 200) from the largest size classes revealed the highest levels of viability among seeds from *C. canadensis* 'Alba', *C. canadensis* 'Oklahoma', and *C. canadensis* (*s.s.*) trees (Fig. 3). Among all trees sampled, *C. canadensis* 'Oklahoma' had the fewest seed that were dead or empty upon dissection (Fig. 4D).

In addition to larvae and adults of *G. mimus*, redbud seeds were also found to include 2 or more species of parasitic Hymenoptera. Cushman (2) reared 3 parasitic hymenoptera from *G. mimus* collected in Texas. Among them were the Braconid, *Glyptolocastes bruchivorus* Cwfd., the Eupelmid *Cerambycobius cyaniceps* Ashm., and a Eulophid, *Horismenus*

sp. At time of submission, the hymenoptera reared from our Redbud Seed Beetles were not identified. Regardless, their emergence would render the seed non-viable. When visible emergence holes of wasp and beetle (Fig. 4B) and dissections of infested seeds (Fig. 4C) were combined, *C. canadensis* 'Oklahoma' was the least preferred host among the abundant seed producers. Overall, *C. canadensis* 'Rubye Atkinson' had the greatest number of emerged or infested seeds (Fig. 4B,C). By contrast, *C. chinensis* 'Avondale' seed was seldom infested and may be an accidental host or non-preferred by *G. mimus*. Too, *C. chinensis* 'Avondale' had the highest proportion of dead or empty seeds indicating either that flowers had not been successfully pollinated or that seed development was frequently aborted (Fig. 4D).

Significance to Industry: Redbud trees produce abundant numbers of seeds, which are easily collected, but trees are notoriously difficult to propagate by stem or root cuttings. Nursery production of named redbud cultivars relies on grafting buds or scions onto seedling rootstocks: a time-consuming and costly process. Eastern Redbud has been chosen as the TenneSelect® 2000 ornamental tree. Increased plant recognition, professional recommendations, and aggressive marketing are likely to stimulate consumer demand for these native trees. *C. canadensis* 'Oklahoma' had the most abundant, viable, and least infested seeds among the species and cultivars examined, followed by seeds of *C. canadensis* (*s.s.*) and *C. canadensis* 'Alba'. Using a U. S. Standard No. 7 Test Sieve, all but the largest redbud seeds of these trees can be separated and discarded. Submerging the larger seeds in water and removing the non-viable, floating seed hulls can further reduce the number of dead or empty seeds. This simple procedure will minimize the amount of time and resources expended on non-viable or empty seeds.

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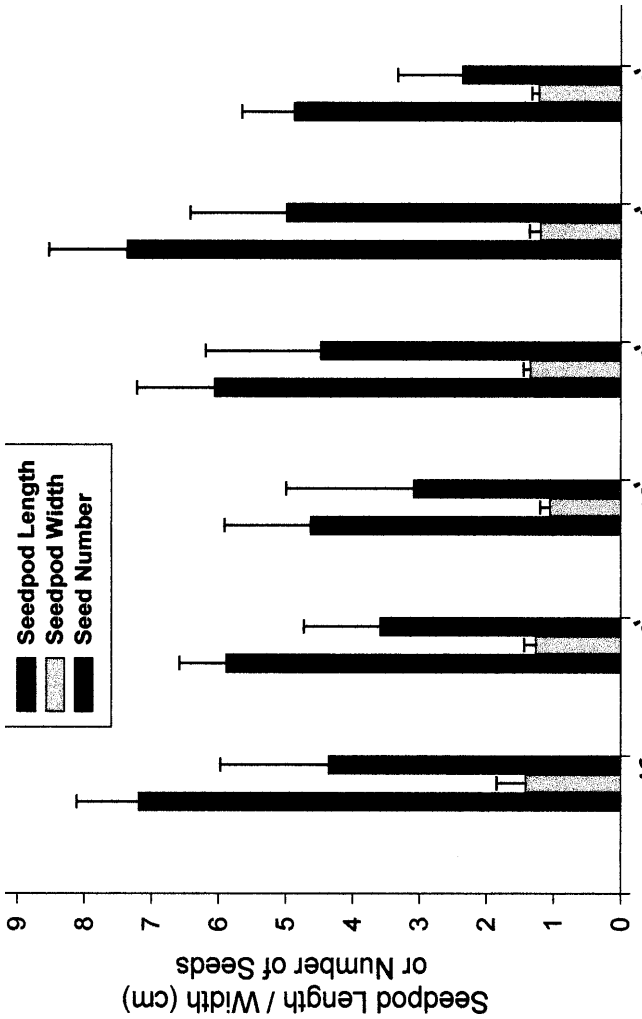

Figure 1. Morphology of Redbud Seedpods. Means (with standard deviation) represent measurements taken on 600 seedpods per species or cultivar

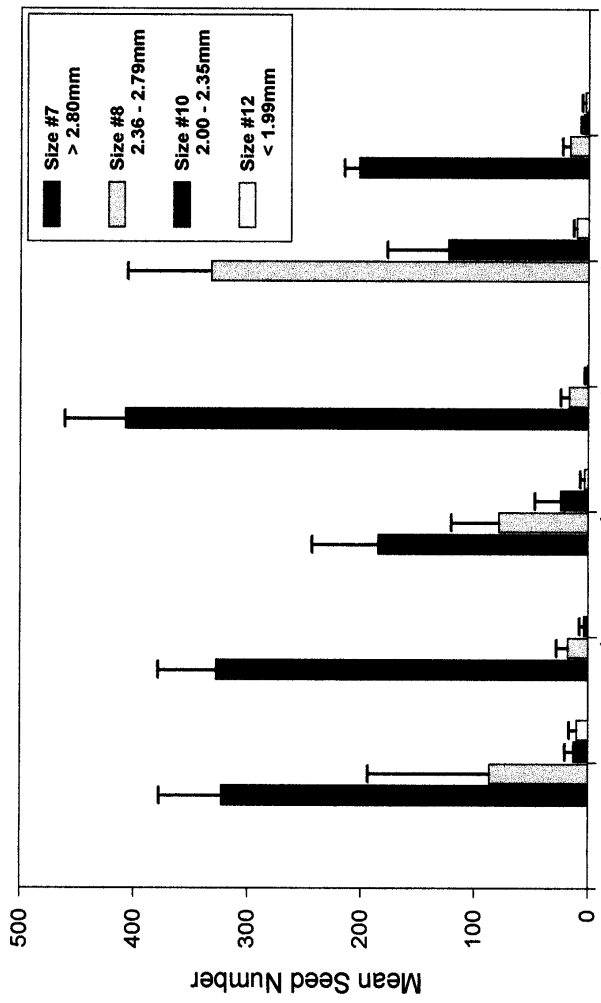

Figure 2. Redbud Seed Number and Size Classes. Means (with standard deviations) represent the number of seeds per 100 seedpods per tree. Six trees of each cultivar or species were sampled, except for *C. canadensis* 'Forest Pansy' (n = 5).

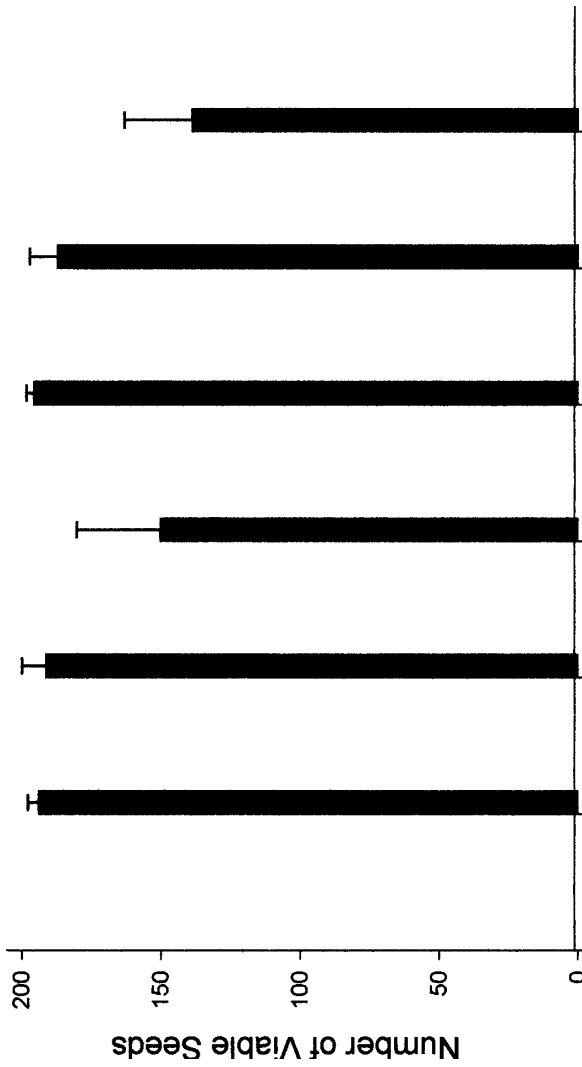

Figure 3. Viability of Redbud Seeds. Means (with standard deviations) represent seed viability among 200 of the largest, non-floating seeds per cultivar or species. Seeds are all Size Class #7 (< 2.80mm), except for *C. canadensis* 'Forest Pansy' (Size Class #8 = 2.36-2.79mm).

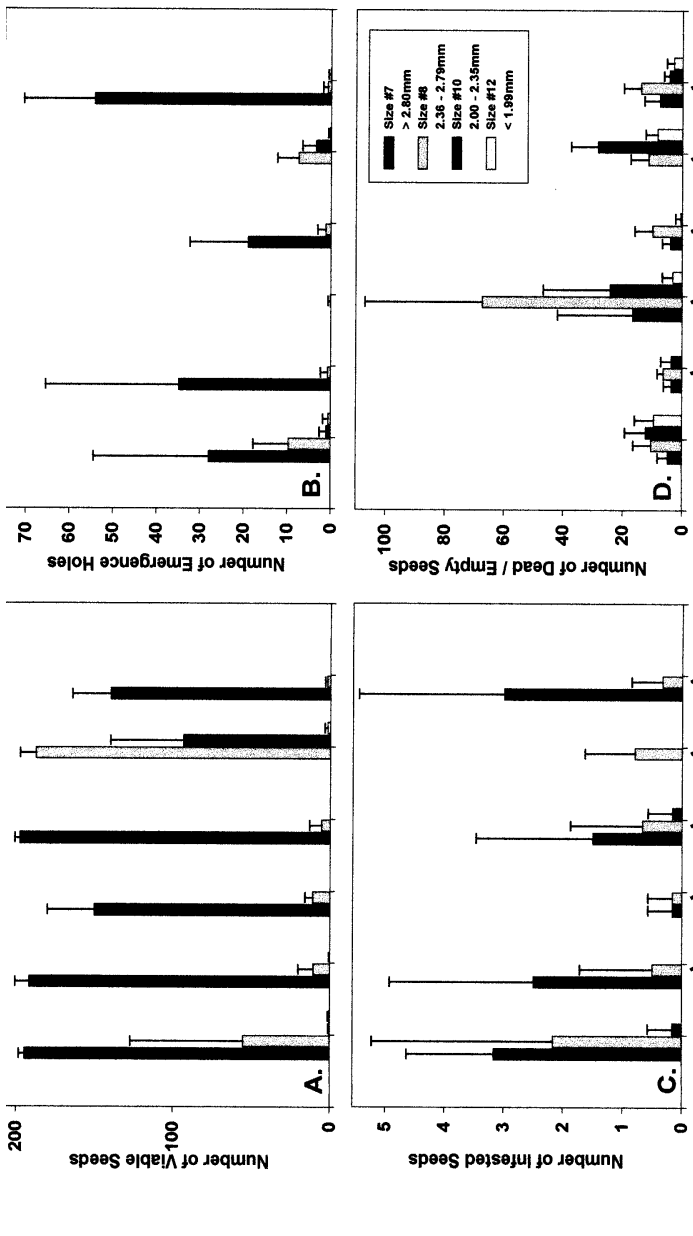

Figure 4. Status of Redbud Seeds. Numbers of viable (A.), emerged (B.), infested (C.), or dead / empty (D.) seeds within each size class. Means (with standard deviations) represent seed status based upon 100 seedpods per tree. Six trees of each cultivar or species were sampled, except for *C. canadensis* 'Forest Pansy' (n = 5).

Stratification of American Hornbeam *(Carpinus caroliniana* L.) Seeds

Lisa E. Richardson-Calfee and John W. Day
Tennessee

Nature of Work: American hombeam *(Carpinus caroliniana* L.) is a small, deciduous tree indigenous to much of the eastern United States. The sinewy texture of its smooth, gray bark resembles that of Fagus (beech) or a twisted, flexed muscle. *C caroliniana* is valued for its ornamental purposes in the naturalized landscape, resistance to insects and disease, and tolerance of wet conditions and various soil types. Its fall color is an "exceptionally showy burnt-orange or red to yellow" (Gilman, *1997).* In *1996,* American hombeam was listed as one of the seventh least available trees and one of the five species with the slowest growth rate among a list of *59* species that municipalities desired for street trees (Gamstetter and Gulick, *1996).* Grown from seed, it is considered one of the most difficult woody species to propagate. The U.S. Forest Service's book Seeds of Woody Plants in the U.S. recommends warm *(15/3* OC) stratification for *60* days followed by cold *(5C)* stratification for *60* days (Rudolf and Phipps, *1974).* Following this method, Bretzloff and Pellett *(1979)* reported 10% *germination.* They determined that lengthening the cold stratification period to *18* weeks increased *germination to 58%* and warm stratification (prior to cold stratification) had no effect on germination. The objective of this study was to compare the effects of various warm and cold stratification periods on germination.

One hundred and seventy *(170)* grams of *C caroliniana* seeds were obtained from F. W. Schumacher Co., Inc. (Sandwich, MA) in October *1996.* Seeds were kept in cold, dry storage *(4C)* until initiation of the experiment. Prior to the experiment, seeds were soaked in tap water at room temperature for *17* hours to separate viable seeds from nonviable seeds (Bretzloff and Pellett, *1979).* Floating seeds were assumed to be nonviable. Viable seeds were dusted with HiYield Captan Fungicide - 5*0%WP* (Voluntary Purchasing Groups, Inc., Bonham, TX) at a rate of *5.3* grams per kilogram of seed (1/2 teaspoon per I lb.) to decrease risk of fungal *contamination.*

Seeds were divided into groups of 10 and placed in sterile petri plates with approximately 1 cm of moistened, sterile sand. Seed treatments were initiated on November *1, 1996* (Table 1). The experimental design was a split plot design with main plots consisting of 4 warm stratification periods (0, 2, 4 or 8 weeks) and subplots consisting of 6 cold stratification periods (4, 8, 12, 16, 20 or 24 weeks). Five replications of each treatment with 10 seeds per experimental unit were employed.

During the warm stratification period, petri plates were kept in a cloth covered plastic tray in the greenhouse where temperatures were maintained between 22-26C. During cold stratification, seeds were stored in a refrigerator at 14C. Seeds were sprayed with sterile, deionized water when needed to reduce desiccation. Upon completion of cold stratification, petri plates were placed on a greenhouse bench where temperatures were maintained between 22-26C. Petri plates were checked daily for newly germinated seeds. To minimize error, germinated seeds were removed from the germination area. On February 17, 1997, several petri dishes were invaded by rodents resulting in loss of some seeds. After 12 weeks, it was assumed that no further germination would occur and the treatment was terminated. Data was subjected to an analysis of variance (ANOVA) procedure to determine the significance of the stratification treatments on germination (SAS Institute, Cary, NC). Means were separated by the Least Significant Difference (LSD) test at the .05% level.

Results and Discussion: Germination percentage increased as the cold stratification period increased and was greatest after 20 or 24 weeks of cold stratification (57 and 61%, respectively) (Tables I and 2). Germination was greater after 16 weeks of cold stratification (38%) as compared to 8 or 12 weeks (13 and 22%, respectively). Eight or 12 weeks cold stratification increased germination compared to 4 weeks of cold stratification (3% germination). Germination of C *caroliniana* seeds was further increased after 4 or 8 weeks warm stratification (prior to cold stratification) compared to 0 or 2 weeks warm stratification (Table 2 and Figure 1).

An interaction occurred between the length of warm and cold stratification period. An increase in the length of the warm stratification period resulted in a decrease in cold stratification required to maintain a specific level of germination (Table 1). The results from this research are consistent with the U.S. Forest Service's Seeds of Woody Plants in the U.S. which recommended a 60-day warm (I 5/30C) stratification period prior to a 60-day cold (5C) period (Rudolf and Phipps, 1974). Bretzloff and Pellett (1979) reported 10% germination under the conditions recommended in the Seeds of Woody Plants in the U.S. Following this schedule, our research resulted in 40% germination (Table 1). Bretzloff and Pellett also determined that lengthening the cold stratification period to 18 weeks increased germination to 58%. After 16-20 weeks cold stratification, germination was 38 and 57% respectively. Bretzloff and Pellett found that 2 weeks of warm stratification (prior to cold stratification) did not increase germination. Our work showed similar results after 2 weeks of warm stratification. However, we concluded that 4 or 8 weeks of warm stratification prior to cold stratification resulted in increased germination.

A similar experiment was repeated during 1998, however, only 3 seeds (of 2000) germinated. Gamstetter and Gulick (1996) listed C. *caroliniana* as being one of the most difficult species to propagate. According to Browse (1979), "hombeam is erratic in its seed bearing habits, producing good crops of sound viable seed very 3 to 4 years". Therefore, a good source of viable seed is a crucial factor in the production of C. *caroliniana.* In conclusion, the greatest germination resulted from 4 weeks warm stratification followed by 20 or 24 weeks cold stratification (66 and 72% germination, respectively).

Significance to the Industry: Although slow growing and more difficult to produce than more popular landscape trees, American hombeam is a desirable tree in the landscape. This study showed that seed germination increased as the cold stratification period increased and was greatest after 20 to 24 weeks. Germination was further enhanced with 4 or 8 weeks of warm stratification prior to cold stratification. Although seed germination was enhanced using the stratification regimes in this study, it is important that growers consider the quality and provenance of seeds.

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Table 1. Effect of warm and cold stratification periods on germination of *Carpinus caroliniana* seeds.

weeks of warm stratification	weeks of cold stratification	average number germinated [z]	percent germinated
0	4	0.0	0
0	8	0.2	2
0	12	0.8[y]	8[y]
0	16	3.0	30
0	20	5.0	50
0	24	6.2	62
2	4	0.0	0
2	8	0.2	2
2	12	0.6[y]	6[y]
2	16	2.4	24
2	20	5.4	54
2	24	5.0	50
4	4	0.2	2
4	8	0.8[y]	8[y]
4	12	4.2	42
4	16	5.0	50
4	20	6.6	66
4	24	7.2	72
8	4	0.8[y]	8[y]
8	8	4.0	40
8	12	3.2	32
8	16	4.6	46
8	20	5.8	58
8	24	6.0	60

[z] 10 seeds per experimental unit, 5 replications

[y] Treatment affected by rodent damage

Table 2. Effect of various warm and cold stratification periods on germination of *Carpinus caroliniana* seeds.

weeks in warm stratification	percent[z] germinated	weeks in cold stratification	percent[y] germinated
0	25b[x]	4	3d
2	23b	8	13c
4	40a	12	22c
8	41a	16	38b
		20	57a
		24	61a

[z] 300 seeds per replication and 10 seeds per experimental unit

[y] 200 seeds per replication and 10 seeds per experimental unit

[x] Means within a column followed by the same letter are not significantly different by LSD at p=0.05.

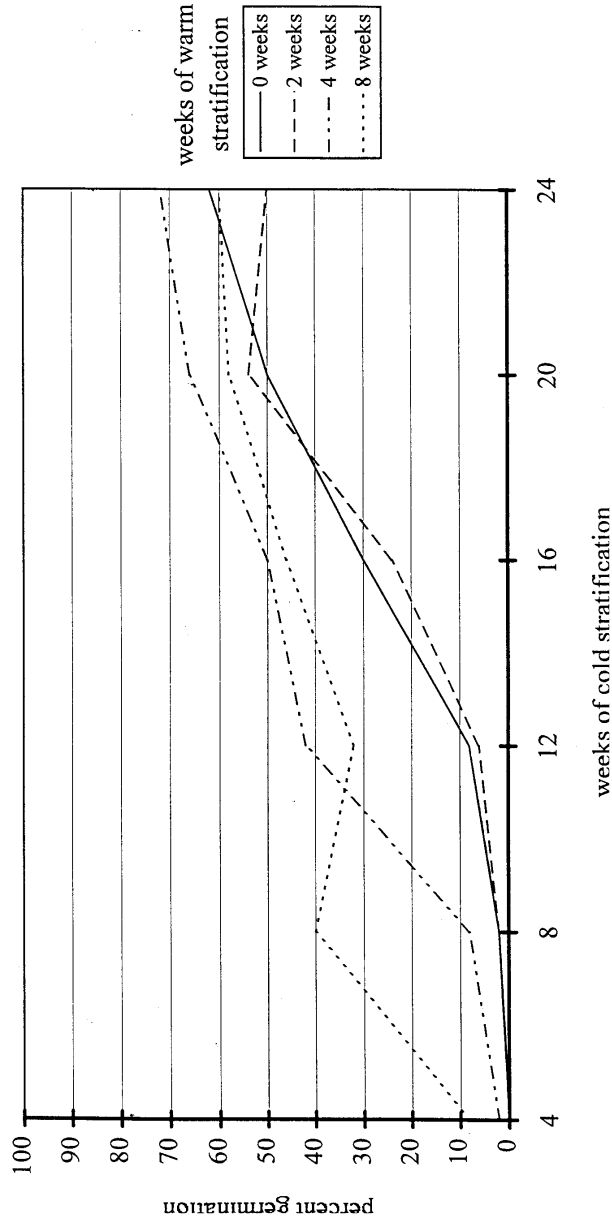

Figure 1. Percent germination of *Carpinus caroliniana* seeds after several warm and cold stratification periods.