Propagation

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Effect of Seed Source on First Year Growth of *Quercus phellos and Q. shumardii*

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Index Words: Provenance, Oak, Seed Source, *Quercus shumardii, Q. phellos*

Nature of Work. Production of oaks for landscape plantings has evolved from digging trees from wild populations near planting sites, to collecting acorns from native sites for local nursery production, to procurement of seed from national dealers. The 1998 Census of Horticulture identified oaks second only to maples in importance as shade trees in the United States. Arborists prefer oak species that develop quickly, as measured by caliper and height, and that are easily transplanted. Susceptibility to pests, poor trunk and canopy development, and labor requirements for selective pruning impact the economics of production both at the nursery and in the landscape. Much consideration has been given to selecting and planting oak species such as *Quercus shumardii* (Shumard Oak), *Q. texana* (Nuttall Oak) and *Q. phellos* (Willow Oak) which have more desirable landscape qualities than old standards like *Q. palustris* (Pin Oak) and *Q. rubra* (Northern Red Oak). Genetic variation in traits associated with adaptation to local conditions, (i.e. temperature, air pollution, pest resistance and water stress) exists not only at the species level, but also at the provenance, family, and individual tree level. By understanding the genetic variation within desirable oak species, it will be possible to identify seed sources and individual trees that are adapted to a variety of climatic conditions and/or better able to cope with environmental changes.

The goal of this study was to test for provenance effects of commercial seed lots on early development of popular oak species. Investigation of potential oak seed sources revealed that two species (*Quercus shumardii, Q. phellos*) produced by Southern nurseries are secured primarily from five commercial seed dealers located in Missouri, Texas, Tennessee and Louisiana. Their collection sites are determined by ease of harvest and yearly crop availability as affected by frost and or drought. Such limited attention to seed source could have significant impacts on the short and long term performance of oaks being planted and maintained in urban environments. The five commercial seed dealers were asked to furnish acorns for which the site of collection (county and/or state) was known.

Seed from all collections were divided and grown in south Mississippi (cold hardiness zone 8a, and heat zone 9) and middle Tennessee (cold hardiness zone 6b, heat zone 7) using the same nursery practices. Seed were planted in tree bands (3-5/8 x 6-inch tall) with pine bark substrate amended with 1.0 lb. Micromax and 4.0 lbs. Osmocote 15-9-12 (12-14 month) per cubic yard during January 2001. The test was irrigated daily and fed weekly beginning 4 June with 200 ppm nitrogen. In mid-September, height, caliper (at soil line), and quality were recorded for each seedling. Quality rating ranged from 1 to 5, with 5 being the highest. Plants were rated on the straightness of the trunk and canopy development. The experimental design was a randomized complete block in two locations and up to 16 single plant replications depending on germination. Six provenances of Willow Oak and five provenances of Shumard Oak were evaluated.

Results and Discussion: Evaluations of first season growth indicated that oaks from the provenances of each species grew differently in Mississippi and Tennessee because there were significant interactions between provenance and location for the three measurements (seedling height, main stem caliper, and quality) used to evaluate growth (Table 1). Most of the differences between the two locations were associated with the worst performing provenances, which grew better in Tennessee than Mississippi. There were only minor differences in height and caliper at the two locations. Since the poorer performance was usually associated with the more northern collection sites, oak seed performance may conform to the same climatic effects observed in pine seed provenances where pine seedlings will survive and grow best if seed come from an area within 5 ^{0}F of the planting site's minimum temperature expectation as reflected in cold hardiness zone maps (2). Seedlings from an area with warmer winters will grow faster than seedlings from local sources; seedlings from an area with cooler winters will grow slower (1).

At the end of one season, differences in provenance performance were evident when evaluating the growth and quality of plants at both locations (Table 1). Willow oak seedlings from acorns collected in Tennessee, Arkansas and North Carolina produced significantly less growth than seed collected from two Louisiana provenances. Shumard oak seed collected from two sites in Tennessee was inferior to seed collected in Missouri and Louisiana. Collection of data will continue to determine if one-year data is a strong indicator of provenance performance during the remainder of the production cycle and under landscape conditions. Statistical models will determine how seed from diverse provenances react to different climatic conditions as reflected in seedling growth and number of culls.

Significance to Industry: Identification of well-adapted seed sources will facilitate the development of higher quality oaks, better suited to changes in climate and environmental stresses than those currently being produced. Shade trees are not marketed based on board feet or weight; however, plantings are subject to the same laws of genetics that have been used by production forestry to improve forest health and vigor. It has been possible for the landscape industry to select and clone elite, mature individuals of easily propagated genera such as *Acer*. Oaks are generally considered to be difficult to asexually propagate so different strategies must be used to emulate the progress achieved in maple. Identification of superior seed sources and developing a better understanding of the environmental adaptations of a species is the logical first step to improvement of product uniformity, pest resistance, and environmental adaptation. Genetically superior trees reduce costs associated with weather and pest damage and can also have aesthetic traits such as improved seasonal color and texture that make our cities more beautiful. Testing provenances is a relatively inexpensive process for improving long-term shade tree performance. After identification of the widely adapted native sites, seed dealers would be encouraged to collect from the elite provenances. By culling undesirable provenances, families and individual trees, test plantings could serve as a source of elite seed. Material from widely adapted provenances would also serve as the best material to screen for choice individuals and efficient cloning methods to reduce dependence on erratic seed crops.

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Table 1. Seedling growth of *Quercus phellos*, Willow Oak and *Quercus shumardii*, Shumard Oak, year 1.

Quercus phellos

Location/Provenance	Height, cm[Z]	Caliper, cm	Quality[Y]
Arkansas 1	63.2ab[X]	0.68a	3.8a
Arkansas 2	51.1c	0.59c	3.3c
Louisiana 1	66.3a	0.71a	3.5b
Louisiana 2	64.3a	0.70a	3.7ab
North Carolina	48.6c	0.62bc	2.7d
Tennessee	60.4b	0.64b	3.5bc
Location	**[W]	NS	*
Rep (Location)	NS	NS	NS
Provenance	**	**	**
Location X Provenance	**	**	**

Quercus shumardii

Location/Provenance	Height, cm	Caliper, cm	Quality
Louisiana 1	73.4a	0.83a	3.8b
Louisiana 2	71.9ab	0.77b	3.9ab
Missouri	69.0b	0.76b	3.8ab
Tennessee 1	60.8c	0.85a	4.0a
Tennessee 2	40.2d	0.68c	3.4c
Location	**	NS	*
Rep (Location)	**	NS	NS
Provenance	**	**	**
Location X Provenance	**	**	**

[Z] Height and caliper were measured in mid-September, 2001.

[Y] Shoot and trunk quality was rated in mid-September 2001 on a scale from 1-5, with 5 being highest.

[X] Means within columns followed by the same letter are not significantly different as determined by Duncan's multiple range test at p.≤ 0.05.

[W] Non-significant (NS) or significant at p ≤ 0.05 (*) or 0.001 (**).

Effect of Soil Water Potential and Mist on Rooting Stem Cuttings of Loblolly Pine (*Pinus taeda*)

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Index words: Vegetative Propagation, Cutting Water Potential, Timber Species, Medium Water Potential

Nature of Work: Loblolly pine (*Pinus taeda* L.) is an important timber species in the southeast United States (7). Vegetative propagation of loblolly pine by stem cuttings is used to multiply superior full-sib families (progeny as a result of controlled pollination where both parents are known) and elite clones within superior families. Seedlings are planted more extensively than rooted cuttings (5) though not necessarily due to convention. Rather, rooted cutting technology needs further refinement for wider use. Recent research with loblolly pine has determined adequate stock plant management techniques (6), proper handling and storage of stem cuttings (4), effect of various auxins on rooting and subsequent root growth (1), and propagation systems necessary for optimum planting stock (2).

One important aspect of vegetative propagation by stem cuttings is the rooting environment. During rooting, stem cuttings experience various stresses such as temperature, moisture, and irradiance. Combinations of such stresses can desiccate cuttings decreasing rooting percentage. Typically, mist application lowers leaf temperature caused by high irradiance and increases the humidity surrounding the cuttings. In turn, water loss from foliage decreases, thus cuttings maintain turgidity. Furthermore, uptake of available water through the base of the stem contributes to overall water status of cuttings. The moisture status of cuttings, collectively termed cutting water potential (Ψ cutting), is an important indicator of rooting success (3). Mist application differs with respect to species, physiological status of the cutting, type of propagation system used, climate, and most importantly, the experience of the propagator. Our objective was to determine the relationship between cutting water potential and rooting percentage that would not rely on propagation systems specifically, but could be applied to rooting stem cuttings of loblolly pine in general.

Two studies were conducted to determine the effect of mist and medium water potential (Ψ medium) on Ψ cutting and rooting percentage. One study used hardwood cuttings (January) and one used succulent, softwood cuttings (June). The experimental design for both trials was a

split-plot. Two mist regimes, a high ("normal") and a low regime consisting of 40% less mist, were the main plots and four Ψ medium treatments were the sub-plots. The Ψ medium treatments were -1.8 kPa (wet), - 2.6 kPa (intermediate), - 3.6 kPa (dry), and a control. They were created using containers of various heights filled with coarse builders' sand and maintained with sub-irrigation controlled by a tensiometer. The control medium consisted of 2 peat : 3 perlite (by volume) placed in a container with a height equal to the intermediate Ψ medium treatment, but sub-irrigation was not applied and the Ψ medium was not maintained. Mist regime was replicated twice and each replication contained two replications of medium water potential treatments. Stem cuttings were a random mix of two full-sib families consisting of approximately 30 clones from each family. Approximately 60 cuttings were placed in each plot. One, 3, and 5 weeks after setting cuttings, Ψ cutting was measured destructively on one cutting per plot at 5 a.m. and 2 p.m. using a pressure bomb. Two and 4 weeks after setting, Ψ cutting was measured every 3 hr beginning at 5 a.m. and continuing until 5 a.m. the following morning (nine measurements). Ψ cutting was averaged over 5 weeks using just the 5 a.m. and 2 p.m. measurements. Ψ medium was also recorded for each plot at 5 a.m. and 2 p.m. for 5 weeks. Values for each plot were averaged over 5 weeks. Rooting percentage was recorded after 10 weeks. Analysis of variance (ANOVA) and regression analysis were used to test the relationships between Ψ medium, Ψ cutting, and rooting percentage.

Results and Discussion: Mean rooting percentage for the January and June experiments was 23% and 48%, respectively. Based on the low rooting percentages and the values of Ψ cutting obtained with hardwood cuttings in the January experiment, both mist regimes were decreased for the June experiment. Mist level, Ψ medium and their interaction had a significant effect on Ψ cutting in January and June (Table 1). In both experiments, cuttings receiving less mist and cuttings in drier media had lower (more negative) Ψ cutting (Table 2). In the January experiment, the effect of Ψ medium on Ψ cutting was strongly dependent on mist level. The increase in stress with drier media was greater in cuttings receiving low mist than in those receiving high mist (Fig. 1). This effect was less obvious in the June experiment, where medium moisture effected Ψ cutting equally in the two mist levels (Fig. 2). These results demonstrate that uptake of water from the rooting medium contributes to the water status of nonrooted cuttings.

In the January experiment, the main effects of mist and Ψ medium did not significantly affect rooting percentage, however the interaction of the two was significant (Table 1). In the high mist treatment, rooting was highest in the dry medium, but in the low mist treatment, rooting was

highest in the wet medium (Table 2). In contrast, in the June experiment, only mist level significantly affected rooting. Rooting percentage was higher in the high mist treatment, regardless of medium moisture treatment.

The relationship of rooting percentage with Ψ cutting depended on the level of moisture stress. Cuttings experiencing moderate to high stress (low mist in winter and both mists in June) showed an increase in rooting as their Ψ cutting increased (decrease in stress) (Fig. 3). In contrast, cuttings under little or no stress (high mist in January) actually exhibited an increase in rooting with decreased Ψ cutting. This may be an indirect effect. For example, cuttings with no stress may also have been experiencing anaerobic medium conditions. However, no basal rotting was observed in the cuttings in these experiments. Thus, the propagator should not endeavor to eliminate all stress in cuttings by misting excessively. In these experiments, rooting was best between -0.4 and -0.65 MPa and was better in treatments in which the cutting surfaces dried between mist applications.

Significance to Industry: Stem cuttings of loblolly pine, regarded as a difficult-to-root species, benefit from a moderate amount of water stress during rooting. Other difficult-to-root conifer species might benefit as well. For example, if the basal portions of hardwood cuttings of *Abies*, *Picea*, or *Chamaecyparus* species rot easily, then a taller container and reduced mist might decrease water-logged conditions, increase survival, and subsequent rooting. On the other hand, if foliage drop occurs frequently due to excessive mist, perhaps a shorter container with infrequent misting might work. As long as adequate moisture is present in the medium, successful rooting of loblolly pine stem cuttings is limited by proper mist application.

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Table 1. ANOVA for effect of medium water potential (Ψ medium) and mist regime on cutting water potential (Ψ cutting) and rooting percentage of hardwood and softwood stem cuttings of loblolly pine.

		Hardwood (January)		Softwood (June)	
Source	df	Ψ cutting	Rooting (%)	Ψ cutting	Rooting (%)
Mist	1	*	NS	*	*
Rep(Mist)	6	*	NS	*	NS
Ψmedium	3	*	NS	*	NS
Mist x Ψmedium	3	*	*	*	NS

NS,* Nonsignificant or significant at $P = 0.05$, respectively.

Table 2. Means for cutting water potential (Ψ cutting) and rooting percentage by mist regime and medium water potential treatment for hardwood and softwood stem cuttings of loblolly pine.

	Hardwood (January)				Softwood (June)			
	Ψ cutting (MPa)		Rooting (%)		Ψ cutting (MPa)		Rooting (%)	
Medium moisture treatment	High mist	Low mist	High mist	Low mist	High mist	Low mist	High mist	Low mist
Control	-0.38	-0.62	21.0	33.6	-0.43	-1.13	51.1	31.1
Dry	-0.42	-0.81	31.5	22.1	-0.63	-1.33	62.4	35.2
Medium	-0.37	-0.85	18.3	14.7	-0.58	-1.24	62.2	32.9
Wet	-0.24	-0.36	5.0	41.7	-0.41	-0.84	64.1	41.3

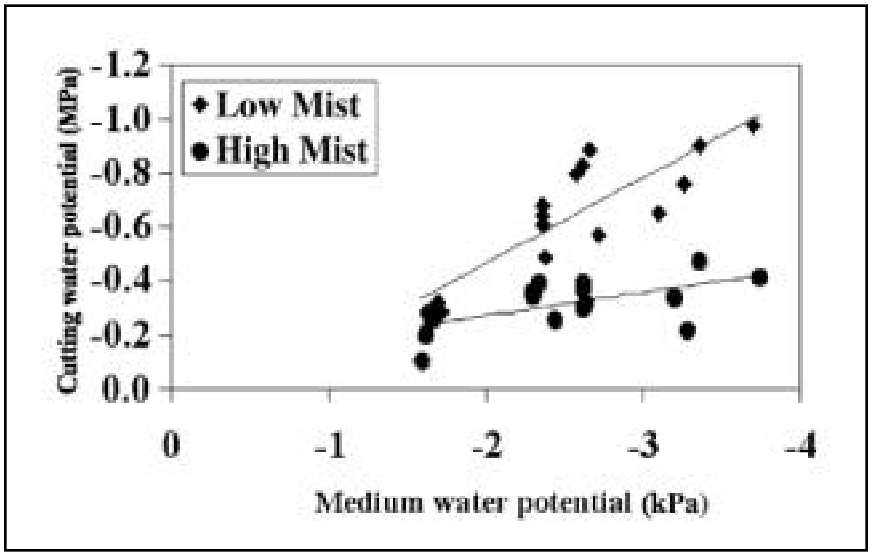

Fig. 1. Effect of medium water potential (Ψ medium) on cutting water potential (Ψ cutting) in January 2001.

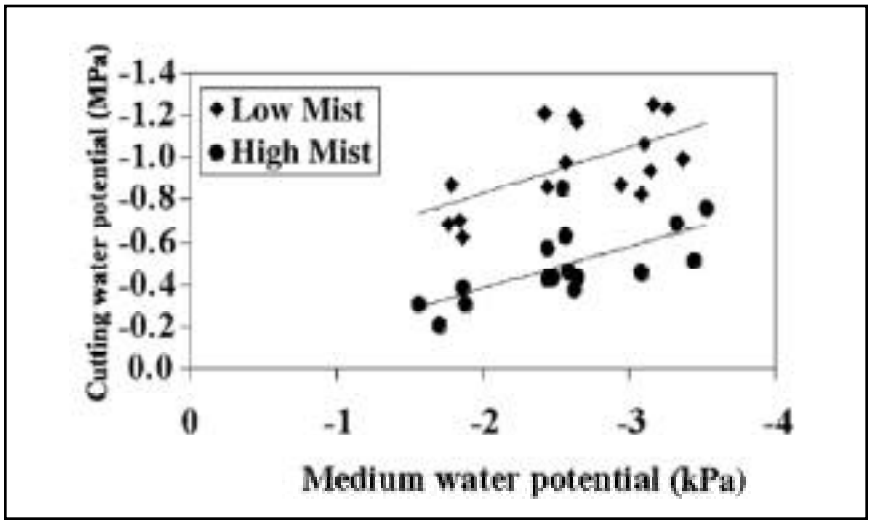

Fig. 2. Effect of medium water potential (Ψ medium) on cutting water potential (Ψ cutting) in June 2001.

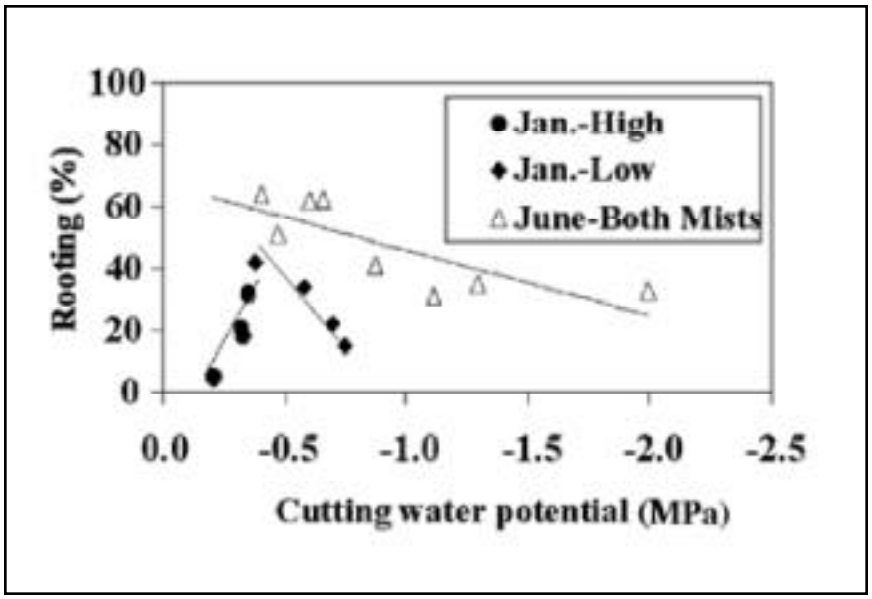

Fig. 3. Effect of cutting water potential (Y cutting) on percentage rooting in two mist regimes in January and June 2001. Symbols are means of four replications of four Ψ medium treatments.

Propagation Of Spineless Wright Acacias

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Index Words: *Acacia wrightii*, Grafting, Rooted Cuttings, Seed Germination, Tissue Culture.

Nature of Work: The southwestern USA has a harsh climate. Landscape plants that would be used in this area need to be able to withstand heat, drought, and grow in dry infertile alkaline soils. Several *Acacia spp.* are adapted to a wide range of environments including acidic, alkaline, saline, or infertile soils, and can be readily established and managed (2). *Acacia* frequently make attractive trees, however, many *Acacia spp.* have spines formed from stipules at the base of a compound leaf. This characteristic may constitute a maintenance liability or pedestrian hazard (1). However, a spineless acacia would be perfect for the landscape. *Acacia wrightii* G. Bentham *ex* A. Gray (wright acacia) is a Texas native with several attractive features that forms a large shrub to small tree which is useful in southwestern USA landscapes (1, 3). This *Acacia* has small, bipinnately compound leaves and white to yellow-white cylindrical flowers that peak in the spring with occasional blooming throughout its growth period (1). Unfortunately, the species type of *A. wrightii* has vicious recurved spines (referred to in the trade as thorns) (1). A newfound genetic variant of *A. wrightii* was expressed as a spineless phenotype. In order for the spineless selections of *Acacia wrightii* to be sold in nurseries, a reliable and efficient propagation method must be determined. The objective of these studies was to compare seed germination, rooted cuttings, grafting, and micropropagation as methods of propagating these spineless selections of *A. wrightii*.

Seeds were collected from the three spineless selections of *A. wrightii* in summer 1999. Seeds were scarified by clipping the seed coat with anvil-type clippers. Seeds were then planted in 15 in x 21 in x 4 in (38cm x 53cm x 10 cm) flats containing a substrate composed of 60% pine bark, 20% peat, 10% vermiculite, and 10% hadite clay. To test germination

and yield of spineless seedlings, in Aug. 1999 69 seeds from *A. wrightii* selection 1, 17 from selection 2, and 290 from selection 3 (max. number of available seeds) were planted, and in May 2001 10 seeds from *A. wrightii* selection 1, 42 from selection 2, and 102 from selection 3 were planted. In both years, germination was recorded after two weeks. Seedlings were then transplanted to 1 gal. (2.8 L) black plastic containers. At seven and ten months after transplanting, plants were observed to confirm the spineless phenotype.

To test for rooting propensity, cuttings of softwood (May 2001), semi-hardwood (Sept. 2001), and hardwood (Feb. 2001) growth stages of *A. wrightii* were taken from selection 3. The basal 0.5 in (1.25 cm) of the 2 in (5 cm) cuttings were dipped for 5 sec. in auxin (2 IBA : 1 NAA, Dip-n-Grow, Astoria-Pacific, Inc., Clackamas, OR) at the rate of 0, 5,000, 10,000, and 15,000 ppm (mg/L). At each cutting stage, three reps of 10 cuttings per treatment (120 cuttings) were placed in rooting flats as described for the germination studies and located under intermittent mist. After 10 weeks, cuttings were measured to determine if they rooted, how many roots were regenerated, and what was the total root length per cutting.

In April 2001, scions from selection 3 were grafted on seedling rootstocks using three different methods. Grafts of whip-and-tongue (40), T-buds (40), and whip-and-tongue with the scion dipped in auxin (20) (Hormex-3, Brooker Chemical Corp., North Hollywood, CA) were compared. All grafts were wrapped with parafilm (American National Can, Neenah, WI). A total of 100 grafts were performed. Grafts were monitored through May 2001.

In the tissue culture experiment, seeds from the *A. wrightii* spineless selection 3 were soaked in sulfuric acid for 30 minutes and then placed in a 30% bleach solution for 30 minutes. The seeds were placed on water agar under sterile conditions for 7 to 14 days. The seeds were then transferred to Murashige and Skoog (MS) or woody plant media (WPM) after approximately 7 days. Effects of the cytokinins, benzyladenine and zeatin, on shoot proliferation were compared at 0, 5, 10, 15, and 20 µM. Shoot proliferation was assessed after 8 weeks.

Results and Discussion: Germination percentages were 33%, 94%, and 58% for selections 1, 2, and 3, respectively in 1999, and 50%, 83%, and 60% for 2001 seeds from selections 1, 2, and 3, respectively. Of these germinated seedlings from 1999, after seven months 30%, 38%, and 14% of the seedlings were spineless for selections 1, 2, and 3, respectively. After ten months, the yields of spineless seedlings were 9%, 13%, and 5%. After losses during container production and delayed

expression of thorns, seed propagation did not yield great enough percentages of spineless seedlings to be a commercially viable method.

Although three different grafting procedures were attempted, whip-and-tongue, T-bud, and a whip-and-tongue with auxin, none were successful. Not a single scion from forty of each type produced a viable union of the rootstock and scion. The color and condition of the grafts suggested possible production of large concentrations of phenolic compounds.

Rooting of hardwood and semi-hardwood cuttings was very low across auxin concentrations (Fig. 1A). Rooting of softwood cuttings was somewhat greater, particularly at high auxin concentrations (Fig. 1A). On average, those hardwood and semi-hard wood cuttings that did root, regenerated one or fewer roots (Fig. 1B) and total root length per cutting averaged less than 2 cm (Fig. 1C). Softwood cuttings had a linear response to auxin concentration, generating as many as nine roots per cutting at 15,000 ppm, whereas the control (0 ppm) cuttings had two or fewer roots. Mean total root length of softwood cuttings responded in an exponential fashion, ranging from an average of about 4 cm for non-treated cuttings to around 12 cm for those treated with 15,000 ppm auxin (Fig. 1C). While a plateau in root regeneration response was not reached (Fig. 1A-C), the cuttings treated with 15,000 ppm did not regenerate roots from the base of the cutting which was necrotic, but rather roots emerged from further up the stem. This suggests that concentrations greater than 15,000 ppm were likely to be toxic.

Preliminary studies to determine tissue culture protocols for micropropagation of *A. wrightii* were more promising than might initially be expected from the literature on other *Acacia spp.* (2). It was possible to obtain sterile propagules from *in vitro* germinated seeds. Initial experiments indicated that 15 to 20 µM zeatin on MS were the most promising treatments for inducing shoot proliferation *in vitro* (Fig. 2).

Significance to Industry: The primary limitation to landscape utilization of *Acacia wrightii* are the presence of vicious thorns. Development of a spineless wright acacia would provide an environmentally friendly adverse site tolerant large shrub or small tree for Texas and the arid southwestern USA. Seed germination, rooted cuttings, and grafting yielded too few of useable spineless plants to be commercially viable. Preliminary tissue culture studies indicated that micropropagation techniques may be useful, and investigations in this area continues.

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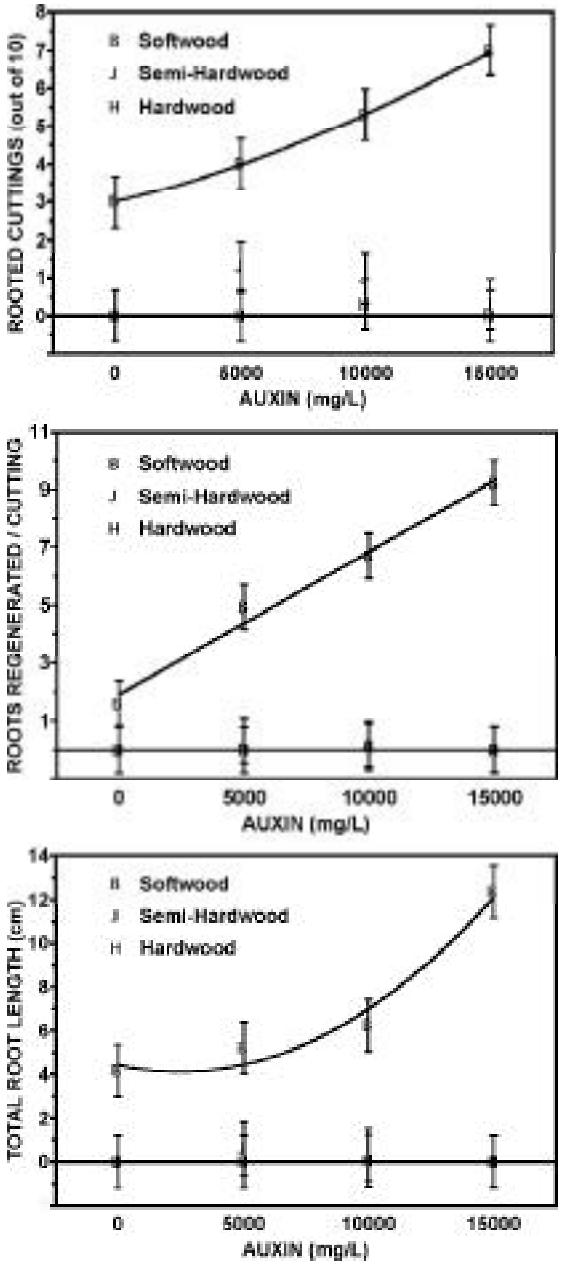

Figure 1. Mean (± standard error) rooting percentages (A), number of roots regenerated per cutting (B), and total root length per cutting (C) of softwood, semi-hardwood, and hardwood cuttings of *Acacia wrightii*; number of observation for A = 3, for B and C = 30 . Regression equations are provided where significant, $P \leq 0.05$.

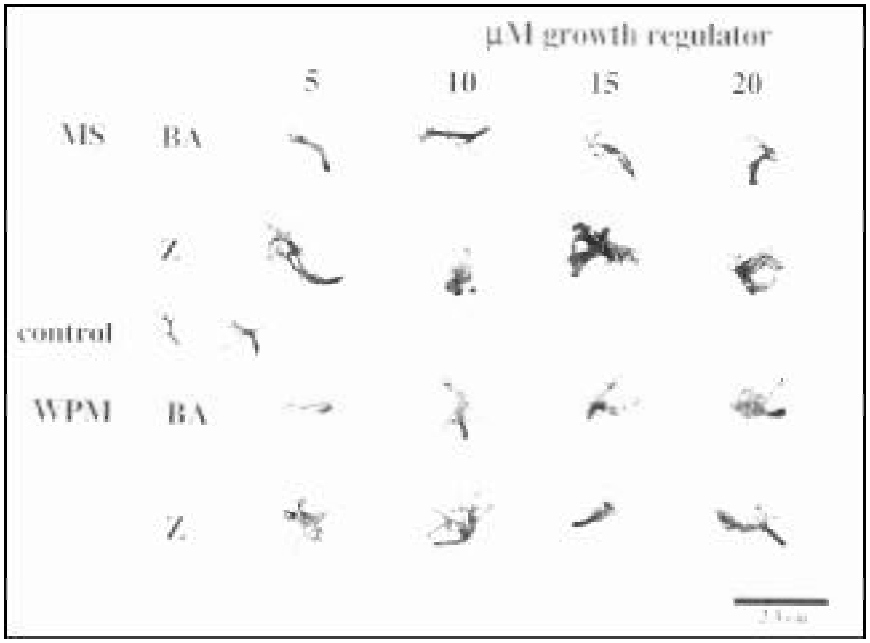

Figure 2. Representative *in vitro* cultures of *Acacia wrightii* grown with MS or WPN media containing 0, 5, 10, 15, or 20 µM of zeatin or benzyladenine.

Influence of Hormone and Timing on Layering Propagation of *Aesculus parviflora*

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Index Words: Aesculus parviflora, Bottlebrush Buckeye, Mound Layering, Propagation

Nature of Work: *Aesculus parviflora* (Bottlebrush Buckeye) has made many recommended lists during recent times. However, few plants are available on a regular basis in the nursery trade. Seed was the main method of propagation until the 1990's when Bir & Barnes(1994) established a protocol for cutting propagation. Fordham (1987), in his discussion of propagation of Bottlebrush Buckeye, devoted his explanation to seed, except for a final comment that root cuttings and root suckers can be a source. Seed availability, timing or facilities may still limit this plant from being propagated in significant numbers by either seed or cuttings.

Layering has been recommended as a form of propagation for plants forming suckers by several authors during the 1900's (Bailey, 1920; Wells, 1985). While addressing layering in one form or another, neither Mahlstede & Haber (1957), Macdonald (1986), Dirr & Heuser (1987), nor Hartman et al. (1998) defines layering as a technique for Bottlebrush Buckeye. Bailey (1920) addresses the benefits of wounding during the layering process. As a means of producing large numbers of Bottlebrush Buckeye with limited facilities and less dependence upon timing, we looked at mound layering. *Aesculus parviflora* were planted on the University of Kentucky Horticulture Farm during the early 1990's in north/south rows. During 1998 the plants were bush hogged to the ground. Multi-stem regrowth occurred during 1999 and 2000. In August 2000 research was initiated in order to determine if rapid propagation could occur by mound layering *Aesculus parviflora*. Sawdust was row mounded eighteen inches deep and three feet wide around 41 plants. From August 2000 until May 2001 three stems on ten randomly selected plants were treated each month. Treatments included cutting into non-rooting one or two year old stems near the base, treating with No. 3 Hormex and keeping the stem gapped with a section of toothpick. A drip irrigation system was installed in the plot, and scheduled to run 20 minutes twice a day at 9:00 a.m. and 2:00 p.m.. One-GPH emitters were spaced every two feet along 2 inch diameter lines. Irrigation was turned off during the dormant months.

Results and Discussion: During March 2001, plants treated the previous 7 months were evaluated for rooting. Plants treated August 2000 had roots formed at the wound site on 29 of 30 stems. Plants treated September 2000 had roots formed at the wound site on 8 of 30 stems. No roots were found on stems treated during October through February. During November 2001, plants were again evaluated for rooting. Rooting had occurred on all plants treated through May 2001 (Table 1). The tendency was for more stems rooting (99%) for months (Aug., Sept., April, May) when treatments were on plants which were in active growth than when treated plants (84%) were in their dormant period (Oct. through March). One plant was left untreated and during March 2001 three plants were completely pruned back to within 3 inches of the ground for comparison to the treated plants. At the November, 2001 harvest time, the unpruned plant had fourteen stems which were rooted, and the three pruned plants generated a total of 68 rooted stems on current season growth. No other wounding or hormone treatment occurred on these four plants. Stems on these plants rooted with just the sawdust treatment of mound layering and irrigation. The other thirty-seven original plants were also producing new stems during 2001. Between untreated old growth stems and new growth stems, an additional six hundred seventeen rooted stems were removed from these thirty-seven plants; an average of 16.7 rooted stems per plant.

Rooted stems had either new coarse or fine roots. Coarse roots were most common and it was suspected that stems with fine roots might not survive. This was not tracked as to root type but survival of rooted stems as liners was recorded. Rooted stems were placed in three quart containers and overwintered in an unheated quonset house. Eighty-three percent of the stems from treated plants leafed out and developed into the liner stage (Table 1). Ninety-three percent of the stems from untreated plants leafed out and developed into the liner stage.

Significance to Industry:

Rapid propagation of *Aesculus parviflora* through mound layering is very feasible. Mound layering without wounding and hormone treatment, will generate rooted shoots. Stems which do not root under normal mound layering techniques will benefit from wounding and hormone treatment.

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Table 1. Stems rooted and successfully established as a liner during month by month treatment, August 2000 – May 2001.

Month	Rooted stems, Nov. 2001	Survival in the liner stage, June 2002
August , 00	30	28
September, 00	30	25
October, 00	25	22
November, 00	26	19
December, 00	25	19
January, 01	27	16
February, 01	25	23
March, 01	23	21
April, 01	30	27
May, 01	29	24

Influence of Selected Surface Disinfestants, Fungicides, and Temperature on Seed Germination of Southern Seaoats (*Uniola paniculata*)

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Index Words: Sand Dune Species, Beach and Dune Restoration, Sexual Propagation, Poaceae

Nature of Work: Two experiments were conducted to investigate the influence of selected surface disinfestants, fungicides, and temperature on seed germination of southern seaoats (*Uniola paniculata* L.)

In the first experiment, seeds of southern seaoats were removed from storage at 4°C (39°F) and treated with the following surface disinfestants and/or fungicides: nontreated (control), 1.3% sodium hypochlorite [NaOCl (chlorine bleach)] 2.6% sodium hypochlorite, RTU®-PCNB (pentachloronitrobenzene), RTU® (thiram + thiabendazole), combinations of 1.3% sodium hypochlorite and RTU®, 2.6% sodium hypochlorite and RTU®, 1.3% sodium hypochlorite and RTU®-PCNB, and 2.6% sodium hypochlorite and RTU®-PCNB. Following treatment, seeds were germinated at an 8/16 hr thermoperiod of 35/20°C (95/68°F). The seed treatments and germination thermoperiod utilized were based on three previous trials that investigated the influence of selected surface disinfestants, fungicides, and temperature on seed germination of the species. Germination was recorded every 3 days for 30 days.

In the second experiment, seeds were removed from storage and treated with the following surface disinfestants and/or fungicides: nontreated (control), 2.6% sodium hypochlorite, RTU®-PCNB, combinations of 1.3% sodium hypochlorite and RTU® , 2.6% sodium hypochlorite and RTU®, 1.3% sodium hypochlorite and RTU®-PCNB, and 2.6% sodium hypochlorite and RTU®-PCNB. Following treatment, seeds were sown in containers filled with a peat-based medium and the containers placed in a growth chamber maintained at an 8/16 hr thermoperiod of 35/20°C (95/68°F) with long day conditions. Emergence data were recorded every 3 days for 45 days. Seedlings were fertilized with a complete nutrient solution which was applied daily after seedling emergence and once the first leaf was visible. After 45 days, the study was terminated and additional data recorded to include plant height (height of main stem), leaf number, length and width of the two longest leaves, and top and root dry weights.

Results and Discussion: Results of the first experiment indicated that seed treatment was highly significant (*P* =0.0001) for both total percentage germination and total percentage of decayed seeds. Germination of nontreated seeds was 45% and four treatments resulted in germination >80% [RTU®-PCNB (81%), 2.6% sodium hypochlorite and RTU® (83%), 1.3% sodium hypochlorite and RTU® (87%), and 1.3% sodium hypochlorite and RTU®-PCNB (89%)].

Data of the second experiment indicted that surface disinfestant and/or fungicide treatments were highly significant (*P*=0.0004). Percentage emergence of the nontreated seeds was 35% and five of the seven treatments resulted in emergence ≥ 75% [2.6% sodium hypochlorite (75%), 1.3% sodium hypochlorite and RTU® (75%), 1.3% sodium hypochlorite and RTU®-PCNB (76.2%), 2.6% sodium hypochlorite and RTU®-PCNB (81.0%), and 2.6% sodium hypochlorite and RTU® (83.3%)] with negligible effects on subsequent seedling growth. There were significant treatment differences regarding some of the variables used to evaluate seedling growth. These differences in most cases were due to seedlings from nontreated seeds having lower values for each measured variable than values for the same variables from nontreated seeds.

Significance to Industry: Seedling transplants of southern seaoats are in great demand for beach and sand dune restoration and stabilization. However, seed decay is a problem that reduces germination and seedling emergence during production of transplants. Results of this research demonstrate the importance of seed treatment of the species and identify surface disinfestant and/or fungicide treatments that will inhibit decay and permit emergence $\geq$75% without adverse effects on subsequent seedling growth.

In Vitro Organogenesis of the Tennessee Coneflower from Hypocotyl, Cotyledon, Leaf and Flower Stalk

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Index Words: *Echinacea tennesseensis*, Organogenesis, Tissue Culture

Nature of Work: The US Fish and Wildlife Service has listed the Tennessee coneflower (*Echinacea tennesseensis* Small) as a protected endangered plant species. It is estimated that there are only 146,000 plants remaining in its native habitat (1). This drought resistant native plant is found exclusively in central Tennessee where it grows on open, well-drained hills, barrens and glades (2). It is the only species of coneflowers that possess petals that do not droop or bend back but spread outward to form an inverted cup-shaped corolla. Flowers ranging in color from purple, rose to white appear in June to September (3). The Tennessee Coneflower can be propagated with seeds or by crown divisions. But, these methods are slow and not productive. This study was undertaken to develop an *in vitro* regeneration system for possible mass production of this rare plant. This report describes a successful regeneration system for *E. tennesseensis*.

Stock plants collected in Middle Tennessee were maintained in a greenhouse at TSU, Nashville campus. Seeds were collected from these stock plants and stored at room temperature until used. Seeds were germinated on a medium containing only 1.0% agar. Hypocotyls and cotyledons for 2 weeks-old seedlings, fully expanded leaves, and flower stalk from stock plants were used as explants. All explants were surface-sterilized by immersion in a 1.0% by volume of sodium hypochloride followed by a 10 sec dip in 70% ethyl alcohol. After 3 rinses in sterile deionized water, hypocotyls and flower stalks were sectioned into 0.8 cm segments and leaves into 1.5 x 1.5 cm sections. All explants were plated onto Murashige and Skoog's medium (4) supplement with 3% sucrose (w/v) and solidified with 0.8% agar (w/v) in disposable Petri dishes. Cultures were maintained at 24C under cool white fluorescent lights (16/8 hr photoperiod at 17 _mol $m^{-2}s^{-1}$ light intensity). To induce shoot formation media with factorial combinations of naphthaleneacetic acid (NAA) ranging from 0.05 to 5.0 mg/l and 6-benzylaminopurine (BA) ranging from 0.05 to 5.0 mg/l were evaluated. The percentage of explants with shoots and shoots per explant were recorded after 2 months of incubation. Shoots were rooted on MS medium supplemented with 2% sucrose (w/v) and 0.05 mg/l indole-3-buteric acid.

Results and Discussion: During the first week of culture, hypocotyl explants expanded and develop a green coloration and within 1 month, adventitious shoot buds and shoots appeared on hypocotyls. Initially buds were reddish in color but turned green as young leaves began to unfold. Shoot formation occurred only in media that contained 0.1 and 0.5 mg/l NAA. BA at 5 mg/l significantly increased the frequency of explants that regenerated shoot buds and the number of shoots per explant. At the low concentration level of 0.05 mg/l NAA, no shoot regeneration was observed regardless of BA concentrations. In media containing higher levels of NAA (1.0 to 5.0 mg/l). calli were formed but no shoot was regenerated. The best frequency for shoot regeneration and number of shoots per explant was obtained with the medium containing 0.1 mg/l NAA and 5 mg/l BA (Table 1).

Results of experiments with cotyledon (Table 2) and flower stalk (Table 3) explants were similar to those obtained with hypocotyls. Fourteen to 54% of cotyledon explants regenerated shoots and the number of shoots per explant ranged from 1.4 to 3.5 when cultured on media containing 0.1 and 0.5 mg/l NAA. With flower stalk explants, the numbers of shoots were twice those obtained from hypocotyl or cotyledon explants. Shoots regenerated from flower stalks grew faster than those regenerated from other tissue.

Leaf explants began to expand in size after 2 weeks in culture in all media. After 3 weeks in culture, adventitious shoot buds formed on leaf edges were visible. Leaf explants responded to a wider range of NAA concentrations than other explants. Shoots regenerated at NAA concentrations ranging from 0.05 to 1.0 mg/l and the best medium for shoot regeneration contained 0.1 mg/l NAA and 5.0 mg/l BA in which 88% of explants produced an average of 12.0 shoots each (Table 4).

To compare the effectiveness of cytokinins on shoot regeneration from leaf explants, 5.0 mg/l BA was replaced with 5.0 mg/l kinetin, zeatin or thidiazuron (TDZ). The frequency of adventitious shoot bud formation was not affected by the different cytokinins. However, the number of shoots per explant was affected. Zeatin slightly increased the number of shoots per explant while TDZ increased it by almost 3 fold as compared with to BA (Table 5). Shoots cultured on MS medium supplemented with 0.05 mg/l IBA initiated roots within 4 weeks of culture. Rooted plants were transplanted into pots containing a mixture of field soil, peat, and perlite (1:1:1) and grown to maturity in the greenhouse.

Significance to the Industry: Tennessee Coneflowers can be regenerated using hypocotyls, cotyledons, flower stalks and leaf sections. MS medium containing 0.1 to 0.5 mg/l NAA and 5.0 mg/l BA or TDZ can be

used for mass propagation of this endangered plant. This plant species has ornamental potential due to its unique persistent flowers, growth habits and drought tolerance. These unique traits can be used for producing new types of inter-specific hybrids.

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Table 1. Effect of NAA and BA on Shoot Formation from *Echinacea tennesseensis'* Hypocotyl Explants.

NAA (mg/l)	BA (mg/l)	Explants with shoots (%)[1]	Number of shoots/explant[1]
0.1	0.05	11.46b	1.8b
0.1	0.1	17.1b	2.2b
0.1	0.5	14.3b	1.8b
0.1	1.0	28.6ab	2.4ab
0.1	5.0	45.7a	3.6a
0.5	0.05	14.3b	2.2ab
0.5	0.1	14.3b	1.6b
0.5	0.5	22.8ab	2.1ab
0.5	1.0	34.3ab	2.6ab
0.5	5.0	37.1a	3.2a

[1] Means within a column followed by different letters are significantly different according to Duncan's Multiple Range Test (P=0.05)

Table 2. Effect of NAA and BA on Shoot Formation from *Echinacea tennesseensis'* Cotyledon Explants.

NAA (mg/l)	BA (mg/l)	Explants with shoots (%)[1]	Number of shoots/explant[1]
0.1	0.05	14.3c	1.4b
0.1	0.1	20.0c	1.9b
0.1	0.5	20.0c	2.1b
0.1	1.0	34.3ab	3.2a
0.1	5.0	54.3a	3.5a
0.5	0.05	17.1c	2.0b
0.5	0.1	20.0c	2.0b
0.5	0.5	25.7bc	1.9b
0.5	1.0	31.4b	1.9b
0.5	5.0	40.1ab	2.3ab

[1] Means within a column followed by different letters are significantly different according to Duncan's Multiple Range Test (P=0.05)

Table 3. Effect of NAA and BA on Shoot Formation from *Echinacea tennesseensis'* Flower Stalk Explants.

NAA (mg/l)	BA (mg/l)	Explants with shoots (%)[1]	Number of shoots/explant[1]
0.1	0.05	11.4d	2.0c
0.1	0.1	20.0d	1.9c
0.1	0.5	20.0d	2.1c
0.1	1.0	34.3c	5.7b
0.1	5.0	72.5a	7.3a
0.5	0.05	17.1d	2.9c
0.5	0.1	42.9bc	3.1c
0.5	0.5	57.1b	2.4c
0.5	1.0	31.4c	6.1b
0.5	5.0	40.1bc	8.4a

Table 4. Effect of NAA and BA on Shoot Formation from *Echinacea tennesseensis'* Leaf Explants

NAA (mg/l)	BA (mg/l)	Explants with shoots (%)[1]	Number of shoots/explant[1]
0.05	0.05	0.0f	0.0e
0.05	0.1	0.0f	0.0e
0.05	0.5	14.4e	3.2d
0.05	1.0	11.4e	3.8d
0.05	5.0	28.6cd	3.7d
0.1	0.05	40.0c	6.4c
0.1	0.1	45.7bc	7.9bc
0.1	0.5	54.3b	10.1ab
0.1	1.0	65.7ab	8.3b
0.1	5.0	88.6a	12.0a
0.5	0.05	34.3cd	5.7c
0.5	0.1	48.6bc	8.4b
0.5	0.5	51.4bc	8.4b
0.5	1.0	40.0c	9.1ab
0.5	5.0	28.6cd	7.0bc
1.0	0.05	14.4e	3.8d
1.0	0.1	8.6ef	1.7d
1.0	0.5	0.0f	0.0e
1.0	1.0	0.0f	0.0e
1.0	5.0	0.0f	0.0e

Table 5. Effect of Cytokinin on Shoot Formation from Leaf Explants of *Echinacea tennesseensis.*

Cytokinin (5.0 mg/l)	Explant with Shoots (%)[1]	Number of Shoots/Explant[1]
BA	85.7a	11.1c
Kinetin	80.0a	11.0c
TDZ	88.6a	32.4a
Zeatin	91.4a	13.5b

[1] Means within a column followed by different letters are significantly different according to Duncan's Multiple Range Test (P=0.05)

Effect of Fungicides on Rooting of Three Ornamental Species

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Index Words: Fungicide, Rooting, Inhibition, Benlate, Benomyl, Captan, Chipco 26019, Iprodione, Daconil, Chlorothalonil, Cleary's 3336, Thiophanate-methyl, Eagle, Myclobutanil, Heritage, Azoxysrtrobin, Terraclor, PCNB, Terrazole, Etridiazole, Reeve's Spiraea, *Spiraea cantoninenses*, Azalea "Pride of Mobile", *Rhododendron indica*, *Viburnum prunifolium*, Blackhaw Viburnum.

Nature of Work: Various fungicides have been shown to inhibit rooting during propagation and this has also been shown to be species specific (2-4). Many species and cultivars have unique characteristics and can be multiplied only by asexual propagation. During cutting propagation, high humidity and mist irrigation are crucial; because these conditions are conducive to many pathogens, common propagation manuals call for the use of foliar fungicides (1). Previous work has been done in this field to a limited extent in the past, mainly focusing on poinsettias (2,4). Unfortunately, little recent work has been and many species, varieties, and cultivars have not been studied in trials, leaving huge gaps in the literature. Since the facts are: some fungicides inhibit rooting, fungicide labels are currently changing rapidly, and many new fungicides are becoming available, a propagator may not know what fungicides are safe on what species.

This project was conducted at Auburn University and studied the effects of nine fungicides and a non-fungicide water treatment on commonly cutting-propagated woody ornamental species. The species *Rhododendron indica* "Pride of Mobile", *Spiraea cantoniensis*, and *Viburnum prunifolium* were chosen due to their common usage in ornamental landscapes, their means of propagation and propagation requirements are all similar, and yet they are all from different families allowing us to explore species specificity. The cuttings were taken early in June and stuck in 2:1 peat and perlite medium and then placed under mist irrigation for ten weeks. Foliar treatments were applied until run-off beginning the afternoon of the first full day of irrigation and repeated weekly; the treatments were the labeled rates of Benlate (Benomyl), Captan (Captan), Chipco 26019 (Iprodione), Daconil (Chlorothalonil), Cleary's 3336 (Thiophanate-methyl), Eagle (Myclobutanil), Heritage (Azoxysrtrobin), Terraclor (PCNB), Terrazole (Etridiazole), and a non-fungicide water treatment. Each treatment for each species was replicated four times

with six samples per treatment. The experiment was repeated twice for a total of three trials over time, totaling 2160 cuttings evaluated.

Results and Discussion: Analysis of the trial, without separating species, showed that Terraclor and Terrazole had significantly fewer rooted cuttings than the other treatments (Fig 4). Individual species analysis showed that azalea rooting was inhibited by Terraclor (Fig 1) and the spiraeas were inhibited by Terraclor and Terrazole (Fig 2). There was no significant difference between treatments for the viburnums (Fig 3), this reiterating the fact that treatments effect species differently.

Significance to Industry: For propagators to make intelligent and proper decisions for disease control using foliar fungicides, they must know about both the species propagated and the fungicide(s) chosen. This study shows that there are large gaps of information in the area of how fungicides effect rooting. Many fungicides may be labeled for ornamental crops that they may inhibit the rooting of. Growers should test the fungicide(s) they plan to use on a small trial crop of the chosen species if better information cannot be found.

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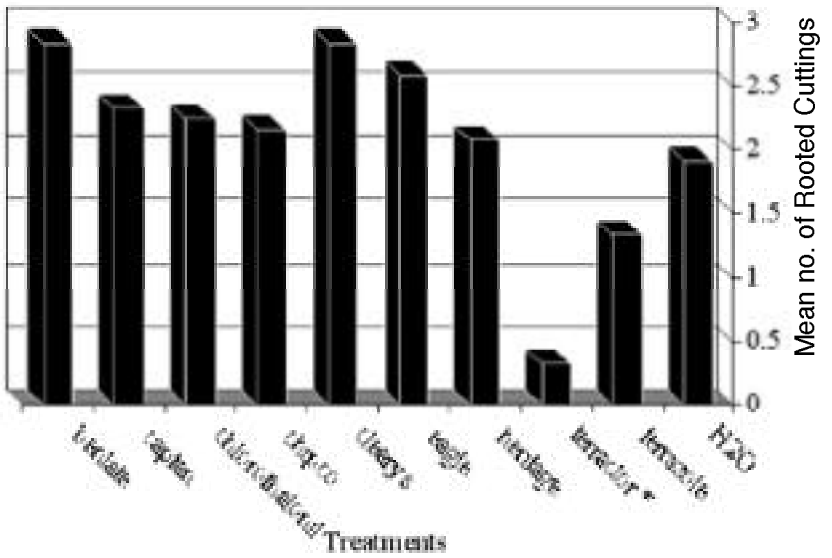
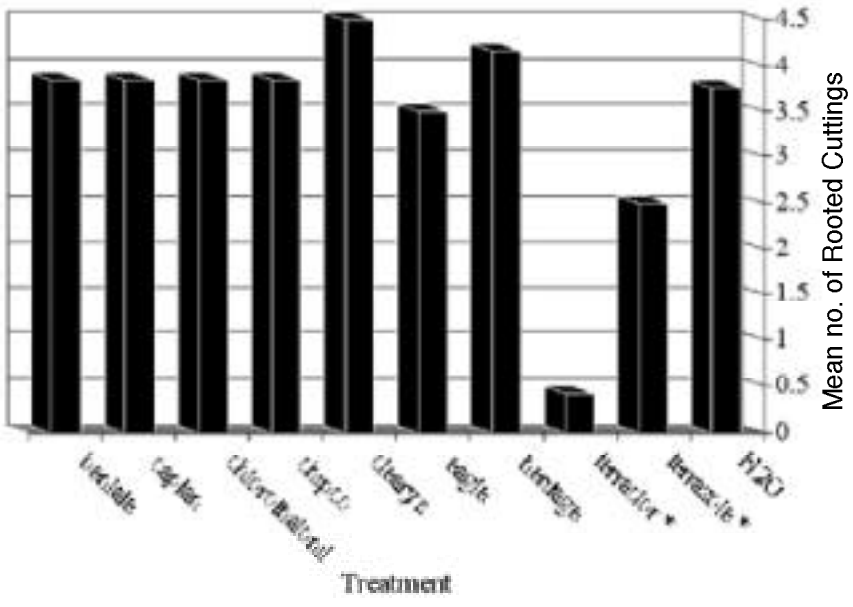


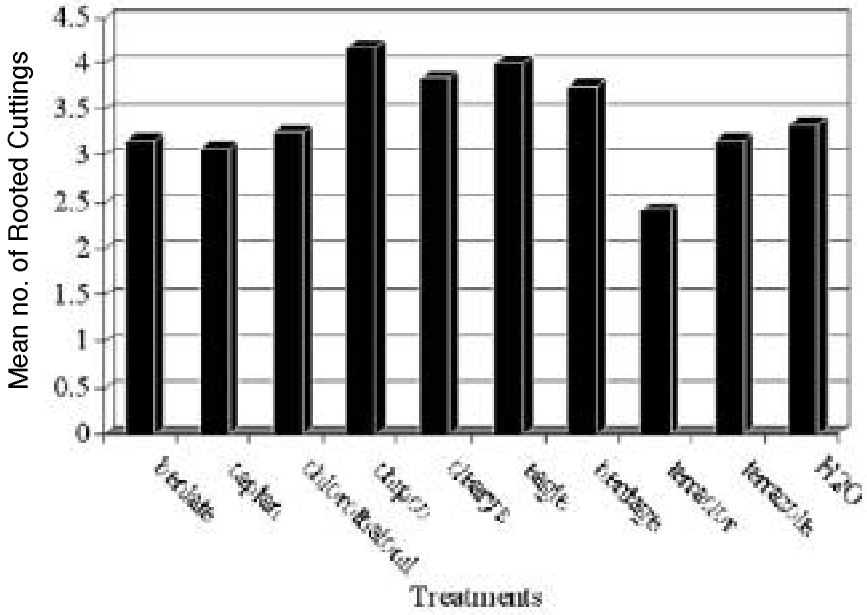
* Indicates significanty fewer rooted cuttings to the 0.05% level

Fig 1–Azalea Rooting Results



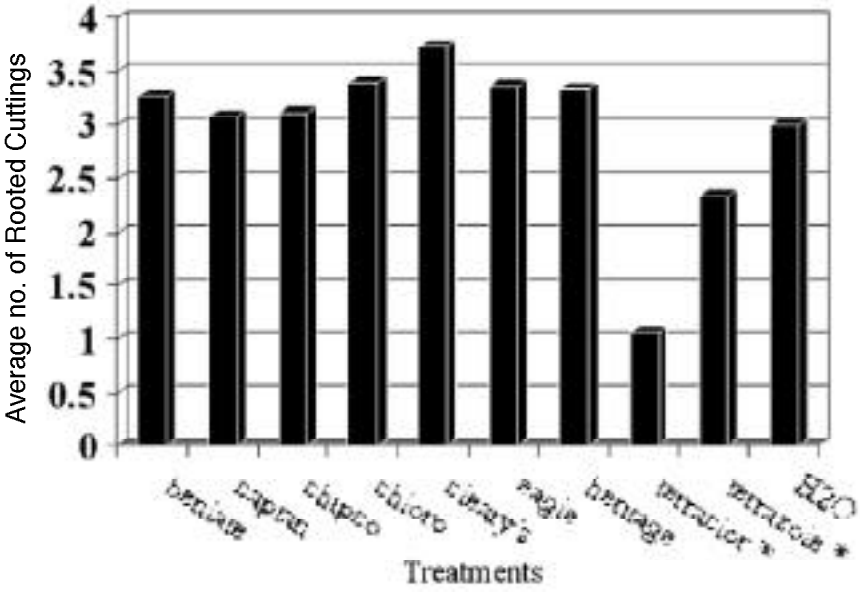
* Indicates significanty fewer rooted cuttings to the 0.01% level

Fig 2–Spiraca Rooting Results



(no significant difference)

Fig 3–Viburnum Rooting Results



* Indicates significanty fewer rooted cuttings to the 0.01% level

Fig 4–Pooled Trial Results

Factors Affecting Rooting of *Vinca minor* Single-Node Cuttings

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Index Words: Vegetative Propagation, Indolebutyric Acid, Naphthalene Acetic Acid

Nature of work: *Vinca minor* is a trailing, evergreen groundcover. The funnelform flowers, about 2 cm in diameter, appear in early spring and are usually blue-lavender or rarely white (1, 2). *V. minor* roots readily from the nodes in the landscape, however there has been mixed success in greenhouse production. This study was undertaken to determine if cultivar, cutting position, hormone concentration, and the time of year the cuttings were taken affected the rooting success of *V. minor*. The cultivars 'Bowles', 'Dart's Blue' and 'Sterling Silver' were used. Single-node cuttings taken from the distal portion of the stems (distal 10cm) and from sections proximal to this were compared. On January 25, March 20, July 15, and October 7, 2001,160 cuttings (80 distal and 80 proximal) were obtained from each cultivar. Rooting hormone solutions were applied as a quick dip (5 seconds) at indolebutyric acid/naphthalene acetic acid (IBA/NAA) concentrations of: 0 (control), 1000/500, 2000/1000, or 3000/1500 ppm. After the hormone treatments, the cuttings were allowed to dry and were then inserted into a rooting medium (MetroMix 360, The Scotts Co., Marysville, OH) in flats. The cuttings were placed under intermittent mist (5 seconds every 5 minutes) during daylight hours, and after seven weeks, evaluated for root numbers, root lengths, and rooting percentages. In general, root number and length responses corresponded closely with rooting percentages unless otherwise stated; therefore, only percentage data are shown here (Tables 1-4). The experiment was a 3x2x4 factorial (3 cvs., 2 stem positions, 4 hormone treatments) arranged in a randomized complete block design with 5 replications, 4 cuttings per treatment per block, on each of the 4 cutting dates. Data were analyzed using ANOVA with mean separation by LSD. Cutting collection dates were analyzed separately.

Results and Discussion: ***January cuttings*:** There was a significant cultivar by hormone level interaction for rooting percent, root numbers and root lengths. 'Darts Blue' rooted well over all three IBA/NAA concentrations, from 75 to 80%. Only the control (0 IBA/NAA) had significantly lower rooting success (43%) (Table 1). 'Bowles' rooted well (80%) only

with IBA/NAA at 1000/500 ppm. 'Sterling Silver' rooted poorly over all hormone treatments in January. Rooting responses for this cultivar increased as hormone concentration increased, with the highest rooting percentage occurring with the 3000/1500 hormone concentration at only 40%. There was little effect on rooting responses due to cutting position in January.

***March cuttings*:** The highest rooting over all treatments for this date occurred with 'Sterling Silver', which had 89% rooting as opposed to 33% and 28% respectively for 'Bowles' and Darts' Blue'. Best rooting for 'Sterling Silver' occurred with IBA/NAA treatments of 1000/500 ppm and 2000/1000 ppm, both of which provided 98% rooting (Table 2). However, even the control (no hormone) provided rooting at 88% although it resulted in fewer roots per cutting (about 2) than the hormone treatments (about 5). 'Bowles' and 'Darts Blue' both rooted poorly with the March cuttings and both cultivars responded almost identically to the hormone treatments (Table 2).

***July cuttings*:** 'Sterling Silver' again had the best rooting performance, with 71% rooting over all treatments and cutting positions. Best rooting percentages for 'Sterling Silver' occurred with no hormone or with the 1000/500 ppm IBA/NAA treatment (about 85%, Table 3), but the low hormone treatment provided more roots per cutting than the no hormone control (7.8 vs. 2.6). Over all cultivars and hormone treatments, more roots per cutting were produced on cuttings from the proximal part of the stem than from the distal portion (4.3 vs 2.6, Table 6). There were also significant cutting position by hormone treatment interactions for root numbers and percentages. The interaction effects were that for proximal cuttings, there were significant increases in root numbers and rooting percentages due to the hormone treatments, but for distal cuttings, there were no increases in these responses due to the hormones (Table 5).

***October cuttings*:** In October, all three cultivars rooted about equally well in terms of rooting percentages (Table 4). All three cvs. with no hormone or treated with 1000/500 ppm IBA/NAA rooted at between 85 and 98%. However, the hormone treatments averaged more roots per cutting than the untreated controls over all cultivars (2.9 vs. 1.3). The higher hormone rates generally gave lower rooting percentages than the 1000/500 ppm rate, with the exception of the 2000/1000 ppm treatment on 'Bowles' which resulted in 90% rooting, equal to the lower hormone rate. Over all cultivars and treatments, the proximal cuttings generally rooted slightly better than the distal cuttings in October (Table 6).

Significance to Industry: The time of year the cuttings were taken and the rooting hormone concentration affected the rooting success of the three cultivars of *Vinca minor* differently. In most cases, optimal hormone concentrations were either 1000/500 ppm or 2000/1000 ppm IBA/NAA depending on cultivar and cutting date. However, in October, all three cultivars rooted in high percentages with the lowest hormone concentration or with no hormones. The root numbers and root lengths were generally consistent with the rooting percentages. There were some differences in rooting due to cutting position on the stem, depending upon cutting date.

Acknowledgements: The authors thank Hanover Farms Nursery, Rockville, VA, for their support of this study.

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Table 1. Rooting percentages of *Vinca minor* single-node cuttings taken January 25, 2001

IBA/NAA conc. (ppm)	*Vinca minor* cultivars		
	'Bowles'	'Dart's Blue'	'Sterling Silver'
0/0	20c[z]	43b	18a
1000/500	80a	75a	28a
2000/1000	53b	80a	33a
3000/1500	50b	75a	40a

[z] Means with the same letter within a column are not significantly different (P= 0.05).

Table 2. Rooting percentages of *Vinca minor* single-node cuttings taken March 10, 2001

IBA/NAA conc. (ppm)	*Vinca minor* cultivars		
	'Bowles'	**'Dart's Blue'**	**'Sterling Silver'**
0/0	18b[z]	15b	88ab
1000/500	58a	50a	98a
2000/1000	35ab	30b	98a
3000/1500	23b	15b	75b

[z] Means with the same letter within a column are not significantly different (P= 0.05).

Table 3. Rooting percentages of *Vinca minor* single-node cuttings taken July 13, 2001

IBA/NAA conc. (ppm)	*Vinca minor* cultivars		
	'Bowles'	**'Dart's Blue'**	**'Sterling Silver'**
0/0	35b[z]	60	85a
1000/500	68a	70	83a
2000/1000	45b	53	63ab
3000/1500	50ab	58	55b
		NS	

[z] Means with the same letter within a column are not significantly different (P= 0.05).

Table 4. Rooting percentages of *Vinca minor* single-node cuttings taken October 7, 2001

IBA/NAA conc. (ppm)	*Vinca minor* cultivars		
	'Bowles'	**'Dart's Blue'**	**'Sterling Silver'**
0/0	85ab[z]	85ab	98a
1000/500	90a	95a	93a
2000/1000	90a	73a	68b
3000/1500	70b	70a	60b

[z] Means with the same letter within a column are not significantly different (P= 0.05).

Table 5. Rooting response interaction effects of cutting position on rooting hormone response for cuttings taken in July.

IBA/NAA Concentration	Root number	Percent rooting
Distal cuttings[z]		
0	1.7	68
1000/500	3.6	63
2000/1000	2.6	43
3000/1500	2.6	50
	NS[y]	NS
Proximal cuttings		
0	1.1c	52b
1000/500	7.2a	83a
2000/1000	5.1b	63b
3000/1500	4.1b	58b

[z] Distal cuttings were single-node cuttings from the distal 10 cm of the stem. Proximal cuttings were taken proximal to the distal 10 cm of the stem.
[y] Means with the same letter within a column are not significantly different (P= 0.05). NS= no significant difference within a column.

Table 6. Comparison of rooting response of *Vinca minor* cuttings taken from the distal 10 cm or proximal to that on the stem, as affected by time of year. Results are combined over three *V. minor* cultivars.[z]

Cutting position	Root number	Percent rooting
July cuttings		
Distal	2.6b[y]	56
Proximal	4.3a	64
		NS
October cuttings		
Distal	2.4b	77b
Proximal	2.8a	85a

[z] *Vinca minor* cultivars included ‘Bowles’, ‘Dart’s Blue’, and ‘Sterling Silver’.
[y] Means with the same letter within a column are not significantly different (P= 0.05). NS= no significant difference within a column.

Live Stakes for Erosion Control

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Index Words: Erosion, Estuary, Live Stakes, *Acer, Alnus, Betula, Cornus, Physocarpus, Platanus, Salix, Sambucus*

Nature of Work: Reducing or preventing erosion along estuaries requires minimal disturbance of adjacent land (1). One solution to this problem is to use live stakes which are " . . . woody plant cuttings capable of quickly rooting in the streamside environment. The cuttings need to be large and long enough to be tamped as stakes" which is usually _ to 2 inches in diameter and 2 to 3 feet long. "Stakes are used on streambanks of moderate slope (4:1) in original soil, not on fill." (2)

The objectives were: 1. to evaluate the influence of IBA treatments on the percentage of stakes surviving in this challenging environment. 2. determine which species are locally adapted to this technique. The site was Codorus loam along a spring fed stream located on the Mountain Horticultural Crops Research Station, Fletcher, NC. Soil was not tilled or fertilized nor was weed or other pest management implemented at any time during the test.

Test 1: Stakes of *Alnus serrulata*, *Cornus amomum* and *Salix nigra* were locally collected on December 18, 2001 and kept moist overnight. On December 19, 2001 stakes were graded for uniformity and cut to length on a table saw. The bottom was pointed and the top cut perpendicular to the stem to facilitate soil penetration and to ensure that proper polarity of the cutting was maintained. Immediately after making a fresh cut, IBA treatments were applied. Treatments were a quick dip of K-IBA/water solution at 0, 1250, 2500 or 5000 ppm IBA with 5 stakes per treatment and 3 replicates. After IBA treatment stakes were driven into the soil at the test site such that at least 6 inches of the stake was above the soil surface and at least one node was below the soil surface. Stakes were on 6 inch centers with treatments randomized within replicates. Replicates were parallel to the stream such that any soil moisture gradients due to flooding or drought would likely occur within replicates.

Test 2: Stakes *of Acer negundo, Betula nigra, Physocarpus opulifolius, Platanus occidentalis,* and *Sambucus canadensis* were prepared as described previously but not treated with IBA. Twelve stakes per replicate with three replicates were employed in the test. Otherwise procedures were similar. Survival was determined by bud break from these dormant deciduous shrub cuttings. Data was collected on April 27, 2002 and again on June 6, 2002.

Results and Discussion:

Test 1: In the IBA test, percentage of plants with living foliage in June was lower than those in April suggesting that some buds broke without a root system to support growth so plants died. No significant difference in the number of stakes with living foliage existed due to treatments. *Alnus serrulata*, a non-recommended estuarine species, had from 13 to 20% of stems with living foliage on June 4, 2002. *Cornus amomum* had from 87 to 100% and *Salix nigra* had from 93 to 100% of stakes with living foliage in June.

Test 2: *Betula nigra, Physocarpus opulifolius, Platanus occidentalis* and *Sambucus canadensis* had 47 % or greater stakes with living foliage on June 4 (Table 1). There was no living foliage on Acer negundo at that date. The change in percentages from April readings to June reflects the differing rates of bud break and survival in these species. The percentage of stakes with foliage breaking continued to increase in *Platanus occidentalis* while it remained essentially the same from April to June in *Physocarpus opulifolius* and *Sambucus canadensis*. The percentage of stakes with living foliage in *Acer negundo* and *Betula nigra* decreased from April to June suggesting that buds broke but that the stakes were unable to support this growth.

Significance to the Industry: 1. There was no benefit to treating live stakes with the concentrations of IBA used in this test. 2. USDA recommended species *Cornus amomum, Salix nigra* and *Sambucus canadensis* all survived at greater than 50%. USDA recommended species *Acer negundo* did not live. 3*. Betula nigra, Physocarpus opulifolius* and *Platanus occidentalis* survived in high percentages and should be added to suggest species lists for the region. *Alnus serrulata* survived at about 20%.

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Table 1. Percentage of live stakes with living foliage.

Plant	Date	
	April 27, 2002	June 4, 2002
Acer negundo	8	0
Betula nigra	72	47
Physocarpus opulifolius	94	94
Platanus occidentalis	53	56
Sambusus canadensis	53	56

Evaluation of Four Different Propagation Mats and Thermostats Temperature Fluctuation Performance And Cost Effectiveness

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Index Words: Propagation

Nature of Work: Medium temperature plays a significant role in successful propagation of many plants. Propagation mats are designed to aid with indoor propagation by keeping the medium consistently warm. Furthermore, it can be greatly influenced by water from mist systems and/or ambient air temperature (1). It may be more cost efficient to control the temperature of the medium with propagation mats at the bench level as opposed to heating an entire propagation house. Every species has an ideal temperature range to initiate roots on cuttings. Just as low medium temperatures may inhibit rooting, excessively high temperatures can cause cuttings to fail. Generally speaking, the optimum medium temperature for propagation of temperate climate plants is 18∞ to 25∞ C (65∞ to 77∞ F) (1). Not maintaining temperatures at an appropriate level for root initiation may result in slow and/ or poor root development, low rooting percentages or complete failure of cuttings (2).

Eight propagation mats and recommended thermostats were purchased from 4 different manufacturers (2 identical mats and thermostats from each manufacturer). Hydrofarm, Olson, Pro-Gro and Redi-Heat were the four brands that were selected for this research. The propagation mats and thermostats were randomly placed on mist benches inside a polyhouse. To simulate a cutting propagation experiment, flats were filled with moistened Morton's Grow Mix #4. One flat was centrally placed on each of the 8 mats (see Table 1 for mat dimensions). Intermittent mist operated for 5 seconds every 10 minutes during daylight hours. A natural photoperiod was provided. Heating and cooling thermostats in the polyhouse were set to maintain the ambient air temperature at 21.1∞ C (70∞ F) +/- 2.9∞ F (+/- 5∞ F). Propagation mat thermostats were set to maintain a constant 25∞ C (77∞ F) temperature. Medium temperature was allowed to regulate for 1 day (24 hours) before data were collected. Spectrum Data Loggers (3) were used to record actual medium temperatures on a continuous hourly basis. Spectrum Temperature Sensors were placed at a depth equivalent to the rooting zone of cuttings.

Results and Discussion: Data were collected for 339 consecutive hours beginning December 7, 2001 and terminating December 21, 2001 (14

days and 3 hours). All data were subjected to an Univariate ANOVA and means were further analyzed using Duncan's multiple range test (4). The main effect was significant at the $p \leq 0.05$ level (Table 2). Mean separation indicated that the temperature of each set of mats were significantly different from one another. Results indicated that the Pro-Gro mats and thermostats maintained a 25∞ C (77∞ F) medium temperature more consistently over the test period than the other 3 mats and thermostats (Table 3). Hydrofarm, Olson and Redi-Heat followed in respective order. Means for the Pro-Gro and Hydrofarm products fell within the manufacturers stated temperature accuracy claims.

Temperature fluctuations will occur in a poly-house environment. Ambient air temperature influenced all four manufacturer's mats, or medium temperature. As ambient air temperatures decreased at night, so did mat/medium temperatures; and as ambient air temperatures increased during the day, mat/medium temperatures increased (data not presented). Temperature accuracy claims varied with each manufacturer (Table 4). Only the Redi-Heat brand maintained the medium temperature at a level that did not decrease below its stated fluctuation claim. However, the Redi-Heat products consistently increased the medium temperature well above the claimed level of temperature fluctuation (Table 4). Excessive heat may not be beneficial to root cuttings (1). The Pro-Gro brand maintained a medium temperature high that did not exceed its stated fluctuation claim, while allowing the medium temperature to decrease below the stated fluctuation claim. Hydrofarm and Olson products each allowed medium temperatures to increase and decrease beyond the stated fluctuation claims.

Hydrofarm retailed at the highest total price of the four brand names with Pro-Gro being the second most expensive (Table 5). However, the suggested thermostat for the Pro-Gro mat has a single electrical outlet capable of regulating one mat, while the suggested thermostat for the Hydrofarm mat has dual electrical outlets capable of regulating two mats. The addition of purchasing two Pro-Gro thermostats to control separate mats makes the total cost of the Pro-Gro and Hydrofarm equipment virtually identical if more than one mat is purchased. The Redi-Heat thermostat was the most expensive of the 4 brands and is capable of regulating 4 mats at once. Redi-Heat mats are the least expensive of the 4 brands. The Olson thermostat was the least expensive and was capable of regulating 1 mat at a time.

Significance to the Industry: Results show that temperature differences exist from one brand of mat and thermostat to the next. Plant propagators typically focus upon rooting hormone compounds, light intensity, medium type and moisture retention. A number of factors can

influence the medium temperature. This research reveled that of the mats and thermostats tested, the more expensive products performed at a more consistent level. Utilizing a product that performs more consistently at the manufacturers claimed level of temperature accuracy may increase the success rate of rooting cuttings.

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Table 1. Propagation Mat Size Dimensions

Mat Brand	Dimensions
Hydrofarm	35” x 48”
Olson	22” x 96”
Pro-Gro	22” x 60”
Redi-Heat	21” x 60”

Table 2. Univariate Analysis of Variance

Source	df	Mean square	F value	Significance
Main Effect	3	2831.41	427.56	*
Error	2708	6.62		

NS, *, ** Nonsignificant or significant at P = 0.05 or 0.01, respectively.

Table 3. Mat Brand, Number of Hourly Readings and Temperature Mean*

Mat Brand	N	Mean
Olson	678	23.38° C (74° F)
Hydrofarm	678	24.56° C (76° F)
Pro-Gro	678	25.06° C (77° F)
Redi-Heat	678	28.17° C (83° F)

* All means were significantly different from each other

Table 4. Manufacturers Temperature Accuracy Claim and Actual Low/ High Mean of Each Brands Mat and Thermostat

Mat Brand	+/- claim	Mean Low/High
Hydrofarm	1.1° C (2° F)	20.60° C / 27.75° C (69° F / 82° F)
Olson	0.5° C (1° F)	16.95° C / 26.00° C (63° F / 79° F)
Pro-Gro	2.2° C (4° F)	21.35° C / 26.60° C (71° F / 80° F)
Redi-Heat	1.6° C (3° F)	24.25° C / 29.60° C (76° F / 85° F)

Table 5. Retail Cost of Mats and Thermostats

Mat Brand	Mats	Thermostats	Total Cost
Hydrofarm	$169.00	$102.00	$271.00
Olson	$99.70	$36.70	$136.40
Pro-Gro	$155.25	$57.80	$213.05
Redi-Heat	$91.80	$104.50	$196.30

In Vitro Regeneration of St. John's Wort and Coneflowers

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Index Words: *Hypericum*, *Echinacea*, Tissue Culture, 2,4-Dichlorophenoxyacetic Acid, Naphthaleneacetic Acid, 6-Benzyl-aminopurine

Nature of Work: *Hypericum and Echinacea* are two of the most widely known and utilized medicinal plants. *Hypericum* (St. John's Wort) is known for its anti-depressant and anti-cancer activity and *Echinacea* (Purple Coneflower) for its enhancement of the immune system. The quality of the product in the botanical supplement industry is important; thus, it is important for growers to have access to a supply of plants that consistently produce high levels of active compounds. Tissue culture is a good method for the propagation of genetically identical plants.

The objective of this research was to develop in vitro propagation protocols for St. John's Wort and coneflower. All plant material used in this study was obtained from sterile seedlings. Seeds of *Hypericum perforatum*, 'Topas' and *Echinacea angustifolia*, *E. pallida*, *E. purpurea* 'Magnus' and 'White Swan' were germinated on moist filter paper in petri dishes. Hypocotyls of *H. perforatum* and leaves, petioles, hypocotyls, and cotyledons of *E. pallida*, *E. angustifolia*, and *E. purpurea* 'White Swan,' 'Magnus' and 'Leuchtstern' were used as explants. To determine the optimum conditions for organogenesis in these two genera, factorial combinations of naphthaleneacetic acid (NAA), 6-benzyl-aminopurine (BA) and 2,4-dichlorophenoxyacetic acid (2,4-D) in Murashige-Skoog (MS) media were tested.

Cotyledon and hypocotyl sections were placed on MS basal medium supplemented with 2% sucrose and varying concentrations of NAA and BAP. All cultures were maintained in a growth chamber at 25^0C and all embryogenic calli and/or shoots that formed were transferred onto fresh media for growth.

Results and Discussion: Hypocotyl sections of *Hypericum* formed calli on MS medium supplemented with 2mg/L 2,4-D after 3 weeks in culture. These calli were subsequently transferred onto a MS medium that contained 0.2 mg/L BA and maintained in complete darkness for three additional weeks. Then, they were cultured under constant light at 25^0C for shoot and root development (Figure 1). Prolific shoot formation was observed on all hypocotyl segments.

Shoot organogenesis occurred from all explants of *Echinacea* with the exception of those taken from hypocotyls and calli formed on all explant types including hypocotyls. The best response was with leaf tissue explants cultured on MS medium supplemented with 1 mg/L BA in combination with either 0.1 or 0.5 mg/L NAA. *Echinacea purpurea*, 'Leuchtstern' produced the highest frequency of shoots (Figure 2). Cotyledon explants from one-week-old *Echinacea* seedlings responded differently to hormones than from other explants. All cultures were examined for shoot and/or callus formation after six weeks of incubation. *Echinacea purpurea* 'White Swan' and 'Magnus' produced very little growth when cultured on a MS medium supplemented with 0.5 mg/L NAA and 5.0 mg/L BAP. On this medium, all explants of *E. angustifolia* formed calli and forty percent of *E. pallida* cultures formed shoots (Figure 3). When the NAA concentration was reduced to 0.1 mg/L, *E. angustifolia* formed shoots from cotyledon explants (30%) while *E. pallida* formed only calli. None of the other *Echinacea* accessions formed shoot or callus. When the concentration of BAP in the MS medium was reduced to 1.0 mg/L, all accessions produced calli from cotyledon explants. *Echinacea purpurea* 'White Swan' formed shoots in 15% of all cultures while *E. pallida* formed shoots in 60%. Hypocotyl explants were not responsive to this treatment.

Leaves obtained from greenhouse grown plants responded more favorably to hormones in all media. All *E. pallida* cultures formed calli regardless of the hormone concentrations used. Over 65% of cultures exposed to 0.1 mg/L NAA and 1.0 mg/L BA formed shoots. Explants of *E. purpurea* 'Leuchtstern' formed shoots more readily than those from *E. pallida*. The frequency of shoot formation ranged from 0 to 90%. The highest frequency of shoot formation occurred on MS medium supplemented with 0.1 mg/L NAA and 1.0 mg/L BAP.

Petiole explants obtained from greenhouse grown *E. purpurea* 'Leuchtstern' produced embryogenic calli after 8 weeks of culture on a MS medium containing 0.5 mg/L NAA and 5.0 mg/L BAP. Upon transfer to a MS medium with a lower concentration of BAP (1.0 mg/L), these calli produced high numbers of shoots after 6 weeks of culture.

Significance to Industry: Medicinal properties of *Hypericum and Echinacea* are due to secondary compounds they synthesize. Examples of secondary compounds with medicinal properties are certain anti-cancer drugs such as Taxol extracted from *Taxus brevifolia*, pain relief medicine from *Salix alba* and eucalyptus fragrances from *Eucalyptus globules*. Secondary metabolites are readily produced in many different types of plants, but the types of metabolites are different. No single plant species or cultivar produces every important compound. While some

compounds are observed in only one cultivar or species, others may be found throughout the genus of that species.

The botanical supplement industry needs plants that dependably produce high levels of high-quality secondary metabolites under a wide range of climatic conditions. To achieve this objective, we have developed a propagation system for *Hypericum* and *Echinacea* that preserves their genetic identity and their ability to produce certain medicinal compounds. The use of tissue culture for propagation ensures a supply of consistent high quality plants to growers and scientists alike. In contrast to field production, in vitro culture techniques are under controlled environmental conditions. These methods provide standardized means to evaluate secondary metabolites production in these clones (Hamill et al. 1987). The understanding of plant regeneration processes in clones enables researchers to investigate processes of secondary metabolites synthesis. Through tissue culture, investigators will be able to identify plants, developmental stages, optimum growth conditions and specific tissue where secondary compounds are synthesized.

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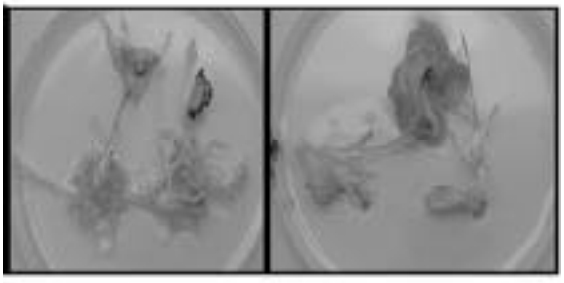
Figure 1. Root and shoot formation from Hypocotyls of *Hypericum*.



Figures 2 and 3. Root and shoot formation from Cotyledons and Leaves of *Echinacea.*

Molecular Identification and Phytochemical Analysis of Eleven *Hypericum* Accessions

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Index Words: St. John's Wort, *Hypericum*, AFLP profiles, HPLC Analysis.

Nature of Work: **S**t. John's Worts (*Hypericum* spp.) contain a range of medicinally important compounds that have antibacterial, antidepressant and anti-inflammatory effects. Because of the importance of these compounds in alternative medicine, there is a vital need to investigate this genus for genetic markers and phytochemical profiles. Development of cultivars with superior phytochemical profiles is facilitated once parental plants have been analyzed for genetic markers associated with phytochemical contents. Such genetic and phytochemical characterizations are fundamental for the rapid identification of offspring that carry genes responsible for phytochemical production following breeding efforts. The objectives of this study were to quantify pharmaceutically important compounds and to identify molecular markers associate with their production in selected *Hypericum* species and cultivars. Leaf samples from eleven species and cultivars of *Hypericum* (*H. androsaemum*, *H. calycinum*, *H. frondosum*, *H. grandiflorum*, *H. inodorum*, *H. monseranum*, *H. olympicum*, *H. patulum*, *H. perforatum*, *H. perforatum* 'Anthos', and *H. perforatum* 'Topas') were used for AFLP (Amplified Fragment Length Polymorphism) and HPLC (High Performance Liquid Chromatography) analyses.

Amplified fragment length polymorphism is a quick and reliable technique that permits for the inspection of a large number of genetic markers with cost and time-effectiveness (Vos *et al.*, 1995). All DNA samples were obtained from leaves collected from one-year old greenhouse grown plants. DNA was isolated from leaf samples with a DNeasy Plant mini extraction kit (QIAGEN, Santa Clara, CA). Presence of DNA was verified in a 2% agarose gel through electrophoresis and concentrations were quantified using a Hoechst-dye based fluorometer (Hoefer Scientific Instruments, San Francisco, CA). AFLP markers were generated by amplification via polymerase chain reaction (PCR) using an AFLP System-Analysis Kit (GibcoBRL, Rockville, MD). AFLP amplification included restriction digestion of the sample DNA, ligation of the adaptors (synthetic oligonucleotides), pre-amplification of adapter-ligated DNA

fragments, and amplification of molecular markers with selective AFLP primers (GibcoBRL, Cat. no. 10544-013). Agarose gel analyses were performed to check for restriction digestion, pre-amplification and amplification of all plant DNA samples. AFLP profiles (DNA fingerprints) were scored by separating each selective amplification products by 6.5% polyacrylamide gel electrophoresis (Qiu, 2001). All AFLP profile images were obtained with an automated DNA analyzer (Global IR^2 DNA Analyzer and Sequencer, LI-COR). Gel analysis software and TreeCon-Dendogram software (Scanalytics Inc., Fairfax, VA) were used to analyze banding patterns on gel images and to graph (clustering method) the genetic distances between each *Hypericum* accessions.

HPLC analysis was used to identify rutin, hypericin, pseudohypericin, and hyperforin. Methanol-extracts of dry leaves were quantified with a C18 (Vydae, Hesperia, CA) column. Fresh shoot tissue obtained from greenhouse-grown plants was dried at 75°C for twelve-hour. Broils' soxhlet extraction method (Broils *et al.* 1998) was used for sample preparation. For extraction of phytochemicals, 1 gram (dry weight) of plant tissue was mixed with 100 ml of 100% methanol and refluxed for 6 hours in a Soxhlet apparatus. The supernatant was cooled to room temperature, diluted to 10 mL in a methanol-water solution (v/v) and filtered through a cartridge-type filtration unit (0.45mm PTFE membrane). A Hewlett Packard 1100 series (Atlanta, GA) equipped with a quaternary pump, autosampler, DAD detector, and gradient pump controller was used for all phytochemical analyses. Analyses were performed at 30°C on a 201 TP 54 C-18 (Vydac, Hesperia, CA) column (4.6 x 250mm). The following protocol was used: flow rate 1.0 ml/min, injection volume 10 ml and total run time 60 minutes. Chromatographic separation was performed using a three solvent gradient: water: phosphoric acid (99.7:0.3), acetonitrile, and methanol.

Results and Discussion: AFLP profiles obtained contained ample polymorphism to distinguish each accession and good DNA fingerprints were obtained with the DNA analyzer. The banding patterns highlighted by the IR2 analyzer were used to compare genetic similarity between each accession. In this investigation, we attempted to draw correlations between phenotypic traits with their corresponding AFLP fingerprints. In many instances, there were similarities between phenotypically similar plants and their AFLP marker profiles. Fingerprints of *H. olympicum* and *H. grandiforum*, two phenotypically similar species, shared identical banding patterns with multiple primer combinations (Figure 1). Also, *H. androsaemum* and *H. inodorum*, two other similar species, also branched together on the dendrogram (Figure 1). Primer pair correlations were observed among *H. monseranum* (a hybrid line) and its parental species *H. patulum* and *H. calycinum*. However, this accession is genetically

more similar to *H. calycinum*, a smaller leaf species with a creeping growth form, than with *H. patulum*. These two accessions also share an upright growth pattern. These observations may explain *H. monseranum*'s leaf variegation since *H. calycinum* also expresses irregular reddish coloration on its foliage. AFLP indicates greater genetic distance between *H. perforatum* 'Anthos' and the other two *H. perforatum* samples. *H. perforatum* 'Topas' and *H. frondosum* were found to be distantly related to *H. perforatum* (species) and *H. perforatum* 'Anthos' (Figure 1). These findings suggest that AFLP technique is able to detect marker differences within genetically similar species.

Polymorphisms revealed by DNA analysis can serve as markers for tracking genetic inheritance within progeny populations and these profiles along with HPLC assessments can be used to select plant for use in breeding efforts. Secondary metabolite or phytochemical levels among *Hypericum* species are frequently higher during flowering. In this study, we evaluated metabolite levels during the vegetative phase. *Hypericum monseranum*, *H. patulum*, *H. calycinum*, *H. inodorum*, and *H. androsaemum* have upright growth form and leaf variegation as their ornamental characters. Broad leaf size appears to conflicts with secondary metabolite production abilities although it would be a more desirable characteristic for ornamental types (Southwell and Campbell, 1991). *Hypericum perforatum* produce more metabolites during flowering. Our results show that it also produces more metabolites during its vegetative state. This study confirmed Kitanov's reports (2001) that *H. olympicum,* another physically non-attractive plant, is a moderate producer of phytochemicals. *Hypericum grandiforum*, which shared the very close genetic relationship with *H. olympicum* (Fig 1), also produces high levels of phytochemicals. *Hypericum grandiforum* also shared a number of banding pattern similarities with *H. moseranum* and *H. patulum*, two upright species that produced elevated secondary metabolites. HPLC analysis of *H. androsaemum* and *H. inodorum,* two phenotypically similar species that branched together on the dendrogram (Figure 1), indicated that they differ in their production of phytochemicals. Only *H. inodorum* was found with elevated levels of phytochemicals during vegetative growth. AFLP profiles paired with metabolite concentrations results will help to identify suitable candidates for marker-assisted breeding. For the perennial plant industry, it would be desirable to develop hybrids that produce metabolite levels similar to small leaf shaped ground cover types but with better ornamental characteristics.

Significance to Industry: The rise in popularity of homeopathic remedies for the treatment of minor medical conditions is opening new marketing opportunities for small farm operators. The development of St. John Wort cultivars with ornamental characteristics as well as high

production of pharmaceutical compounds would provide small farm operator with new niche plants. These plants could be marketed as ornamentals as well as medicinal herbs. In this study we compared 11 *Hypericum* accessions based on their AFLP and marker phytochemical profiles. AFLP markers associated with each accession can be used to identify plants and to determine if they can be associated with specific characteristics. Molecular methods used in this research can easily be adapted for genetic linkage and marker assisted breeding studies of *Hypericum* as well as other plants (Arnholdt-Schmitt, 2000). Plant breeders and horticulturists could use these methods for trueness to type determinations and for identification of progenies that have high phytochemical production potentials early in the selection process. This would reduce the time and inputs spent in selecting parents and judging for the presence of desirable characteristics in progenies.

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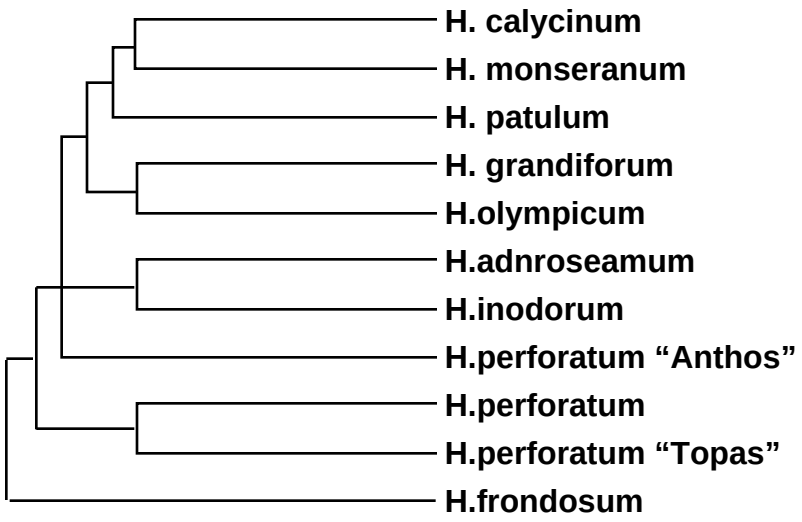

Figure 1: Genetic distances among 11 *Hypericum* accessions as depicted by a TreeCon-Dendogram software program (Scanalytics Inc., Fairfax, VA, USA).

Evaluation of an Alternative Method of Auxin Application in Cutting Propagation

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Index Words: ***C****hrysanthemum pacificum*, *Dendrathema pacificum*, *Rosa* x 'Red Cascade', Auxin Application, Auxins, Cutting Propagation, Rooting Hormones

Nature of Work: For many years, the use of auxins in cutting propagation has focused on its physical application to stem cuttings as a quick basal dip (using liquid or powder formulations) or an extended basal soak (using liquid formulations). Current Worker Protection Standards require that each employee involved in the use of such chemicals must receive specific safety training and wear required safety equipment, which in the case of auxin formulation for cuttings include protective gloves, eyewear, and clothing. Employees often note that the equipment is uncomfortable, and may also be concerned about their exposure to the chemicals. If an alternative means of auxin application were available that could reduce the number of nursery employees who must handle the auxins or reduce the amount of time that each employee must work with the chemicals, nursery safety could be enhanced. Spray applications of auxins for rooting stem cuttings may be one alternative.

Scientific literature contains little mention of spray applications of auxin for rooting stem cuttings. Kroin (1992) reported that certain cuttings could be rooted by spray treatment of cuttings, but provided no data from research studies. Chadwick and Kiplinger (1938) noted that chrysanthemum cuttings rooted better with a 24-hour basal dip than with a foliar spray using very low concentrations of IBA, but provided no data. Van Bragt et al. (1976) determined that cuttings of various woody species rooted better when immersed in a solution of auxin for two minutes in comparison to a basal dip in an auxin powder.

The objective of this experiment was to determine whether a foliar spray application of the auxins indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) as a dilution of Dip 'N Grow® rooting hormone (Dip 'N Grow, Inc., Clackamas, OR) would be as effective as a quick basal dip application (standard industry protocol) for rooting terminal cuttings of *Dendrathema pacificum* (*Chrysanthemum pacificum*)

and *Rosa* x 'Red Cascade'. One-inch terminal cuttings of chrysanthemum and 0.75 inch single-node cuttings of rose were stuck into Fafard 3B mix (peat/perlite/vermiculite/pine bark) (Conrad Fafard, Inc., Agawam, MA) in bedding packs. Cuttings in treatment one were basally dipped for one second in a solution of 1,000 ppm IBA + 500 ppm NAA. Cuttings in all other treatments were sprayed to the drip point using a plastic hand spray bottle with IBA + NAA concentrations of 0 + 0, 0.5 + 0.25, 1.0 + 0.5, 2.5 + 1.25, 5.0 + 2.5, 10.0 + 5.0, or 50.0 + 25.0 ppm, respectively. Cuttings were stuck and sprayed in the late afternoon and allowed to dry overnight. Chrysanthemum cuttings were placed under a greenhouse mist system providing overhead mist for 6 seconds every 16 minutes during daylight hours for a rooting period of 18 days. Rose cuttings were placed inside a high-humidity enclosure within a greenhouse for a rooting period of 23 days. A completely randomized design was utilized with four replicates (bedding packs) per treatment and eight subsamples (cuttings) of chrysanthemum and ten subsamples of rose per replicate.

Results and Discussion: In response to a foliar application of auxin on the chrysanthemum cuttings, regression analysis indicates that rooting percentage did not vary by auxin concentration, while the number of roots and the total root length per rooted cutting were greater with increasing auxin concentration. Rooting percentages for cuttings sprayed with auxins at all concentrations greater than 0 were similar to cuttings receiving a quick basal dip (Table 1). The number of roots per rooted cutting was the same for cuttings sprayed with 50 ppm IBA + 25 ppm NAA as for cuttings treated with the quick basal dip, but was lower for all other spray treatments. Total root length per rooted cutting was similar to the basal dip for spray treatments of 2.5 ppm IBA + 1.25 ppm NAA and above.

In response to a foliar application of auxin on the rose cuttings, regression analysis indicates that rooting percentage decreased at higher auxin concentrations, the number of roots and the total root length per rooted cutting did not vary by auxin concentration, and the shoot length per rooted cutting decreased at higher auxin concentrations. Rooting percentages for rose cuttings sprayed with 50 ppm IBA + 25 ppm NAA were lower than for cuttings receiving a quick basal dip (Table 2). Number of roots and total shoot length per rooted cutting was lower for cuttings in all treatments compared with the quick basal dip. Shoot length per rooted cutting was lower for cuttings sprayed with 50 ppm IBA + 25 ppm NAA than for cuttings treated with the quick basal dip.

Results indicate that a single spray application of 50 ppm IBA + 25 ppm NAA after sticking is as effective as a quick basal dip in 1000 ppm IBA + 500 ppm NAA prior to sticking for rooting terminal cuttings of *D.*

pacificum, while a basal dip is more effective than a spray for rooting cuttings of *Rosa* x 'Red Cascade'.

Significance to the Industry: Propagation employees' exposure to chemicals could be reduced if an alternate (and economical) method of applying auxin to cuttings can be developed that provides results equivalent to the standard quick basal dip. While some crops may respond well to a foliar spray application, many respond better to the standard basal dip treatment (unpublished data). This study provides a starting point for investigation of methods other than the standard basal dip for applying auxin in cutting propagation.

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Table 1. Rooting response of *Dendrathema pacificum* (*Chrysanthemum pacificum*) cuttings to IBA + NAA (Dip 'N Grow) applied as a foliar spray and a basal dip.

Auxin Treatment (ppm)	Rooting Percentage	Mean Number of Roots[y]	Mean Total Root Length (mm)[y]
0 IBA + 0 NAA Spray	78.1% *[z]	12.7 *	730 *
0.5 IBA + 0.25 NAA Spray	93.8%	14.8 *	788 *
1 IBA + 0.5 NAA Spray	96.9%	13.5 *	755 *
2.5 IBA + 1.25 NAA Spray	90.6%	15.6 *	814
5 IBA + 2.5 NAA Spray	93.8%	15.1 *	819
10 IBA + 5 NAA Spray	93.8%	15.6 *	845
50 IBA + 25 NAA Spray	90.7%	21.7	1054
1000 IBA + 500 NAA Basal Dip	100.0%	23.3	1060

[y]Means calculated using rooted cuttings only.
[z]Means followed by * within a column are significantly lower than the mean for the basal dip treatment according to Dunnett's Test (a=0.05).

Table 2. Rooting response of *Rosa* 'Red Cascade' cuttings to IBA + NAA (Dip 'N Grow) applied as a foliar spray and a basal dip.

Auxin Treatment (ppm)	Rooting Percentage	Mean Number of Roots	Mean Total Root Length (mm)[y]	Mean Shoot Length (mm)[y]
0 IBA + 0 NAA Spray	95.0%	4.1 *[z]	116 *	15.6
0.5 IBA + 0.25 NAA Spray	100.0%	4.1 *	139 *	23.2
1 IBA + 0.5 NAA Spray	97.5%	4.1 *	132 *	21.9
2.5 IBA + 1.25 NAA Spray	100.0%	4.3 *	139 *	18.5
5 IBA + 2.5 NAA Spray	100.0%	4.0 *	143 *	22.7
10 IBA + 5 NAA Spray	92.5%	4.2 *	141 *	17.0
50 IBA + 25 NAA Spray	62.5% *	4.5 *	139 *	3.0 *
1000 IBA + 500 NAA Basal Dip	100.0%	5.6	238	23.7

[y]Means calculated using rooted cuttings only.
[z]Means followed by * within a column are significantly lower than the mean for the basal dip treatment according to Dunnett's Test (a=0.05).

Using Sequential Digital Images Captured With A Flat Bed Scanner To Evaluate Woody Plant Seeds With Different Germination Requirements

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Index words: Imaging, Honeylocust, *Gleditschia triacanthos*

Nature of work: Several methods have been used to capture germinating seed images including video and still cameras (1, 5) or flat bed scanners (2, 4). Video and still camera usage is relatively expensive and requires specialized lighting and camera equipment. Flat bed scanners allows for economical and high quality digitization of seed images (4). Geneve and Kester (2) developed a simple Petri dish germination system that would be amenable to automated capture of sequential digital images in real time. The objective of the current study was to demonstrate how sequential digital images could be captured during seed germination using a flat bed scanner interfaced with a computer. The power of this technology will be demonstrated by evaluating imbibition in honeylocust (*Gleditsia triacanthos* L.) seeds following physical or acid scarification.

Honeylocust is a large leguminous tree native to North America. Seeds have physical dormancy with a hard seed coat that is impervious to water and gases. The seed coat must become permeable to allow for moisture and gaseous uptake and consequent seed germination. Hardseededness may be due to a compact arrangement of cellulose micro fibrils in the cell wall, involving an irreversible change in micellar structure during maturation and dehydration of the seed (6). Liu *et. al.* (3) noted that water impermeability in honeylocust seeds was due to the cuticle covering the macrosclereid cells at the seed coat surface. Also, the rate of imbibition of water into the seed determines the rate at which the embryo hydrates and subsequent radicle emergence.

Seeds of honeylocust were either acid scarified in concentrated H_2SO_4 for 60 minutes or physically scarified by nicking the center of the seed using a file. Two seeds were placed in 6 cm diameter plastic Petri dishes containing one piece of transparent cellulose film (Celorey-PUT, Cydsa Monterrey, Mexico). The cellulose film allows for uniform distribution of water throughout the Petri dish and being transparent allows uninterrupted image capturing by the flat bed scanner (2). Honeylocust seeds were surface sterilized in 10 % Clorox® solution for 10 minutes and

washed in distilled water before being placed in a Petri dish containing 3 ml of distilled water. Petri dishes were sealed with Parafilm® and placed in the flat bed scanner (HP Scanjet 5370 C with transparency adapter).The scanner was controlled using a SigmaScan Pro 5.0 for Windows (SPPC Science, Chicago, IL) macro written in Visual Basic which allowed for timed interval scans. For this experiment, scans were taken at hourly intervals. Gray scale images (stored as .tif files) were analyzed using another SigmaScan® macro which allowed for batch processing of the various images in a short period of time. Data was recorded for percentage increase in seed size till the time of radicle emergence.

Results and Discussion: Seeds treated with concentrated H_2SO_4 showed faster water uptake compared to physically scarified seeds (Figure 1). Acid treated seeds reached 50% of their final size within 11 hours after imbibition while physically scarified seeds required 20 hours (Figure 1). Uniform removal of the waxy coating and etching of the seed coat by acid treatment results in more rapid water uptake. This process as compared with nicking created a single point of entry of water on the seed coat. Acid treated seeds showed asymmetric water uptake across the seed with more water initially entering at the seed poles (chalazal and micropylar ends) producing a “dumbbell” shaped appearance (Figure 2). Physically scarified seeds showed initial water uptake at the point of nicking with water spreading from the center of the seed to the opposite ends of the seed or from one end to the other end of the seed depending on the initial nicking point (Figure 2)

The time required for radicle protrusion was about 20 hours less in acid treated seeds compared to physically scarified seeds (Figure 1). Acid treated seeds also attained a larger overall size prior to radicle emergence compared to physically scarified seeds. At the time of radicle protrusion, acid treated seeds had increased approximately 200% of their initial size, while physically scarified seeds only increased by 165%.

Significance to the industry: Sequential digital images captured with the flat bed scanner allowed for easy identification and analysis of water entry into seeds. This technique revealed changes in seed morphology that were previously undocumented for seeds with physical dormancy. Continued research will provide additional morphological details for seeds with other types of dormancy including physiological and morphological dormancy. The use of sequential imaging also holds promise for an automated system to assess seed quality in seed lots. This will be important for determining initial seed quality after seed harvest and for evaluating quality in stored seeds that are experiencing deterioration.

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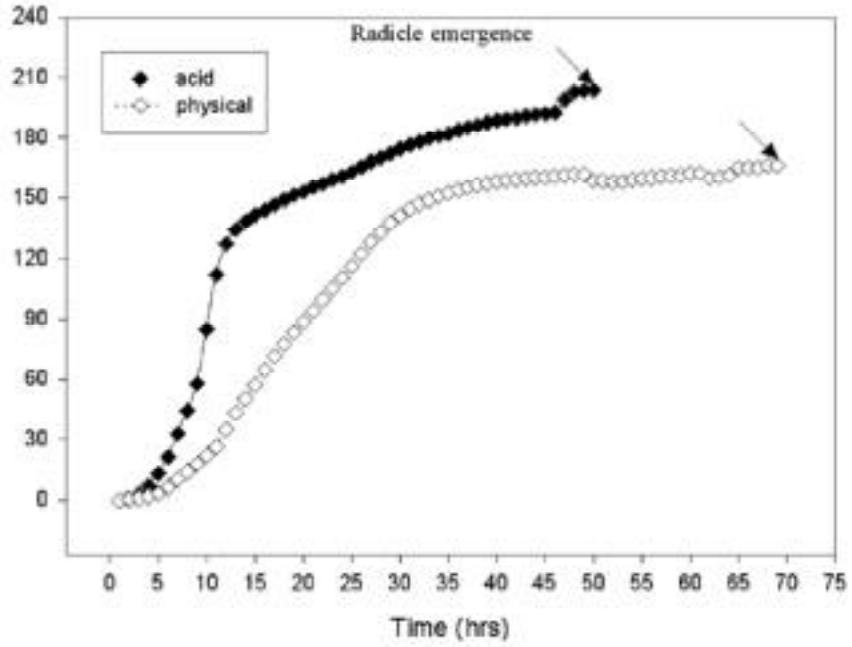

Figure 1: Imbibition following acid or physical scarification in honeylocust seeds.

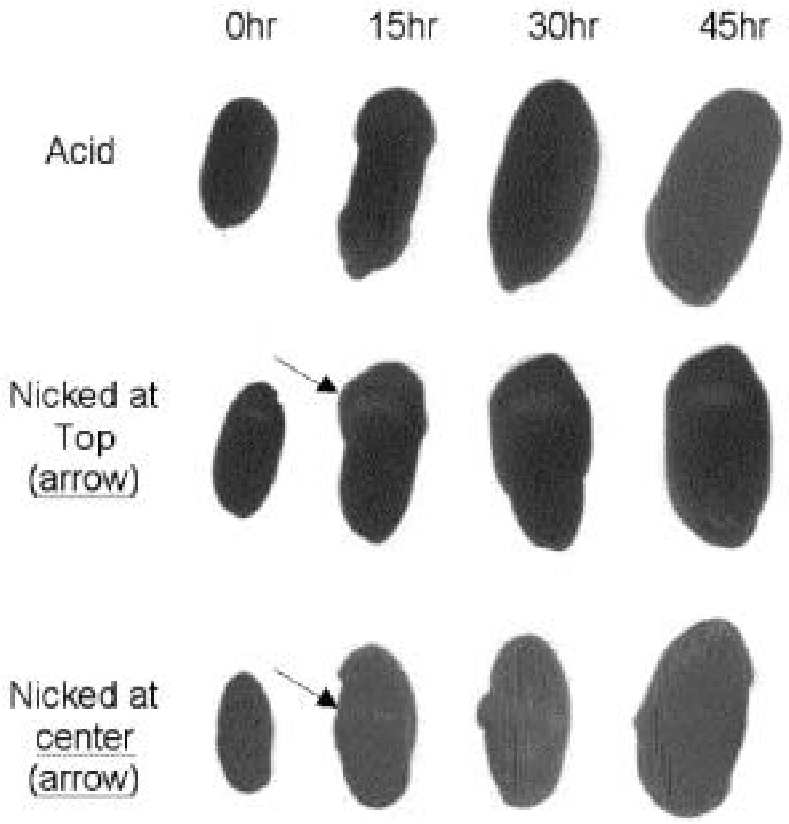

Figure 2: Water entry over the first 45 hours in seeds treated with acid or physically scarified by nicking the seeds at the top or center of the seed (micropylar ends face bottom).

Genotypic Differences of *In Vitro* Propagated Sea Oats (*Uniola paniculata* L.)

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Index Words: Micropropagation, N^6-benzyladenine,Iindole-3-acetic Acid, Dune Restoration

Nature of Work: Destabilization and erosion of coastal beach and dune systems by natural forces or man-made activities have increased the risk of catastrophic, ecological and economic damage following storm events. Reversal of coastal erosion is usually accomplished by beach nourishment through the addition of sand followed by dune stabilization. Stabilization is attempted by planting bare areas with native dune species (4) such as sea oats (*Uniola paniculata* L.). Sea oats are routinely propagated as nursery liners from field-collected seeds (1). However, dwindling natural seed donor stands, genetic diversity concerns, and the potential use of poorly adapted ecotypes at planting sites have prompted restrictions on the source and use of field-collected sea oats seeds. Consequently, efficient alternative methods to propagate diverse sea oats genotypes are needed. The application of plant tissue culture technology for sea oats vegetative propagation provides an opportunity to select, store and rapidly produce diverse genotypes with ecologically valuable characteristics. This approach has been explored with other dune species (2).

Preliminary studies (unpublished) were conducted to establish 28 different sea oats genotypes *in vitro* using micropropagation techniques. Significant variability in plantlet survivability occurred when attempting to acclimatize Stage II (unrooted) or Stage III (rooted) microcuttings to greenhouse conditions (Stage IV). The severity of these problems appeared to be genotype dependent. The objective of this study was to characterize differences in Stage II shoot multiplication rates between sea oats genotypes as affected by N^6-benzyladenine (BA) concentration.

Single shoot explants were harvested from sea oats shoot cultures (established from plants collected from Egmont Key National Wildlife Refuge, Pinellas County, FL) of both a difficult and easy to acclimatize genotype (EK 11-1 and EK 16-3, respectively). Shoot explants were individually cultured in 40 mm X 125 mm glass flat-bottom culture tubes

containing 30 mL media consisting of mineral salts (3), 100 mg/L (ppm) myo-inositol, 0.4 ppm thiamine-HCL, 30 g/L (3 %) sucrose, supplemented with 0, 0.5, 0.75, 1, 1.25, 1.5 or 1.75 ppm BA and solidified with 8 g/L (0.8 %) TC Agar (*Phyto*Technology Laboratories, Shawnee Mission, KS). Media were adjusted to pH 5.7 before addition of agar and autoclaving. For both EK 11-1 and EK 16-3 genotypes, each BA treatment was replicated 7 times. Cultures were maintained for 28 days at 22°C (72°F) in incubators under a 16-hr photoperiod provided by cool white fluorescent tubes at 40 mmol m^{-2} s^{-1} PPF (photosynthetic photon flux) as measured at culture level. A second experiment was prepared simultaneously to compare the treatments described above with 0, 0.5 and 0.75 ppm indole-3 acetic acid (IAA) media supplementation. The first experiment was a completely randomized factorial design. Statistical analysis of BA and genotypic effects was conducted using two-way ANOVA (analysis of variance). Mean separation among treatments was performed using LSD (least significant difference) at the 5% significance level. One-way ANOVA was used in the second experiment to evaluate the effects of IAA on treatments of both genotypes containing the BA concentration selected as optimal for shoot multiplication.

Results and Discussion: There was a significant effect of BA supplementation ($F=7.47$, df=6, $p<0.0001$) and genotype ($F=29.38$, df=1, $p<0.0001$) on sea oats shoot number and on leaf length ($F=18.71$, df=6, $p<0.0001$, and $F=29.85$, df=1, $p<0.0001$, respectively). Medium supplementation with BA was necessary to promote shoot production (Table 1). Shoot production in all EK 11-1 BA treatments were not significantly different, whereas shoot production in EK 16-3 was significantly enhanced in media containing 0.5, 0.75 or 1 ppm BA. For both genotypes, BA supplementation inhibited leaf length. Leaf length was greater for EK 16-3 than EK 11-1 plantlets at the 0, 1 and 1.25 ppm BA levels. Based on quantitative and visual assessments, the optimal BA concentrations for EK 11-1 and EK 16-3 genotypes were in the range of 0.5 -1.75 ppm BA and 0.5-1.0 ppm BA, respectively. The criterion for selection of a suitable BA concentration was based on regeneration responses, *i.e.* shoot number, leaf length and quality of plantlets of both genotypes. Summarizing, supplementation with 0.5 ppm BA was beneficial for regeneration of both sea oats genotypes.

Effects of IAA in combination with 0.5 ppm BA were also examined. There was no significant effect of IAA on shoot number of EK 11-1 plantlets (Table 2). IAA supplementation inhibited shoot production in EK 16-3 plantlets and leaf elongation of EK 11-1 plantlets. There was no significant effect of IAA on leaf length of EK 16-3 plantlets. These results indicate that optimal regeneration rates for both genotypes could be obtained without IAA supplementation.

Significance to Industry: The commercial application of micropropagation for sea oats production for habitat/dune stabilization requires that diverse genotypes be reliably propagated *in vitro*. Based on present and previous studies in our laboratory, Stage II medium supplemented with 0.5 ppm BA alone can be used to commercially micropropagate a wide range of sea oats genotypes. However, the physiological basis for observed differences in acclimatization capacity among sea oats genotypes must still be elucidated before a commercially viable micropropagation protocol can be defined.

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Table 1. Effects of BA supplementation and genotype on shoot number and leaf length (mm) of sea oats after 28 days in culture.

Genotype	BA (ppm)	Shoot number	Leaf length (mm)
EK 11-1	0	2.6 fg[z]	122.6 b
	0.5	9.4 abcd	58.6 def
	0.75	10.1 abc	71.4 cdef
	1	12.6 a	49.7 f
	1.25	10.7 ab	51.9 ef
	1.5	11.3 ab	50.3 f
	1.75	9.4 abcd	47.6 f
EK 16-3	0	1.1 g	204.9 a
	0.5	10.3 abc	87.3 bcde
	0.75	7.3 bcde	105.0 bc
	1	6.4 cdef	92.1 bcd
	1.25	5.7 def	113.1 b
	1.5	3.7 efg	37.6 f
	1.75	2.7 fg	69.6 cdef

[z]Least square means for BA and genotypes for multiple comparison. Means followed by the same letter are not significantly different at $p \leq 0.05$.

Table 2. Effects of IAA supplementation on shoot number and leaf length (mm) of sea oats genotypes cultured in medium containing 0.5 ppm BA after 28 days of culture.

IAA (ppm)	Shoot number		Leaf length (mm)	
	EK 11-1	EK 16-3	EK 11-1	EK 16-3
0	9.4 ab[z]	10.3 a	58.6 a	87.3 a
0.5	6.9 b	4.1 b	42.5 b	54.3 b
0.75	10.3 a	9.1 a	40.8 b	98.1 a
ANOVA[z]	NS	* *	* *	NS

[z]Non-significant (NS) or significant (**) at $p \leq 0.01$. Means followed by the same letter are not significantly different at $p \leq 0.05$.

Enhancing Germination of *Echinacea* Species

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Index Words: *Echinacea purpurea*, *E. tennesseensis*, *E. angustifolia*, *E. pallida*, stratification, Liquid Smoke

Nature of Work: *Echinacea* species are grown for ornamental uses as well as medicinal purposes. An obstacle for large-scale production of *Echinacea* species is erratic seed germination (Smith-Jochum and Albrecht, 1987; Smith-Jochum and Albrecht, 1988). This erratic germination can be the result of low viability due to dormancy issues and/or poor storage conditions after harvesting. Cold stratification has traditionally been recommended to break the after-ripening dormancy common in *Echinacea* species (Li, 1998). Stratification times can vary with *Echinacea purpurea* requiring 40 days at 2C (Shalaby *et al*., 1997) and *E. angustifolia* up to 84 days at 5C (Baskin *et al*., 1992). Kidwell *et al* (2001) reported a commercial liquid smoke product enhanced the germination of *Echinacea purpurea* with maximum germination occurring 7 days earlier than the controls. The objective of this research was to compare cold stratification and liquid smoke application on germination of four *Echinacea* species.

The experiment was conducted at the Nursery Research Services Center at Tennessee Tech University in Cookeville, Tennessee. Based on previous results four species of *Echinacea* were selected and included *Echinacea angustifolia, Echinacea pallida, Echinacea tennesseensis,* and *Echinacea purpurea* 'Bravado'. Seeds of each species (Johnny's Select Seeds, Albion, ME) were sown into 128 cell plug trays having a volume of 2.82 in^3/cell (TLC Polyform) using Promix BX media. The 128 cell plug trays were cut into quarters resulting in trays having 32 cells. Seed treatments included 1) control (CON), 2) cold stratification (STRAT), 3) liquid smoke application (LS), and 4) cold stratification and liquid smoke application (STRAT+LS). Seeds of each species were one seed/cell. Each treatment was replicated three times with 32 seeds per replicate. Cold stratification consisted of 20 days at 36F. Liquid smoke was applied at 100 ml/m^2 using a pump sprayer to the appropriate treatments. All treatments were placed under mist applied every 6 minutes from dawn to dusk using a controller. Treatments were arranged using a completely randomized design (CRD). Germination was recorded daily beginning 8 until 21 days after treatment (DAT) for all species. Seeds were considered germinated when the cotyledons

emerged from the media. All data were analyzed using analysis of variance (ANOVA) and means were separated using least significant difference (LSD), *P*=0.05.

Results and Discussion: Though the four species are from the genus *Echinacea* there were distinct germination responses to the treatments. *Echinacea purpurea* 'Bravado': The greatest germination 9 DAT was observed in STRAT (64%) followed by STRAT_LS (53%), LS (18%), and CON (7%) (Figure 1.). By 11 DAT germination of STRAT, STRAT_LS, and LS were similar and all were greater than the CON. All treatments were similar 16 DAT through 21 DAT. These results were similar to those reported by Kidwell *et al*. (2001). *Echinacea tennesseensis*: The pattern of germination was similar to that of *E. purpurea* 'Bravado'. The STRAT treatment had the greatest germination through 12 DAT and 17 DAT when the STRAT_LS and LS treatments were similar, respectively (Figure 1). The CON treatment had the lowest germination percentage through the study. *Echinacea pallida*: All treatments were similar through 12 DAT (Figure 1). The greatest germination occurred in the CON and LS treatments that were similar and the lowest germination rates were in the STRAT and STRAT_LS treatments from 13 DAT through the end of the study. The non-responsiveness observed was similar that reported by Shalaby *et al*. (1997) in which stratification did not enhance germination. *Echinacea angustifolia*: Germination percentages were greatest for the STRAT and STRAT_LS treatments through 9 DAT, after which were no differences between treatments (Figure 1).

Significance to the Industry: Germination of three of the four *Echinacea* species was enhanced by treating the seed with cold stratification treatments and/or liquid smoke application compared to the controls. While cold stratification resulted in the greatest germination percentages liquid smoke application could be a viable tool for growers who either can't stratify or do not want to spend the extra time stratification requires. The fact that all of the species within the *Echinacea* genus did not respond similarly indicates that more research is needed to fully evaluate the usefulness of these management tools.

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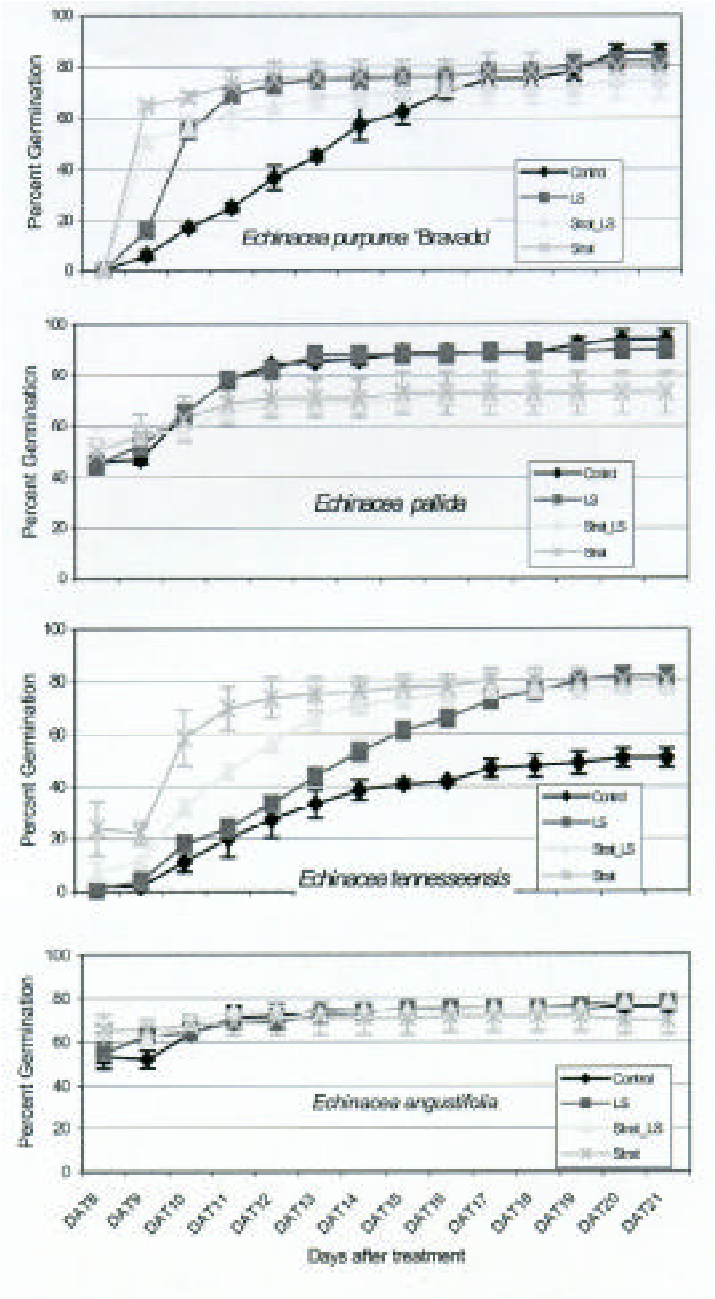

Figure 1. Germination of *Echinacea purpurea* 'Bravado', *E. pallida*, *E. tennesseensis*, *E. angustifolia* in response to application of liquid smoke and/or cold stratification treatments. Vertical bars represent standard error, n = 3.

In Vitro Storage of Hosta Micropropagules – Effect of Media Sucrose on Post-Storage Recovery

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Index words: Sucrose-free Storage, Commercial Micropropagation, Photoautotrophy

Nature of work: In vitro storage under growth retarding conditions delays the necessity for frequent transfers to fresh media and allows flexibility in meeting market demand leading to efficient management of labor. Storage conditions should preserve the post-storage quality and regrowth potential of in vitro plants. Wilson et al. (1998) reported that low light [photosynthetic photon flux (PPF) of 5-7 mmol m^{-2} s^{-1}] in storage improved post-storage quality and recovery potential of in vitro plantlets. In our research, increasing media sucrose to 5% or 7% during the multiplication phase (stage II) increased the internal sugar levels, biomass, and quality in Hosta micropropagules. When these cultures were transferred to rooting phase (stage III) in a media containing 3% sucrose and subsequently stored for 5 weeks at 10∞ C, under a PPF of 5 mmol m^{-2} s^{-1}, plantlets from the 5% or 7% sucrose media were of better quality than the plantlets from 1% or 3% sucrose (unpublished data). Data suggests that sucrose loading during multiplication phase had positive influence on post-storage plant quality (unpublished).

Sugar-free micropropagation holds significance in commercial micropropagation because sucrose-free medium reduces media contamination and consequent loss (Kozai, 1991). Therefore, the objective of this investigation was to examine if sucrose loading during multiplication phase allows in vitro rooting and storage in sucrose-free medium.

This study was conducted with two cultivars of Hosta: *Hosta tokudama* Tratt. 'Newberry Gold' and Hosta 'Striptease'. Stage II Hosta buds cultured in 5% media sucrose were procured from Southern Sun Propagation Systems, Norris, SC. Plants were transferred to stage III for rooting (on sorbarod plugs) (Ilacon Industries, UK) in magenta boxes (Magenta Corp. Chicago IL) containing modified Murashige and Skoog (1962) liquid medium. During stage III plants were cultured in media with 3% sucrose (photomixotrophic cultures) or without sucrose (photoautotrophic cultures) for four weeks at 25±2 °C under a PPF of 150 mmol $m^{-2}s^{-1}$. Nine buds of 'Newberry Gold' were cultured in each culture vessel while six buds of 'Striptease' (due to larger bud size) were cultured per

vessel. Following Stage III, cultures were stored, in the same culture vessel with residual medium, for 7 or 14 weeks under 5 mmol m^{-2} s^{-1} at 10° C, with and without a 2-week intermittent period of darkness during the final phase of storage to simulate shipping. Plantlets (four vessels from each treatment) were harvested for dry weight at the end of stage III (prior to storage) and after storage. Post-storage plantlets, from five culture vessels of each treatment, were transferred (after removal of necrotic leaves) to 606-cell packs containing a commercial potting mix (Superfine germinating mix, Fafard, Anderson, SC) and grown under mist for 4 weeks for acclimatization. After 4 weeks under the mist, mortality of the plantlets was recorded. Data were analyzed by ANOVA and treatment differences were separated using LSD at P = 0.05. Efficacy of sucrose loading on sucrose-free storage was assessed by comparing post storage survival and plant quality in both photoautotrophic and photomixotrophic cultures.

Results and Discussion: Presence of media sucrose led to significantly higher shoot and root biomass in both the cultivars at the end of stage III (Fig.1 A & B). In ‘Striptease’, the root biomass of photomixotrophic cultures increased by 70% during 7 weeks of storage, while in photomixotrophic cultures under continuous illumination, the root biomass remained unchanged thereafter. But in ‘Striptease’ photomixotrophic cultures that were stored for 14 weeks dark period had a negative influence on the root biomass (Fig 2A). However, in ‘Newberry Gold’, no change in root biomass occurred during 7 weeks of storage but a significant root growth occurred between 7 and 14 weeks of storage (Fig 2B). Incidence of shoot apex necrosis was higher in photoautotrophic cultures of both the cultivars, consequently, reflected by poorer percentage of survival in the greenhouse compared to the photomixotrophic plantlets (Table 1). Both photoautotrophic and photomixotrophic ‘Striptease’ cultures stored for 7 or 14 weeks recovered in the greenhouse but quality of photomixotrophic cultures were better than photoautotrophic cultures (data not shown). Photoautotrophic ‘Striptease’ cultures had a significantly greater percentage of mortality (about 25%) compared to photomixotrophic cultures (0%) after 7 weeks of storage. Photoautotrophic and photomixotrophic ‘Newberry Gold’ cultures stored for 7 weeks recovered in the greenhouse but extending the storage duration to 14 weeks led to further decline in the greenhouse survival of the photoautotrophic plantlets reflected by high percentage of mortality (Table 1). Overall, sucrose-free medium during rooting and storage led to poor post-storage recovery in both the cultivars, while extending storage duration led to further deterioration in ‘Newberry Gold’. Results indicate between-cultivar differences in rooting and post storage recovery; ‘Striptease’ can survive better in photoautotrophic cultures than ‘Newberry Gold’. Media sucrose during rooting stage and storage

contributed towards enhanced post storage survival, by offering nutritional support for improved rooting and maintenance of growing shoot apex during storage.

Significance to Industry: In vitro techniques are being increasingly used in the large-scale production of uniform disease free propagation material. Demand for propagation materials is often seasonal and therefore production peaks strive to match demand peaks. Employing sufficient labor exclusively during production peaks is impractical, because micropropagation involves expensive trained labor. Developing techniques for in vitro storage and subsequent shipping enables efficient utilization of labor, thereby, bringing down production cost in commercial micropropagation. Our study demonstrates that supplementing media with sucrose improves rooting and provides sustenance through out low temperature storage, enabling prolonged storage, as well as ensuring enhanced post storage survival.

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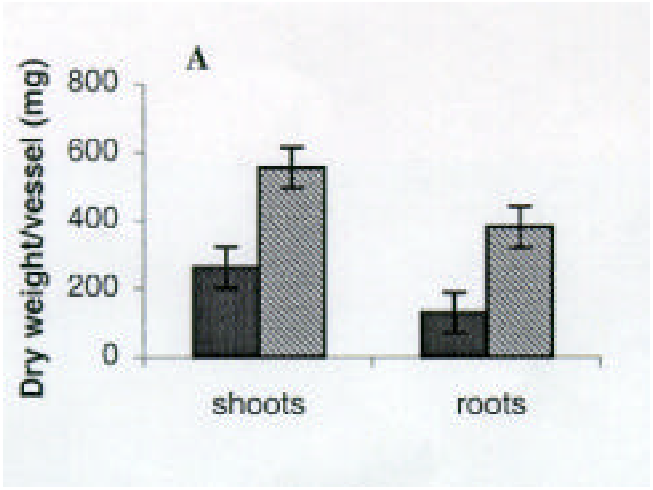

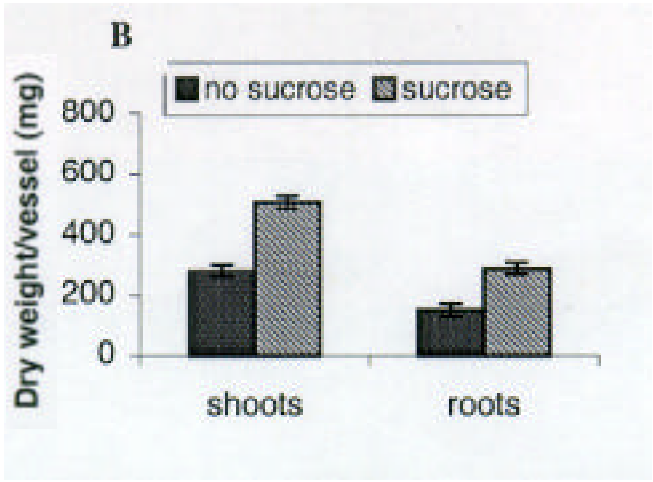

Figure 1. Shoot and root biomass per vessel in the photoautotrophic (no sucrose) and photomixotrophic (3% sucrose) cultures of Hosta 'Striptease' (A) and *Hosta tokudama* Tratt. 'Newberry Gold' (B) at the end of stage III (prior to storage). Means ± S.E. are shown

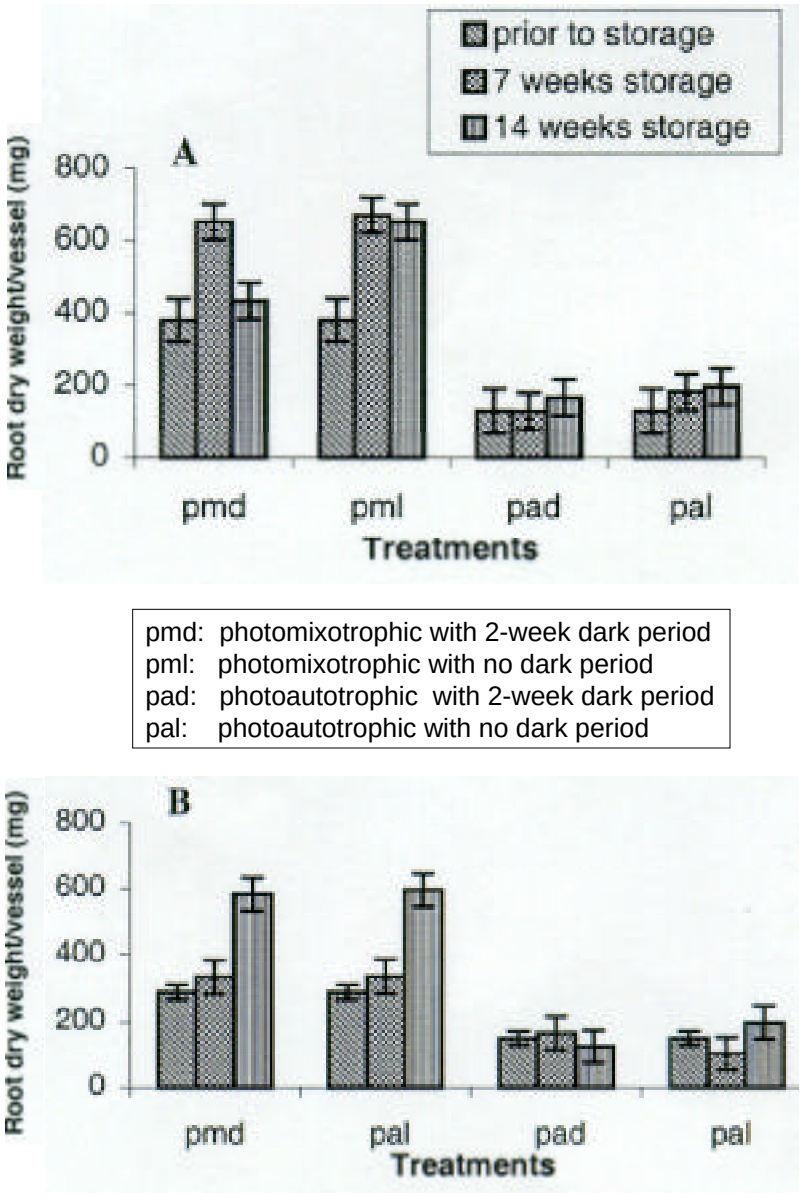

Figure 2. Post-storage root biomass in Hosta 'Striptease' (A) and *Hosta tokudama* Tratt. 'Newberry Gold' (B). Cultures were transferred to rooting in photoautotrophic (no sucrose) or photomixotrophic (3% sucrose) media and subsequently taken to storage for 7 or 14 weeks at 10 ^{o}C under 5 µmol m^{-2} s^{-1} of PPF with or without an intermittent dark period during storage. Means $\pm$ S.E. are shown

Table 1. Percentage of plantlet mortality in the photoautotrophic and photomixotrophic cultures of *Hosta tokudama* Tratt.'Newberry Gold' and Hosta 'Striptease' following 4 weeks post-storage recovery in greenhouse. Means with the same letter are not significantly different within each cultivar.

Treatments	% Post acclimatization mortality			
	Newberry Gold		Striptease	
	7 weeks	14 weeks	7 weeks	14 weeks
Photomixotrophy with 2-week dark period	0% c	2.2% c	0% b	20.8% ab
Photomixotrophy with no dark period	0% c	0% c	0% b	10% ab
Photoautotrophy with 2-week dark period	35.5% b	88.9% a	26.7% a	33.3% a
Photoautotrophy with no dark period	35.5% b	84.4% a	25.0% ab	20.0% ab

Winter Greenhouse Propagation of *Clematis* x *jackmanii* with Capillary Surface Material Fibers

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Index Words: Perennials, Cuttings, Ebb and Flow, Dlematis

Nature of Work: An experiment was conducted to examine effects use of capillary surface material (CSM) in a commercial soilless media for propagation of *Clematis* x *jackmanii*. CSM are synthetic fibers specifically designed to move water in bulk mixes in consumer hygiene and industrial chemical processes.

Clematis cuttings were rooted under intermittent mist for ten days to initiate roots. Two node cuttings with root initials were placed into commercial media on March 4, 2002. The commercial media used was Farfard 3-B, which is primarily comprised of pine bark, peat moss, and perlite. Cuttings were placed into two soil treatments of commercial media and 8 grams/per liter Capillary Surface Material Fibers (CSM) added to commercial media. Flats with 48 cells per flat were used for the treatments. Twelve cuttings per treatment were placed into time controlled intermittent mist (On two hours before dawn, off two hours after dusk, sixteen minute pulses, eight second pulses. No fertilizer was applied) with bottom heat applied (25 C) and overhead water breaker nozzle (fertilizer injected water application that was checked on a daily basis with equal application being applied as needed) water applications. Twenty-four cuttings were placed in a time controlled Ebb and Flow bench (On at 10:00 am and 4:00 pm for three minute intervals and fertilizer was suspended in the water solution) during the same time of the two previous treatments. After three weeks from root initiation, the plants were removed from the cells, soil washed away from the roots, and scanned using Epson Expression 1680 color scanner. The images were downloaded into WinRhizo image analysis software to collect and evaluated data in quantified areas of root length, root surface area, and number of root tips (Regents Instruments, Inc., Quebec: Bauhus and Messier 1999). The data was analyzed in JMP 3.2 software (SAS Institute, Cary NC).

Results and Discussion: Rooting of *Clematis* x *jackmanii* was excellent (100 percent) in all of the prescribed treatments. After two weeks in the greenhouse, roots were emerging from the cells and shoots were growing.

On March 25, three weeks after initiation, the vegetative lengths and numbers of nodes were recorded and the roots were removed from the crown. The roots were scanned and analyzed. Clematis mean root length with CSM in the medium was 1.7 times greater and number of root tips was 2.1 times more than the control. These are two important factors in the establishment of plant material (and may prevent transplant shock associated with potting up or planting into a landscape). Of the roots grown of Clematis, the mean surfaces area of the CSM media was 1.2 times larger then the control. This is related to the area of fertilizer and water uptake for the root and the recuperative capabilities of roots that ensure good survival and overall vegetative and floral growth. It's clear the addition of CSM has improved root growth.

The other treatment evaluated in this experiment was the method of fertigation. The effects of CSM were greatest in hand water irrigation (overhead irrigation with water soluble fertilizers) where extended levels of air existed in the soil profile. In general, the mean number of tips and root lengths were 1.75 more with CSM in the medium.

Table 1. Root characteristics of *Clematis x Jackmanii* following three weeks of greenhouse growth under different fertigation systems. Cuttings were grown in a commercial soilless media (Fafard 3-B) with and without CSM fibers.

Fertigation	CSM	Root Morphometry		
		Length	Surface Area	# of Tips
Ebb and Flow	Y	167±8	46±2	409±31
	N	127±8	45±2	324±32
Mist	Y	313±12	58±3	710±44
	N	234±12	55±3	394±44
Pressure breaker Nozzle	Y	340±12	54±3	1068±44
	N	101±12	33±3	287±44
ANOVA - Prob.> F				
Fertigation		<.0001	<.0001	<.0001
CSM		<.0001	0.0002	<.0001
CSM*Fertigation		<.0001	0.0011	<.0001

These observations suggest a practical use of CSM incorporated media in large-scale production of plant material. Property of water and fertilizers availability with CSM in the media profile needs to be studied further. Current information of CSM fibers in media show positive effects on rooting and plant growth and deserve attention to an impact it could make on horticulture.

Significance to Industry: Cutting propagation is the most important method for clonal propagation of woody plant materials to the public. The clonal propagation is carried out in a modified environment for woody species. One of the key elements within the environment is the medium in which adventitious roots form and develop to ultimately produce a sellable plant.

Current propagation methods for adventitious rooting rely primarily on media with a mixture of soilless components. This report demonstrates CSM can be included in the soilless components of rooting medium, thus increase water distribution, enhance root growth, and allow less frequent watering. With CSM in the media, there were increases in overall root length, surface area, and numbers of tips for the propagation systems tested. The greatest improvements were noted in treatments with low available water and adequate fertilization.

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