

Plant Propagation

Gene Blythe

Section Editor

Germination of Scarified Seeds of *Neptunia lutea* and *Mimosa* (*Schrankia*)

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Significance to Industry: Native groundcovers may offer low input plants for locations where irrigation water is limited. Use of native cultivars would also reduce the potential for escape of exotic species to sensitive ecosystems. One of the first requirements for introduction of a species into the nursery trade is a reliable means of propagation. This study provides recommendations for germinating two taxa of native groundcovers.

Nature of Work: Invasive species have become a hot topic in the Green Industry and appropriate native species have been offered as a potential alternative (2). One challenge is that many of our exotic species have been gleaned from among thousands of potential species for their particularly appealing ornamental attributes and wide environmental adaptability. This is often not the case with our native species, where breeding and selection programs are less common. *Neptunia lutea* (Leavenw.) Benth. and *Mimosa* L. (*Schrankia* Willd.) offer fine-textured foliage, a low spreading growth habit, excellent drought and heat tolerance, and attractive season-long flowering with yellow or pink flowers, respectively (1). The first step to bringing these taxa into cultivation is to determine propagation methods. Scarification requirements are presented in this report.

Seeds of *Mimosa* sp. were collected in September 2006, in College Station, TX. Seeds were separated from the pods by hand and were allowed to dry at room temperature (22°C, 71.6°F) until treatments were applied. Six pre-germination treatments were applied to the seeds starting on 23 May 2007. Seeds were treated with a 15, 30, 60, or 120 min H₂SO₄ soak (96.1% solution; Mallinckrodt Baker, Inc., Phillipsburg, NJ.), mechanical scarification with sandpaper, or a nontreated control. Seeds were then soaked for 24 h in 50 ml of 22°C (71.6°F) water. Germination was carried out in Petri dishes [9 cm (3.5 in) dia.] with a triple layer of moist filter paper under constant light and at 27°C (80°F). Each dish contained 10 seeds. Three dishes per treatment combination randomly arranged within the growth chamber were evaluated (10 seeds per Petri dish per pregermination treatment). Water [5 ml (0.17 oz)] was added to the Petri dishes as needed to keep the filter paper wet. A count of germinated seeds was made daily for the first 14 d and then at 21 d. A seed was counted as germinated when the radicle protruded at least 2 mm (0.08 in).

Seeds of *N. lutea* were collected in June 2006, in College Station, TX. Seeds were separated from the pods by hand and were allowed to dry at room temperature (22°C, 71.6°F) until treatments were applied. Six pre-germination treatments were applied to the seeds starting on 22 May 2007. Seeds were treated with a 15, 30, 60, and 120 min H₂SO₄, mechanical scarification with sandpaper, or a nontreated control. Seeds were then soaked for 24 h in 50 ml of 22°C (71.6°F) water. Germination was carried out in Petri dishes [9 cm (3.5 in) dia.] with a triple layer of moist filter paper under constant light and at 27°C (80°F). Each dish contained 10 seeds. Three dishes per treatment combination randomly arranged within the growth chamber were evaluated (10 seeds per Petri dish per pregermination treatment). Water [5 ml (0.17 oz)] was added to the Petri dishes as needed to keep the filter paper wet. The number of seeds germinated was counted daily for the first 14 d and then at 21 d. A seed was counted as germinated when the radicle protruded at least 2 mm (0.08 in). Results of each experiment were separately analyzed using SAS (version 9.2; SAS Institute Inc., Cary, NC).

Results and Discussion: Mechanical scarification did not improve imbibition of seeds of *Mimosa* compared to non-scarified controls; however, 15 to 120 min of acid scarification increased imbibitions of seeds from 57% for the controls to 100% for chemically treated seeds (Table 1) during the first 24 hr after treatment. This suggests that for *Mimosa* some barrier to water uptake other than the seed coat may be present that is removed with acid exposure. In contrast, barriers to imbibition of *N. lutea* seeds were likely due to the mechanical barrier of the seed coat as few imbibitions occurred without scarification, whereas exposure to mechanical scarification or 30 to 120 min of acid scarification of the seed coats resulted in 100 % imbibition (Table 1) within 24 hr of treatment. Incomplete scarification occurred with 15 min acid exposure for *N. lutea* (Table 1).

Germination occurred rapidly for both species, and then neither species had additional germination from 8 to 21 days after treatment (Fig. 1). Germination of both species occurred mostly within the first two to three days (Fig. 1). Thereafter few additional *N. lutea* seeds germinated (Fig. 1B), while an additional 10% to 20% of the seeds germinated for *Mimosa* from day 3 to 8 (Fig. 1A). With *Mimosa*, nearly 100% germination was obtained with 15 to 30 min exposures to concentrated sulfuric acid, while 60 to 120 minutes resulted in imbibition (Table 1), but severely inhibited germination (Fig. 1A). Mechanical scarification increased germination of *Mimosa* by about 10% over controls, which had about 60% to 70% germination with no scarification treatment (Fig. 1A). In contrast, *N. lutea* germination was less than 10% without scarification and was greatest with mechanical scarification at about 95% (Fig. 1B). A 15 min acid exposure to seeds of *N. lutea* was slightly less effective than mechanical scarification and, although all chemical scarification treatments were better than the control, increased exposure beyond 15 min did begin to inhibit germination to some extent (Fig. 1B).

Barriers to germination of *N. lutea* appeared to be due almost entirely to the presence of an intact seed coat, while over half of the seeds of *Mimosa* imbibed without pretreatment scarification; however, scarification did increase imbibition. Germination of *Mimosa* was optimal with a 15 to 30 min exposure to concentrated sulfuric acid and was superior to mechanical scarification. While mechanical scarification by hand produced the greatest

germination with *N. lutea*, exposure to sulfuric acid for 15 min was nearly as effective and would require less labor. Exposure to concentrated sulfuric acid should not exceed 15 min for *N. lutea* or more than 30 min. for *Mimosa* based on these experiments. Imbibition alone was not a reliable predictor of germination rates for either species.

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Table 1. Imbibed *Neptunia lutea* and *Mimosa (Schrunkia)* seeds within 24 hr following mechanical scarification (seed coat abrasion) or 0 to 120 min. of concentrated sulfuric acid scarification.

Taxa	Scarification	Imbibed seeds (%)
<i>Mimosa (Schrunkia)</i>	None (control)	57 ^z b
	Sulfuric acid 15 min.	100 a
	Sulfuric acid 30 min.	100 a
	Sulfuric acid 60 min.	100 a
	Sulfuric acid 120 min.	100 a
	Mechanical	63 b
<i>Neptunia lutea</i>	None (control)	7 c
	Sulfuric acid 15 min.	80 b
	Sulfuric acid 30 min.	100 a
	Sulfuric acid 60 min.	100 a
	Sulfuric acid 120 min.	100 a
	Mechanical	100 a

^zMeans within a taxon and column followed by the same letter do not differ at $P \leq 0.05$ using least squares means comparisons. Values represent means of three replicates, each containing ten treated seeds.

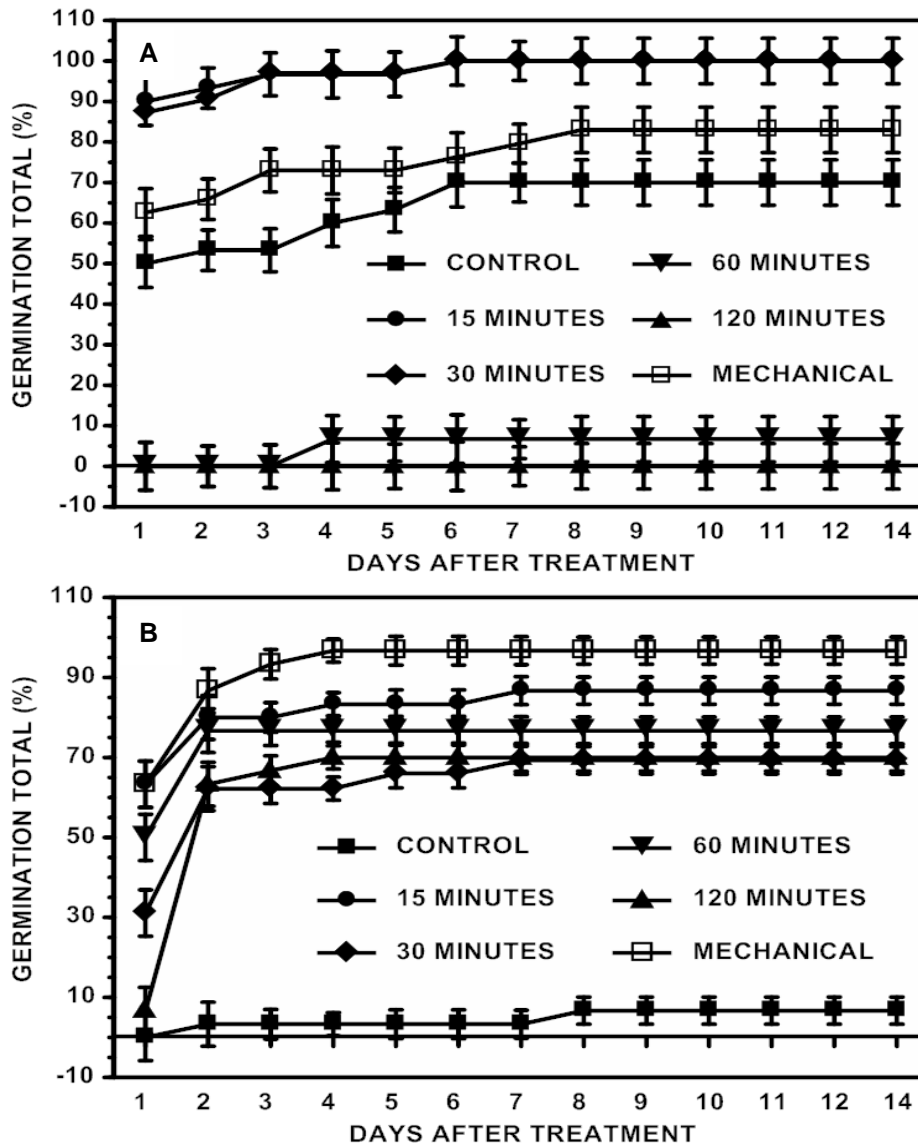


Figure 1. Mean (\pm SEM) total germination percentage from 1 to 14 days after treatment with mechanical scarification, 15 to 120 min. exposure to concentrated sulfuric acid, or no scarification (control) of seeds of (A) *Mimosa* or (B) *Neptunia lutea*. Values are means of three replicates of ten seeds each. No additional germination had occurred by day 21 when the experiment was terminated.

Rooting Stem Cuttings of Woody Ornamentals in a Cedar Amended Substrate

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Index Words: cutting propagation, eastern redcedar, *Thuja* 'Green Giant', *Ulmus parvifolia*

Significance to Industry: Stem cuttings of *Thuja* 'Green Giant' and *Ulmus parvifolia* 'Emerald Prairie' were rooted in a series of substrates containing increasing concentrations of eastern redcedar chips as well as a standard propagation substrate. As expected, cuttings of *Thuja* 'Green Giant' rooted well in the standard mix. Cuttings rooted as well in the 0% and 25% redcedar amended substrates ($\geq 91\%$ rooting) with decreasing rooting as redcedar content increased. Stem cuttings of *Ulmus parvifolia* 'Emerald Prairie' did not root as well as anticipated and rooting was negatively affected by the increasing redcedar content in the substrates. With both species, root number and root dry weight was not impacted by the increasing redcedar concentration. Results suggest redcedar may be an acceptable peat moss replacement for species that root relatively easily.

Nature of Work: Utilizing locally available materials for substrates during the nursery production process can enhance profitability and sustainability. Eastern redcedar (*Juniperus virginiana* L.), which grows throughout the eastern half of the United States may be one such material. Recent work has suggested that, when processed properly, eastern redcedar chips may be successfully incorporated into a container substrate for production of woody and herbaceous plants (4,5). However, as a propagation substrate, eastern redcedar has been virtually unexplored. Many propagation substrates use a blend of perlite and peatmoss to achieve proper water holding capacity and porosity (1). Peatmoss is a non-renewable resource and its increased usage has led to an increase in cost and concerns regarding its sustainability. This study seeks to expand the potential uses for eastern redcedar chips beyond a container substrate component into the propagation side of the nursery.

Chipped eastern redcedar trees from Queal Enterprises (Pratt, KS) were obtained and further ground with a hammer mill (Model 30HMBL; C.S. Bell Co., Tifton, OH) to pass a 3/16-in (4.76 mm) screen. The processed redcedar was then blended with perlite to form five substrates consisting of 0%, 25%, 50%, 75%, and 100% redcedar (by vol.). A standard 75% perlite:25% peat (by vol.) substrate was utilized for comparison. Substrates were then placed into 36-cell propagation trays with a cell volume of 7.5 oz (220 ml) and depth of 5 in (13 cm) (Stuewe and Sons Inc., Tangent, OR.).

On the morning of July 6, 2011, terminal stem cuttings of *Thuja* 'Green Giant' ('Green Giant' arborvitae) were collected from three different trees growing at the Kansas State University John C. Pair Horticultural Center (Haysville, KS). Cuttings were trimmed to a length of 6 in (15.2 cm) and the basal 0.5 in (1.3 cm) was dipped in 5000 ppm (0.5%) K-IBA (potassium salt of indolebutyric acid). The cuttings were allowed to air dry for 5 min and were then inserted into the substrates to a depth of 2.0 in (5.0 cm). Trays were then placed on a greenhouse bench under intermittent mist that operated for 6 sec every 6 min from 7:00AM to 8:00PM. On the morning of July 7, 2011 terminal cuttings of *Ulmus parvifolia* 'Emerald Prairie' ('Emerald Prairie' lacebark elm) were collected from stock plants growing at the John C. Pair Horticultural Center. The cuttings were processed identically to 'Green Giant' arborvitae with the exception that elm cuttings were treated with 10,000 ppm (1.0%) K-IBA. Cuttings were harvested after allowing 9 weeks for root development and evaluated for percent rooting, number of roots per rooted cutting, and root dry weight. A cutting was considered rooted if it had at least one root at least 0.04 in (1.0 mm) in length.

The experimental design was a randomized complete block design with six substrate treatments and six cuttings per treatment (subsamples). The treatments were replicated six times resulting in a total of 36 cuttings per treatment. Data were subjected to analysis of variance (ANOVA) and trend analysis. Where appropriate, means were separated with Fisher's protected LSD ($P \leq 0.05$). Each species was analyzed as a separate experiment.

Results and Discussion: Previous work has indicated that 'Green Giant' arborvitae is easy to propagate from stem cuttings (2), making it an ideal candidate for testing a new propagation substrate. As expected, stem cuttings of 'Green Giant' arborvitae rooted well in the standard perlite/peat substrate (92%). Cuttings rooted equally as well with 0% or 25% redcedar amended substrate, suggesting that redcedar could replace peatmoss for rooting stem cuttings of 'Green Giant' arborvitae. However, rooting was reduced when the redcedar content of the substrate was 50% or greater (Table 1). The number of roots was unaffected by redcedar content and averaged 12.7 roots per rooted cutting. Root dry weight was also unaffected by redcedar content and averaged 0.002 oz (55 mg) per rooted cutting.

'Emerald Prairie' lacebark elm has also been shown to root well as a semi-hardwood stem cutting (3). However, overall rooting in the current study was lower than expected, including using the standard perlite/peat substrate (67% rooting). The addition of redcedar into the rooting substrate decreased percent rooting in a linear fashion suggesting that redcedar negatively affected the rooting potential of the stem cuttings (Table 1). However, redcedar did not affect the average number of roots produced per rooted cutting (5.6). Root dry weight per rooted cutting was not influenced by the redcedar content of the rooting substrate and averaged 0.0007 oz (20 mg). However, in the standard perlite/peat substrate, root weight was improved to 0.002 oz (50 mg). The data herein suggests that processed redcedar may be an acceptable amendment to a propagation substrate for species that root easily from stem cuttings. However, as a propagation substrate amendment for other species, redcedar should be carefully evaluated.

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Table 1. Rooting percentages for stem cuttings of *Thuja* 'Green Giant' and *Ulmus parvifolia* 'Emerald Prairie' in substrates containing increasing concentrations of cedar or a perlite:peat standard.

Perlite:Redcedar	<i>Thuja</i>	<i>Ulmus parvifolia</i>
	'Green Giant'	'Emerald Prairie'
	Rooted (%)	Rooted (%)
100:0	94 a ^z	50 ab
75:25	91 ab	36 bc
50:50	67 c	25 c
25:75	78 bc	22 c
0:100	64 c	17 c
Linear	**y	**
Quadratic	NS	NS
Perlite:Peat	Rooted (%)	Rooted (%)
75:25	92 ab	67 a

^zMeans followed by the same letter within a column are not statistically different based on Fisher's LSD where $P \leq 0.05$; n=36.

^yTrend analysis: ** (highly significant; $P \leq 0.01$) or NS (not significant).

Response of Azalea Cuttings to Leaf Damage and Leaf Removal

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Index Words: cutting propagation, vegetative propagation, leaf removal, azalea web blight, *Rhizoctonia*, thermotherapy

Significance to Industry: Binucleate *Rhizoctonia* species, the pathogens that cause azalea web blight, can be carried on stem cuttings, perpetuating the disease through subsequent crops. Previous studies have demonstrated that submerging *Rhizoctonia*-infested stem pieces of 'Gumpo White' azalea in 122°F (50°C) water for 20 minutes can eliminate the pathogen without causing damage to leaf tissue. Extending the treatment duration to 60 or 80 minutes demonstrated that some cultivars, but not all cultivars, could be injured with extended submersion in hot water. An important point was that a 20-minute-plus margin of error exists where damage would be relatively minor if distracted workers accidentally left stem cuttings in 122°F water longer than intended. In the present study, an incremental increase in leaf damage from hotter water temperatures resulted in incremental reductions in final root development and final leaf count. Conversely, increasing percentage of physical leaf removal caused no reduction until at least 75% of the leaf area was removed. This demonstrates two important points: 1) water hotter than 122°F can severely impact the vigor and survival of stem cuttings, and 2) azalea stem cuttings generally can recover from partial leaf loss if given proper care.

Nature of Work: Sanitation is a well proven and cost effective approach for limiting the entry of pathogens into a propagation facility (3, 4, 5). Hot water treatment, a form of thermotherapy, is a method of sanitation that has been used for seeds, tubers, and vegetables. Until recently, hot water treatment had not been reported for use with stem cuttings.

In our previous study (1), hot water treatment [submersion in 122°F (50°C) water for 21 minutes] eliminated *Rhizoctonia* from azalea stem pieces that had been inoculated and colonized by *Rhizoctonia* AG-P, whereas chemical disinfectants and fungicides were ineffective. In the same study, when terminal leafy cuttings of 'Gumpo White' azalea were treated with hot water at 122°F for 20 or 40 minutes, only minor leaf damage was noted on the cuttings. In a subsequent study (2), these same hot water treatments were found to be safe using terminal stem cuttings of 12 commonly grown cultivars of azalea. To understand the risk of damage in the event that a distracted worker accidentally left stem cuttings in hot water longer than intended, the hot water treatment was extended

to 80 minutes. As the duration of submersion extended past 20 minutes to 40, 60, and 80 minutes, the range of damage increased respectively on the twelve cultivars, from none to minor, minor to severe, and moderate to severe. All cultivars could tolerate 40 minutes, which is a fairly large margin of error, and cuttings of a few cultivars exhibited little adverse response even at 60 and 80 minutes.

Since little is known about the rooting response of azalea following different types of tissue damage, the current study was conducted to evaluate root development and subsequent leaf production on cuttings of two cultivars in response to incremental reductions in photosynthetic leaf area resulting from either heat-induced leaf tissue damage or physical leaf removal.

Terminal cuttings of 'Gumpo White' (Satsuki) and 'Roblel' [Autumn Debutante™ (Encore)] were collected, prepared, and treated from 10 June to 18 June 2009. Cuttings of both cultivars were submerged in 131 to 140°F (55 to 60°C) water for 7 to 43 min. Cuttings were temporarily inserted into a peat and pine bark substrate (Fafard 2B, Conrad Fafard) in propagation trays and placed under intermittent mist to allow development of the initial leaf damage from the hot water treatment. Between 15 June and 24 June, leaves were assessed for precision to 25%, 50%, 75%, and 100% leaf area damage per cutting. Cuttings with respective levels of damage were transferred to 72-cell plug trays using the same substrate.

Additional cuttings with comparable reductions in photosynthetic leaf area (but no hot water treatment) were prepared between 16 June and 18 June by physically removing leaves from the cuttings. Cuttings of 'Roblel' azalea each had approximately 8 leaves of uniform size, so 0%, 25%, 50%, 75%, and 100% leaf area removal was achieved by retaining 8, 6, 4, 2, and 0 leaves, respectively, per cutting. 'Gumpo White' azalea cuttings had small, medium, and large leaves with a mean area of 48, 73, and 210 mm², respectively. For 'Gumpo White' cuttings, 0%, 25%, 50%, 75%, and 100% leaf area removal was achieved by retaining sets of (8,2,3), (6,2,2), (5,2,1), (4,1,0), and (0,0,0) (small, medium, large) leaves, respectively, per stem cutting. Cuttings with each of the five degrees of damage were inserted into plug trays as noted above and placed under intermittent mist. The experimental design was a randomized complete block with twelve replications.

Cuttings were assessed for root development and total leaves per cutting between 31 Aug. and 2 Sept. 2009. Root development was assessed on a scale of 0 (no roots) to 10 (extensive root development). Data was analyzed with linear mixed models using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Least square means and pairwise differences adjusted for multiple comparisons were calculated for a heteroscedastic model that accounts for non-constant denominator degrees of freedom (Satterthwaite method).

Results and Discussion: Root development generally decreased with increasing percentage of induced leaf damage (Table 1). Despite this general trend, cuttings of

'Roblel' and 'Gumpo White' responded differently to the increasing percentage of leaf damage induced from submersion in hot water ($P = 0.0066$) and by leaf removal ($P < 0.0001$). 'Roblel' was more tolerant to a moderate degree of leaf damage. With both cultivars, leaf damage due to hot water treatment more severely impacted rooting than leaf removal. Generally, incremental increases in hot-water-induced leaf damage were associated with incremental decreases in final leaf count and root development, whereas 75% of the leaf area had to be physically removed before causing a decline in the final leaf count and root development with both cultivars. As expected, a 100% reduction in photosynthetic leaf area from either hot-water-induced leaf damage or physical leaf removal resulted in either poor root development and low leaf counts or death of the stem cutting with both cultivars. The results imply that water temperatures hotter than 122°F caused physiological damage beyond simply the loss of photosynthetic area related to leaf removal. It is interesting that cuttings were fairly tolerant to a simple loss of photosynthetic area from leaf removal, and apparently could recover from up to a 75% leaf loss by generating new root and leaf growth.

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Table 1. Mean leaf count and root development on cuttings of two azalea cultivars in response to selected percentage levels of induced leaf damage resulting from either hot water treatment or physical leaf removal, after a rooting period of 10 weeks (n=12).

Leaf damage type	Induced leaf damage (%) ^z	'Gumpo White'		'Roblel'	
		Leaves (no.)	Root development ^y	Leaves (no.)	Root development ^y
Hot water	0	18.0 a ^x	7.8 a	13.9 ab	9.9 a
	25	15.3 ab	4.9 b	17.8 b	9.8 a
	50	8.6 c	2.6 bc	10.8 a	6.7 b
	75	0.6 d	0.6 c	8.5 a	4.0 b
	100	0.0 d	0.5 c	0.0 c	0.5 c
Leaf removal	0	20.2 a	8.9 a	11.3 a	9.4 a
	25	18.5 a	8.1 a	11.2 a	8.9 ab
	50	15.5 ab	8.2 a	11.0 a	9.0 ab
	75	12.8 b	4.9 b	10.5 a	6.7 b
	100	0.0 d	0.5 c	0.4 c	0.6 c
Significance:					
Damage type		<0.0001 ^w	<0.0001	0.0621	0.0145
Damage percentage		<0.0001	<0.0001	<0.0001	<0.0001
Damage type × percentage		<0.0001	<0.0001	0.0014	0.0026

^zPercent leaf area damaged either from submergence in 131°F to 140°F water or by removal of a respective number of leaves representing an equivalent leaf area.

^yRoot development was assessed on a scale of 0 (no roots) to 10 (extensive root development).

^xBased on multiple pairwise comparison, means followed by the same letter down a column are not significantly different ($\alpha = 0.05$).

^wProbability (based on type III sums of squares) using the MIXED procedure of SAS with heterogeneous variances models to test the null hypothesis that all factor levels are equal.

Propagation of *Osmanthus heterophyllus* 'Variegatus' by Stem Cuttings

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Index Words: adventitious rooting, auxin, indolebutyric acid, holly osmanthus, false holly, holly tea olive

Significance to Industry: The most consistent rooting (> 70%) of stem cuttings of *Osmanthus heterophyllus* 'Variegatus' was achieved with nontreated softwood cuttings collected in late May to early June. Auxin treatment of softwood cuttings does not stimulate rooting and is unnecessary. Rooting of semi-hardwood cuttings is variable from year to year, and rooting of hardwood cuttings is negligible.

Nature of Work: 'Variegatus' osmanthus [*Osmanthus heterophyllus* (G. Don) P.S. Green 'Variegatus' (Oleaceae Hoffmanns. & Link)] is one of many cultivars of *Osmanthus heterophyllus* (holly tea olive, holly osmanthus, false holly) (3). The cultivar is an extremely attractive, slow growing, evergreen shrub having leaves similar in morphology to the species. However, what makes 'Variegatus' osmanthus so attractive is the creamy white margins on the leaves. The striking foliage and the upright growth habit contribute to it being an outstanding landscape plant that can reach heights ≥ 3.0 m (8 to 10 ft) (3).

The various cultivars of *O. heterophyllus* are generally propagated by stem cuttings, but propagation information regarding particular cultivars is lacking. Blazich and Acedo (2) reported nontreated semi-hardwood and hardwood cuttings of 'Ilicifolius' osmanthus rooted in high percentages (> 80%), whereas comparable results were only noted for hardwood cuttings of 'Rotundifolius' osmanthus. Semi-hardwood cuttings of 'Rotundifolius' did not root. The response of both cultivars following treatment with the free acid of indolebutyric acid (IBA) at 2500 to 10,000 mg·L⁻¹ (ppm) was variable and often inhibited rooting. Blazich and Acedo (2) did not attempt to root softwood cuttings of either cultivar.

On several occasions the authors have tried to root semi-hardwood and hardwood cuttings of 'Variegatus' osmanthus. Results with hardwood cuttings have been consistently poor, leading to the conclusion they do not root. On the other hand, results with semi-hardwood cuttings taken in late August to mid-September have been mixed. Some years, semi-hardwood cuttings root well; in other years they root poorly. Lack of consistent results resulted in the following research with the objective to investigate propagation of 'Variegatus' osmanthus by softwood cuttings as influenced by auxin treatment.

Two hundred terminal softwood cuttings approximately 7 to 9 cm (2.8 to 3.5 in) in length were collected on June 2, 2009, and May 31, 2010, from each of two plants of 'Variegatus' osmanthus growing under uniform fertility on the grounds of a private residence in Raleigh, NC. The plants were large, exceeding 3.7 m (12 ft) in height and 2.4 m (8 ft) in width. The stems of the cuttings were light to pale green in color and, when pressure was applied to a cutting, the stem broke but the pieces remained attached at the point where pressure was applied.

Following collection, cuttings from both plants were pooled. Cuttings were trimmed from the bases to lengths of 6 to 8 cm (2.4 to 3.1 in). They were then treated with solutions of the potassium (K) salt (K-salt) of indolebutyric acid (IBA) at 0, 500, 1000, 1500, or 2000 mg·L⁻¹ (ppm). Solutions were prepared by dissolving reagent grade K-IBA in distilled water. When treating cuttings with K-IBA, the basal 1 cm (0.4 in) was dipped into the K-IBA solution for 2 sec followed by 20 min of air drying before insertion into the rooting medium. After auxin treatment, cuttings were inserted to a depth of 2 to 3 cm (0.8 to 1.2 in) in individual plastic Anderson bands (Anderson Tool & Die, Portland, OR) [6.0 x 6.0 x 12.7 cm (2.4 x 2.4 x 5.0 in)] held in deep propagation flats/trays [40.6 x 40.6 x 12.7 cm (16 x 16 x 5 in)] with 36 bands per flat (6 rows x 6 columns). The rooting medium was 1 peat : 1 perlite (by vol.).

The trays were placed under natural photoperiod and irradiance on a single raised bench in a glass covered greenhouse on the campus of NC State University, Raleigh. Day/night temperatures were approximately 23.9 ± 5.6/21.1 ± 2.8C (75 ± 10/70 ± 5F). Intermittent mist operated 4 sec every 5 min from 7:00 am to 8:30 PM daily. The experimental design was a randomized complete block using 12 cuttings per treatment with six replications.

Fifteen weeks after the rooting studies were initiated, cuttings were harvested and data recorded. Data included the number and length of primary roots > 1 mm (0.04 in). Any cutting having one or more roots was classified as rooted. Data were subjected to analysis of variance procedures and regression analysis.

Results and Discussion: For both years, 2009 and 2010, nontreated softwood cuttings of 'Variegatus' osmanthus rooted at > 70% with the influence of K-IBA treatment being generally nonsignificant (Table 1). The only significant response ($P < 0.10$) to K-IBA treatment was a linear decrease in mean root length for cuttings taken in 2009. Results for 2009 and 2010 indicate auxin treatment of softwood cuttings of 'Variegatus' osmanthus is of questionable value. In a previous study, Blazich and Acedo (2) investigated propagation of *O. heterophyllum* 'Illicifolius' and 'Rotundifolius' by stem cuttings. They reported nontreated semi-hardwood and hardwood cuttings of 'Illicifolius' osmanthus and hardwood cuttings of 'Rotundifolius' osmanthus rooted in high percentages (> 80%). Response to treatment with solutions of the free acid of IBA at 2500 to 10,000 ppm was variable, and generally inhibited rooting.

Prior to beginning rooting studies with softwood cuttings of 'Variegatus' osmanthus, the authors attempted in three different years to root hardwood cuttings, with negligible results. This led to research with softwood and semi-hardwood cuttings. Attempts to root softwood cuttings of 'Variegatus' osmanthus began in early June 2007 and this was done at the same time each year through late May 2010. For brevity, only results for 2009 and 2010 are presented (Table 1), however, results for 2007 and 2008 were similar (data not presented). Treatment with K-IBA had little or no statistically significant affect on percent rooting, mean root length, or mean root number.

During 2007 to 2010 we also attempted to root semi-hardwood cuttings of 'Variegatus' osmanthus taken in late September to early October. These cuttings were treated similarly to the softwood cuttings and rooted under similar greenhouse conditions. In 2007 and 2008 nontreated semi-hardwood cuttings rooted at > 70%, and K-IBA treatments were generally ineffective (data not presented). However, in 2009 and 2010 rooting was poor with the best rooting approximately 20% for nontreated cuttings. Why rooting of semi-hardwood cuttings was so variable during this 4-year period is unknown and might be related to the number of growth flushes during the growing season.

Growth is uniform in the spring when the first flush of growth of 'Variegatus' osmanthus occurs. The terminal vegetative buds of all shoots break and produce a flush of growth. Following shoot elongation, growth ceases and the new shoots lignify (harden). Sometimes, a second flush occurs in late summer. This growth, however, will not be uniform throughout a plant. Some shoots produce a second flush of growth; others do not. Consequently, some cuttings representing the first flush appear to fit the description of semi-hardwood cuttings and cuttings of the second flush appear to be softwood (4). If both types of cuttings are taken and kept separate for rooting, rooting of each type is generally poor. On the other hand, if the plant has not produced a second flush of growth and all the cuttings are semi-hardwood, rooting is generally similar to results presented in Table 1 for softwood cuttings. Perhaps when a second flush of growth occurs and the growth is not uniform throughout the plant, the physiology of the plant is altered, resulting in a situation where softwood cuttings of the second flush and semi-hardwood cuttings of the first flush do not root as well as softwood cuttings taken after the first flush. As reported by Hartmann et al. (4), "August softwood cuttings in the Southeastern United States are not physiologically the same as June softwood cuttings". This could explain why rooting of softwood cuttings from the first growth flush is generally much better than rooting of cuttings from the second flush. Owing to the unpredictable rooting response of semi-hardwood cuttings and softwood cuttings following a second flush of growth, the most consistent rooting of stem cuttings of 'Variegatus' osmanthus is usually achieved with softwood cuttings following the first flush of growth. In Raleigh, NC, this condition normally occurs in late May to early June.

Genotype variation with respect to adventitious rooting has been reported for many species (1, 6, 7, 8, 9, 11), but appears to not be an issue with the many cultivars of *O. heterophyllus*, which are clones. However, another aspect of this phenomenon which has not received much consideration is genotypic variation with respect to the growth stage of the stock plant (stem tissue maturity). Thus, not only do clones of some

species exhibit variation in adventitious rooting, but the growth stage of the clones that is also most conducive to rooting can vary by genotypes, as has been reported (5, 8, 10, 11). This also seems to apply to some cultivars of *O. heterophyllus* when one compares results herein with those of Blazich and Acedo (2). For example, in the present investigation, optimum rooting of stem cuttings of 'Variegatus' osmanthus occurred with softwood cuttings collected in late May or early June following the first flush of growth, whereas rooting of semi-hardwood cuttings taken later in the growing season was inconsistent, and rooting of hardwood cuttings was negligible. On the other hand Blazich and Acedo (2) reported nontreated semi-hardwood and hardwood cuttings of 'Illicifolius' osmanthus rooted in high percentages (> 80%) whereas comparable results with 'Rotundifolius' osmanthus occurred only with hardwood cuttings. They did not, however, attempt to root softwood cuttings of these cultivars.

Similar to other cultivars of *O. heterophyllus* such as 'Illicifolius' and 'Rotundifolius', adventitious roots of 'Variegatus' osmanthus are coarse and brittle, even when rooted in a medium of 1 peat : 1 perlite (by vol.). Therefore direct rooting/sticking should be employed when rooting stem cuttings of 'Variegatus' to reduce possible transplant shock.

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Table 1. Influence of K-IBA treatments on the rooting of softwood cuttings of *Osmanthus heterophyllus* 'Variegatus'.

Treatment	Rooting (%) ^z		Roots (no.) ^y		Root length (mm) ^y	
	6/2/2009	5/31/2010	6/2/2009	5/31/2010	6/2/2009	5/31/2010
Nontreated	84.7	73.6	6.0	9.6	37.4	31.4
500 ppm K-IBA	79.2	83.6	6.4	8.4	36.3	34.1
1000 ppm K-IBA	63.9	81.9	4.9	7.7	37.0	34.9
1500 ppm K-IBA	76.4	58.3	5.9	4.8	32.0	33.7
2000 ppm K-IBA	62.5	80.6	4.3	7.3	30.9	35.7
Significance:						
Linear	NS ^x	NS	NS	NS	*	NS
Quadratic	NS	NS	NS	NS	NS	NS

^zEach value is based on 72 cuttings.

^yEach mean value is based on the number of cuttings which rooted for a particular treatment.

^xNS (nonsignificant) or * (significant) at $P < 0.10$.

Micropropagation of *Acer platanoides* L. 'Crimson Sentry'

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Index Words: Norway maple, basal salts, cytokinins, auxins, autopolyploids, regeneration

Significance to Industry: Norway maple (*Acer platanoides* L.) is a valuable landscape tree known for its attractive foliage and architecture. Since its introduction to North America by Bartram in 1756 (10), Norway maple has played a major role in replacing the American elm following Dutch elm disease (13, 14). Pest and disease resistance, and tolerance of poor soils have made Norway Maple a popular choice as a municipal street tree. However, Norway maple has become invasive in disturbed forests, along roadside edges, and within intact forests bordering ornamental plantings in the Northeastern and Midwestern United States (3, 12, 13). Inducing autopolyploids may improve ornamental features of Norway maple and provide an opportunity to develop triploid, seedless cultivars. In this study, in vitro regeneration protocols were developed for micropropagation and as a platform for future ploidy manipulation.

Nature of Work: In vitro regeneration provides an excellent method for rapid propagation. In vitro shoot regeneration studies for *A. platanoides* have primarily focused on wild types or the popular cultivar 'Crimson King' (4, 7, 8). These studies investigated different combinations of basal salts such as Murashige and Skoog (MS) (9), Linsmaier and Skoog (LS) (5), and Lloyd and McCown (WPM) (6) and cytokinins such as zeatin, kinetin (Kin), thidiazuron (TDZ) and 6-benzylaminopurine (BAP) to maximize in vitro shoot regeneration (1, 2, 4, 7, 8). Further in vitro rooting studies with *A. platanoides* have investigated low salt media supplemented with the synthetic auxin 3-indolebutyric acid (IBA) (1, 8). Thus, the objectives of this research were to develop in vitro multiplication and rooting protocols for *A. platanoides* 'Crimson Sentry'.

Stock explants for all experiments were maintained in vitro on *Acer* medium containing MS basal salts and vitamins, 2 μM BAP, 100 $\text{mg}\cdot\text{L}^{-1}$ myo-inositol, 100 $\text{mg}\cdot\text{L}^{-1}$ MES buffer, 30 $\text{g}\cdot\text{L}^{-1}$ sucrose, pH 5.75, and solidified with 7.5 $\text{g}\cdot\text{L}^{-1}$ agar in 180 cc jars. All samples were incubated at 23°C under a 16-h photoperiod (cool white 40W fluorescent lamps with PPFD 30 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

The effect of MS, WPM, and Quoirin and Lepoivre (QL) (11) basal salts in factorial combination with the cytokinins BAP, TDZ, 2-isopentenyladenine (2iP), meta-topolin (*mT*), and Kin on in vitro growth of 'Crimson Sentry' was examined. All media treatments were supplemented with 100 $\text{mg}\cdot\text{L}^{-1}$ myo-inositol, 100 $\text{mg}\cdot\text{L}^{-1}$ MES buffer, 30 $\text{g}\cdot\text{L}^{-1}$

sucrose, $7.5 \text{ g}\cdot\text{L}^{-1}$ agar, and $2 \text{ }\mu\text{M}$ of cytokinin with media pH adjusted to 5.75. Six replicates, each with 5 shoots (subsamples), were incubated on each media composition under standard culture conditions using a completely randomized design. After 5 weeks, data were collected on the number of shoots, shoot length (of the longest shoot), number of leaves, and number of nodes (on the longest shoot). Data sets were subjected to analysis of variance (ANOVA) using the GLM procedure of SAS (version 9.1; SAS Institute Inc, Cary, NC) (Table 1).

In a separate experiment, the optimum concentration of BAP for shoot multiplication was evaluated. Media containing MS basal salts and vitamins, $100 \text{ mg}\cdot\text{L}^{-1}$ myo-inositol, $100 \text{ mg}\cdot\text{L}^{-1}$ MES buffer, $30 \text{ g}\cdot\text{L}^{-1}$ sucrose, and $7.5 \text{ g}\cdot\text{L}^{-1}$ agar with a pH of 5.75 was supplemented with BAP at 0, 2, 4, 8 or $16 \text{ }\mu\text{M}$. Seven replicates per treatment with 5 subsamples (2- to 3-cm shoots) were arranged in a completely randomized design under standard culture conditions. After 5 weeks, data were collected on the number of shoots, shoot length (of the longest shoot), number of leaves, and number of nodes (on the longest shoot). Data were subjected to trend analysis using the GLM procedure of SAS (Table 2).

For studies on in vitro rooting, media consisted of half-strength WPM basal salts and vitamins and $30 \text{ g}\cdot\text{L}^{-1}$ sucrose, supplemented with 0, 5, 10, 20, 40 or $80 \text{ }\mu\text{M}$ IBA. All media were adjusted to a pH of 5.75. Each treatment consisted of 6 replications. Five subsamples (1- to 2-cm shoots) were assigned to each treatment, and all were arranged in a complete randomized design under standard culture conditions. Following 8 weeks of growth, data were collected on rooting percentage and root length (longest root) (Table 3). Data were subjected to trend analyses using SAS (Table 3).

Results and Discussion: Shoot regeneration was achieved with all treatments of basal salts and cytokinins. ANOVA results showed that basal salts, cytokinins, and their interactions significantly affected both shoot length ($P \leq 0.01$) and number of nodes ($P \leq 0.05$) (Table 1). The combination of MS and $2 \text{ }\mu\text{M}$ BAP produced the longest mean shoot length ($30.6 \pm 1.4 \text{ mm}$), and there was a general trend among all media types of BAP producing longer shoots. The combination of MS + BAP and QL + BAP also produced the most nodes (3.6 ± 0.2 and 3.5 ± 0.2 , respectively). Shoot number was influenced only by cytokinin type ($P \leq 0.01$). In general, BAP and *mT* produced the highest number of shoots across all basal salt treatments. Both medium and cytokinin type significantly influenced mean leaf number ($P \leq 0.01$ and 0.01 , respectively) but not their interaction.

In the second experiment, trend analysis of shoot data revealed that shoot length has a negative linear response to BAP concentration ($P \leq 0.01$) (Table 2). There was no significant effect of BAP concentration on shoot number. However, multiplication rate and number of 20 mm explants obtained per replicate per 6 weeks exhibited a quadratic response to BAP ($P \leq 0.01$) with the best BAP concentration estimated at $6 \text{ }\mu\text{M}$.

For the rooting experiment, *in vitro* root formation was observed in all treatments after 8 weeks. Trend analysis showed percent rooting, number of roots per rooted cuttings, and root length of microcuttings of *A. platanoides* 'Crimson Sentry' all exhibited a quadratic response to IBA concentration ($P \leq 0.01$, 0.01, and 0.05, respectively) (Table 3). Based on trend analysis, the highest percent rooting and longest roots was achieved using between 5 and 10 μM IBA. The highest number of roots produced per microcutting was achieved using between 5 and 40 μM IBA.

This study demonstrated that there are several different media components that interact to influence *in vitro* growth of *A. platanoides* 'Crimson Sentry'. Based on the results, MS media supplemented with 6 μM BAP provided high shoot proliferation, while half-strength WPM supplemented with 10 μM IBA produced the best rooting results. Protocols developed in this study will be used in future experiments focused on the *in vitro* development of autopolyploids.

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Table 1. Effect of media composition and cytokinin on in vitro growth of *Acer platanoides* 'Crimson Sentry'.

Media	Cytokinin	Shoots (no.) ^z	Shoot length (mm) ^z	Leaves (no.) ^z	Nodes (no.) ^z
MS	BAP	3.2 ± 0.3ab	30.6 ± 1.4a	8.1 ± 1.0ab	3.6 ± 0.2a
	TDZ	0.9 ± 0.04c	10.8 ± 0.5g	2.4 ± 0.4g	1.2 ± 0.1h
	2iP	1.9 ± 0.7d	12.0 ± 0.9fg	6.3 ± 1.1cde	2.2 ± 0.2ce
	mT	3.1 ± 0.2ae	21.4 ± 2.4bc	7.5 ± 0.6ac	2.9 ± 0.2b
	Kin	1.8 ± 0.2d	9.9 ± 1.1g	5.5 ± 0.6de	1.9 ± 0.2ef
WPM	BAP	3.9 ± 0.5ab	22.1 ± 1.8bc	7.8 ± 0.8ac	2.9 ± 0.2b
	TDZ	0.9 ± 0.03c	12.0 ± 0.9f	2.4 ± 0.4g	1.4 ± 0.1gi
	2iP	2.1 ± 0.3df	15.0 ± 0.8ef	4.9 ± 0.2ef	2.1 ± 0.1de
	mT	2.9 ± 0.4af	19.7 ± 3.1cd	6.8 ± 0.5bcd	2.6 ± 0.2bc
	Kin	2.3 ± 0.1def	10.9 ± 0.5g	4.5 ± 0.2ef	1.8 ± 0.1e
QL	BAP	3.5 ± 0.2AB	24.8 ± 3.0b	8.9 ± 0.5a	3.5 ± 0.2a
	TDZ	0.9 ± 0.1c	10.3 ± 1.6g	2.2 ± 0.4g	1.1 ± 0.2i
	2iP	1.7 ± 0.3cd	9.2 ± 0.2g	3.7 ± 0.5fg	1.6 ± 0.1fgh
	mT	2.5 ± 0.3def	16.9 ± 1.5de	5.9 ± 0.6de	2.4 ± 0.2cd
	Kin	2.4 ± 0.2def	10.1 ± 0.4g	3.6 ± 0.3fg	1.8 ± 0.2eg
Analysis of Variance:					
Media		NS ^y	*	**	*
Cytokinin		**	**	**	**
Media x Cytokinin		NS	**	NS	*

^zValues represent means ± SEM. Means followed by different letters within columns are significantly different, P ≤ 0.05.

^yNS, *, **: Nonsignificant or significant at P ≤ 0.05 and 0.01, respectively.

Table 2. Influence of BAP^z concentration on in vitro shoot growth of *Acer platanoides* 'Crimson Sentry'.

BAP (μM)	Shoots (no.) ^y	Shoot length (mm) ^y	Multiplication rate (explants per jar after 6 weeks) ^y
0	1.4 \pm 0.2	17.4 \pm 0.4	7.0 \pm 0.8
2	2.3 \pm 0.4	27.4 \pm 2.1	14.2 \pm 0.3
4	2.5 \pm 0.2	25.5 \pm 1.7	14.2 \pm 1.7
8	2.0 \pm 0.1	13.3 \pm 1.0	9.2 \pm 0.8
16	2.4 \pm 0.2	12.9 \pm 0.8	6.0 \pm 0.4
Trend analysis:			
Linear	NS ^x	**	*
Quadratic	NS	NS	**

^zBAP = 6-benzylaminopurine.^yValues represent means \pm SE.^xNS, *, **: Nonsignificant or significant at p=0.05 and 0.01, respectively.Table 3. Influence of IBA^z concentration on in vitro rooting of *Acer platanoides* 'Crimson Sentry'.

IBA (μM)	Rooting (%) ^y	Roots (no.) ^y	Root length (mm) ^y
0	0 \pm 0	0.0 \pm 0.0	0.0 \pm 0.0
5	77 \pm 13	2.6 \pm 0.8	17.1 \pm 3.1
10	70 \pm 12	3.0 \pm 0.9	17.9 \pm 3.5
20	53 \pm 13	1.8 \pm 0.7	11.9 \pm 2.7
40	63 \pm 10	3.4 \pm 1.0	11.5 \pm 1.4
80	13 \pm 7	0.2 \pm 0.1	3.7 \pm 1.7
Trend analysis:			
Linear	NS	NS	NS
Quadratic	**	**	*

^zIBA = indolebutyric acid.^yValues represent means \pm SE.^xNS, *, **: Nonsignificant or significant at p=0.05 and 0.01, respectively.

Media and Cutting Type Impact Stem Cutting Propagation of *Stevia rebaudiana*

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Keywords: stevia, cutting propagation, clonal propagation, sugar substitutes, sweeteners

Significance to the industry: Stevia [*Stevia rebaudiana* (Asteraceae)] is becoming a major part of the natural sweetener industry in the United States. This is leading to higher demand for production, although limited information on production is available. Labor costs have been shown to be a limiting factor in production by cuttings, the main method by which stevia is propagated (1). Lowering these labor costs by propagating this plant more efficiently is one goal of this research. Toward this end, a cutting propagation study was conducted to evaluate the effects of substrate and cutting type on propagation of stevia. Among the substrates tested, the greatest root length was produced in a pine bark/sand substrate. Cuttings in a sand/vermiculite substrate produced the greatest number of shootbreaks over 1 inch long. Additional shootbreaks lead to a denser plant with more leaves for harvest. Compared with rooted terminal cuttings, rooted medial cuttings had healthier and greener leaves among the old and new foliage by the end of the study.

Nature of work: Foods such as honey and fruit have historically been used for their sweetening properties. At present, cane sugar serves as the main source of sugar, with beet sugar providing a small percentage. Overconsumption of these sugars has shown to have negative health effects by contributing extra calories, which can lead to obesity, a contributing factor to chronic diseases such as *diabetes mellitus*, cardiovascular diseases, and hypertension. Advances have made it possible to offer consumers sweetness without calories (4). Among these advances is the use of the plant *Stevia rebaudiana* (5). Stevia is a native plant from the south-central South American country of Paraguay. The plant is native to the Rio Monday Valley of the Amambay Mountain region where it grows as a perennial (5). Stevia grows best in its native soil composed of infertile acid sands or mucks, yet it will grow well on a wide range of soils given a consistent supply of moisture and adequate drainage (3). Production requirements for stevia show it could possibly be a sugar replacement crop in the southeastern United States. Stevia could potentially be rotated with crops such as sugar cane, sweet corn (which requires fertile soils and a long, warm growing season), and tobacco. Stevia can produce good yields on soils and in climates not suited for standard row crops and with far fewer pesticides and less fertilization per unit of sweetening power. Stevia could also replace certain crops, such as tobacco. Stevia grows well in areas with rainfall amounts of about 54 inches (1). Alabama's yearly rainfall is 54 in. along with Mississippi, Georgia, Louisiana and Florida having rainfall amounts in the range of 49 in. to 60 in., all sufficient for production of stevia.

Propagation of stevia is typically accomplished using stem cuttings. Rooting of cuttings can sometimes be promoted by the use of growth regulators (2). Cuttings treated with IBA, IAA, and a combination of both at 1000 ppm caused injury due to the high concentration of growth regulators, whereas paclobutrazol (a plant growth retardant and triazole fungicide) at 50 or 100 ppm showed promise in inducing roots and sprout from stem cuttings (3). This process increases costs of producing stevia by cuttings. With limiting information on cutting propagation of stevia available, the current study examined cutting propagation of stevia cuttings using four different substrates without amendments.

Cuttings were taken from container-grown stock plants on June 22, 2011. Cuttings were prepared with two nodes using sterilized pruners and placed into bags by cutting type (terminal and medial). The cuttings were then placed into a cooler to transport them to the mist system. No leaves were stripped from the cuttings and no growth regulators were used. All cuttings were planted in 32-cell packs ($2\frac{3}{8}$ " x $2\frac{3}{8}$ " per individual cell with a depth of $2\frac{1}{4}$ "") with one cutting per cell at the Plant Sciences Greenhouse in Auburn, AL. Four different types of media were used: 1 part pine bark to 1 part sand by volume, 1 part peat moss to 1 part perlite by volume, 100% sand, and 1 part sand to 1 part vermiculite by volume. Cuttings were placed under intermittent mist with mist applied for 15 seconds every 10 minutes for the first 4 weeks of the study. During the remaining four weeks of the study, the cuttings were misted for 5 seconds every 10 minutes. Data collected consisted of foliar color rating of the old foliage (foliage present when cuttings were taken) and new foliage (growth after cuttings were planted) using a 1 (dead) to 5 (green) scale, counts of new shoot breaks over 1 inch long, and root length of the longest root.

Results and Discussion: The greatest root length occurred with cuttings propagated in pine bark/sand, whereas those in peat moss/perlite had the shortest root lengths (Table 1). Cutting type had no effect on root length. When rating the leaves of old foliage, medial cuttings had healthier, greener leaves compared to leaves on terminal cuttings (Table 2). With new foliage, the medial cuttings also showed higher ratings. Cuttings seemed to produce more shootbreaks in sand/vermiculite and the least in peat moss/perlite. Both sand based substrates produced good results for shootbreaks and root length for this plant.

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Table 1 . Rooting and shootbreaks influenced by cutting type and substrate.

Treatment	Shootbreaks (no.) ^Z	Root length (cm)
<u>Substrate</u>	<u>Mean</u>	
50:50 pine bark:sand	0.67 ^Y	20.89
50:50 peat moss: perlite	0.46	15.66
100% sand	0.56	17.89
50:50 sand:vermiculite	1.14	17.87
<u>Cutting type^X</u>		
Medial	1.17	18.97
Terminal	0.242	17.22
<u>Significance</u>		
Substrate	0.0063	0.0005
Type	0.076	<.0001
Substrate x type	0.3833	0.1371

^ZShootbreaks that grew over 1 inch long were counted for analysis.

^YData were analyzed using ANOVA.

^XCuttings were taken with two nodes per cutting.

Table 2. Foliar color rating of *Stevia rebaudiana* cuttings.^z

Method	Old Foliage Rating y				
	1	2	3	4	5
Medial	0a ^x	26a	69a	27a	2a
Terminal	1b	51b	62b	13b	1b
Method	New Foliage Rating w				
	1	2	3	4	5
Medial	2a	11a	58a	47a	6a
Terminal	1b	27b	63b	26b	3b

^zFoliar color was based on a 1-5 scale (1-dead; 5-green)

^yTwo node cuttings were planted and old foliar color was based on the foliage present when cuttings were taken.

^xMean separation was done using ANOVA

^wTwo node cuttings were planted and new foliar color was based on new growth after cuttings had been planted.

Micropropagation of *Ortegocactus macdougallii* Alexander, a Threatened Mexican Cactus

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Index Words: *Ortegocactus macdougallii*, biznaga pistache de Chico Ortega, micropropagation, in vitro propagation, Cactaceae.

Significance to Industry: *Ortegocactus macdougallii*, the "biznaga pistache de Chico Ortega", is an endemic Mexican member of the Cactaceae. The monotypic genus *Ortegocactus* includes only the species *O. macdougallii* (4). Unfortunately, native populations have a tendency to disappear since they are under severe pressure caused by soil degradation and human activities, especially over-collection of fruits and seeds, seedlings, and adult plants, which are coveted by collectors because of the plant's unique morphological traits (yellow flowers, tiny size, and the olive green of the stems) (3). Other critical problems related to the plant's biology and propagation are its very slow growth rate, reduced shoot production, low germination, and incompatibility that result in a poor fruit and seed set, and low survival of seedlings growing in natural habitats (2, 3).

Recently, the Mexican government has classified *O. macdougallii* in the category of a threatened plant species (NOM-059-ECOL-2010) (2). Because of its threatened status, it is urgent that scientists study the biology and physiology of this species with the objective to establishing the basis and strategies for protecting and rescuing native populations. Research on asexual reproductive protocols through tissue culture will provide knowledge for sustainable conservation and commercial production, which are important to ensure not only plant availability for reforestation of native communities but also to supply material for collectors who pay from 5.00 to 20.00€ for plants 2-3 cm in height. With this in mind, we performed this research with the main objective to study the particular conditions for establishing an efficient and reliable micropropagation protocol via organogenesis through the meristem activation of areoles. The nursery industry in general and the ornamental industry in particular may benefit from this micropropagation protocol.

Nature of Work: For the initiation stage of micropropagation, a series of experiments were run to study the response of explants to the plant growth regulators 1-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP) at different concentrations (0.3, 1, and 3 mg·L⁻¹) as supplements to Murashige and Skoog (MS) culture medium (1), giving a total of 12 experimental treatments. During the shoot

proliferation stage, we set up two experiments in order to compare the best treatment obtained from the initial cultures ($1 \text{ mg}\cdot\text{L}^{-1}$ NAA + $3 \text{ mg}\cdot\text{L}^{-1}$ BAP) with two additional BAP concentrations (4.5 and $6 \text{ mg}\cdot\text{L}^{-1}$) and the same concentration of NAA. For the rooting stage, four treatments were tested, which resulted from the reduction of the original formulation of the MS medium (one-quarter and one-half strength) plus the addition of IBA (0.4 and $1.2 \text{ mg}\cdot\text{L}^{-1}$). The regenerated plantlets of *Ortegocactus macdougallii* were transplanted and acclimatized for 6 days on a bench with low light conditions ($400 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of photosynthetic photon flux density (PPFD) prior to transfer to a different installation with 30% UV filtration with $800 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of PPFD to evaluate plant performance and survival.

Results and Discussion: Data obtained during the initiation stage of micropropagation demonstrated that plant management and culture conditions in the greenhouse prior to explant dissection, cleaning, and disinfection procedures followed during *in vitro* establishment were successful, resulting in low rates of contamination and tissue oxidation. After 116 days of initial culture in stage I, we observed two morphogenic responses in the explants: differentiation of disorganized structures (callogenesis) and differentiation of organs (organogenesis) into roots and shoots. Callus was differentiated from cells of both epidermis and parenchymatic tissues located at the edge of the cut area in all media treatments that included auxin (NAA), independent of the concentration and the presence of cytokinin. However, we observed that medium of high auxin concentrations (1 and $3 \text{ mg}\cdot\text{L}^{-1}$) produced root differentiation. When BAP interacted with NAA in an adequate balance ($0.3 \text{ mg}\cdot\text{L}^{-1}$ NAA + $3 \text{ mg}\cdot\text{L}^{-1}$ BAP and $1 \text{ mg}\cdot\text{L}^{-1}$ NAA + $3 \text{ mg}\cdot\text{L}^{-1}$ BAP), the explants produced both callus and shoot primordia after 56 days of culture. Detailed stereoscopic observations of the cultures allowed us to identify the tiny adventitious shoot primordia, which apparently originated from cells of the epidermis of the explants and the basal zone (Fig. 1a,b). The second group of shoots regenerated at this stage was produced through the axillary meristem activation of areoles and were visible after 56 days of *in vitro* culture (Fig. 1c). At this stage, shoot production was considered to be very poor and the treatments with media supplemented only with BAP (0.3 , 1 , and $3 \text{ mg}\cdot\text{L}^{-1}$) were not able to break the dormancy of axillary buds as was expected. In regard to the third response produced by the treatments in these initial cultures, we observed that three treatments [treatment 1: $0 \text{ mg}\cdot\text{L}^{-1}$ NAA + $0.3 \text{ mg}\cdot\text{L}^{-1}$ BAP; treatment 2 (NAA $0 \text{ mg}\cdot\text{L}^{-1}$ + BAP $1 \text{ mg}\cdot\text{L}^{-1}$), and treatment 3 ($0 \text{ mg}\cdot\text{L}^{-1}$ NAA + $3 \text{ mg}\cdot\text{L}^{-1}$ BAP)] were able to differentiate adventitious roots, whereas treatments 5 ($0.3 \text{ mg}\cdot\text{L}^{-1}$ NAA + $1 \text{ mg}\cdot\text{L}^{-1}$ BAP), 7 ($1 \text{ mg}\cdot\text{L}^{-1}$ NAA + $0.3 \text{ mg}\cdot\text{L}^{-1}$ BAP), and 10 ($3 \text{ mg}\cdot\text{L}^{-1}$ NAA + $0.3 \text{ mg}\cdot\text{L}^{-1}$ BAP) also induced callus formation in addition to root formation. With these six treatments we were able to regenerate whole plants at this stage. Detailed observations regarding the origin of the adventitious roots revealed that they were differentiated from parenchymatic cells and pith ground tissues in the neighborhood of vascular tissues (Fig. 1d).

In general, shoot proliferation during subculturing seems to be controlled by an adequate balance between auxin and cytokinin. The optimum concentration of NAA was

1 mg·L⁻¹, while concentrations of 3, 4.5, and 6 mg·L⁻¹ BAP produced an average of 5 to 7 shoots (Fig. 1e). Despite this, the treatment containing 3 mg·L⁻¹ of BAP could be considered superior to the others since it resulted in longer shoots (5 cm). Because *Ortegocactus macdougallii* is a slow-growing species, the adequate time between subcultures was 110 days. As was previously observed during stage I, shoots originate through areole activation and *de novo* differentiation.

Whole plantlets were easily obtained through adventitious root formation when shoots were cultured on any of the following three media: MS medium at one-quarter or one-half strength, by using MS medium at one-half strength plus NAA (1 mg·L⁻¹) and BAP (3 mg·L⁻¹), or with low IBA concentrations (0.4 and 1.2 mg·L⁻¹). However, it was clear that the addition of IBA (1.2 mg·L⁻¹) enhanced the rhizogenic process because, as noted, with significantly greater numbers of roots (8 on average) and a higher percentage of rooted shoots (75%) compared with other treatments. We recorded 90% of plantlet survival after transplantation (Fig. 1f).

Based on data obtained in this research, we can conclude that we successfully established an efficient and reliable micropropagation system for *O. macdougallii*. The complete cycle of micropropagation requires about 13 months of culture to produce 625 plantlets after three rounds of subcultures; however, if we want to increase the proliferation rate with 5 rounds of subcultures, the culture time would be increased to 21 months, but 6,250 plants would be produced.

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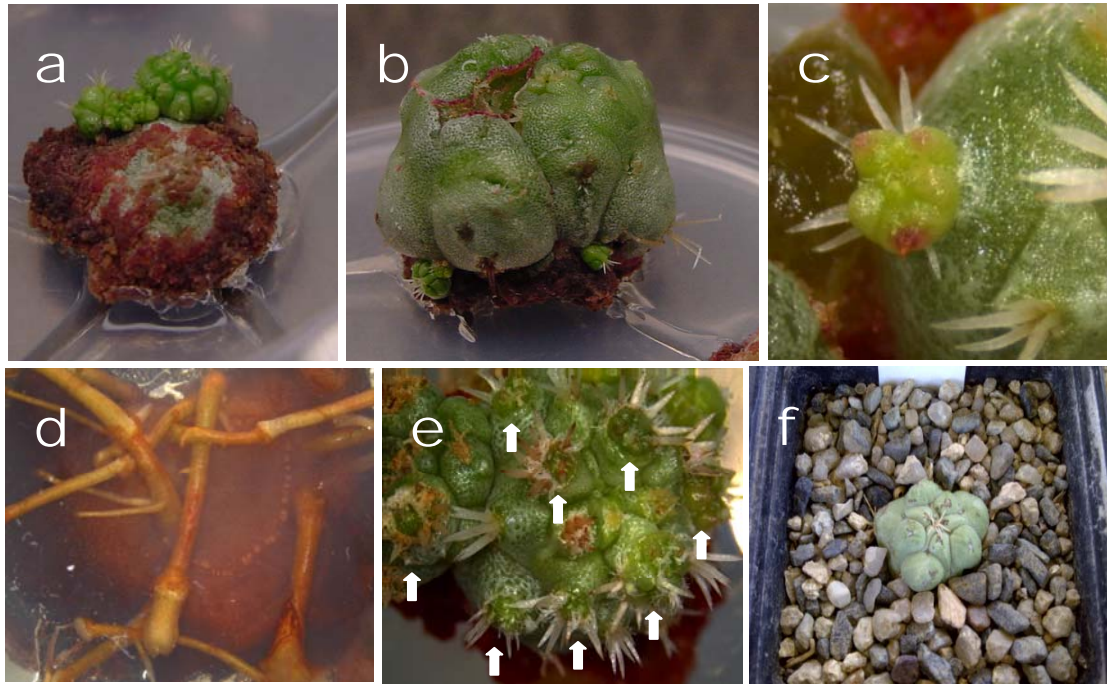


Figure 1. Micropropagation of *Ortegocactus macdougallii* showing: adventitious shoots differentiated from the epidermis (a) and the base of the explant (b); shoot differentiated from an activated areole (c); adventitious roots differentiated in the neighborhood of vascular tissues (d); multiple shoots (arrows) growing in an explant from areole activation (e); and a regenerated plantlet 90 days after transplantation to ex vitro conditions.

In Vitro Regeneration of *Solanum aethiopicum* L. (scarlet eggplant), an African Vegetable Crop with Potential Ornamental Value

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Index Words: African eggplant, aubergine, garden egg, Pumpkin-on-a-stick, scarlet eggplant, *Solanum aethiopicum*

Significance to Industry: *Solanum aethiopicum* L. (scarlet eggplant) is native to sub-Saharan Africa and is now found throughout tropical Africa, South America (mainly Brazil), and occasionally other areas such as southern France and Italy (10). Domesticated *S. aethiopicum* is the result of crossing wild *Solanum anguivi* Lam. and semi-domesticated *Solanum distichum* Schumach. & Thonn (10). *S. aethiopicum* has several recognized cultivar groups including Gilo, Kumba, Shum, and Aculeatum. These groups are defined by specific plant characteristics such as prickly leaves and stems, leaf shape, fruit shape and taste, and environmental growing requirements. The fruit, leaves, shoots, and roots are used for both food and medicinal purposes, and the specific use depends on the geographic area and/or plant type. *S. aethiopicum* is sometimes used as an ornamental and also as a rootstock for tomato and common eggplant because of its resistance to certain pathogens such as *Fusarium oxysporum* and *Ralstonia solanacearum* (2, 5, 6) and higher tolerance to drought and heat (7).

Nature of Work: *S. aethiopicum* is a vegetable crop mostly grown for its edible fruits. The fruits can be eaten raw or cooked like the common eggplant and are awakening a growing interest in the specialty and exotic vegetable markets of the world (4, 8). In addition to the attributes mentioned above (tolerance to some biotic and abiotic stresses), *S. aethiopicum* has the potential to be a valuable ornamental crop because of the tremendous diversity found in the color and shape of its leaves, stems, and fruits (10) (Fig. 1). Considerable variance also exists within scarlet eggplant varieties, so selection of outstanding plants can yield more productive materials (4). However, *S. aethiopicum* is highly cross-pollinated (8, 9), so the selected individuals may not breed true. Therefore, clonal propagation of the selected plants would yield materials with improved productivity and uniformity (4). Additionally, gene transfer through genetic transformation could be used to improve *S. aethiopicum*. Development of an efficient and dependable regeneration system is a prerequisite for genetic transformation. This micropropagation system could also be used for rootstock propagation. To our knowledge, there is only one report on in vitro culture of *S. aethiopicum* (4), and only two accessions were evaluated in that study. In vitro regeneration is usually highly genotype dependent, so there is a need for evaluating more *S. aethiopicum* genotypes. The purpose of this study was to develop an efficient and dependable tissue culture system for *S. aethiopicum*.

For this study, we used two types of media, M1 and M2, and four *S. aethiopicum* accessions from USDA-ARS (PI 194166, PI 374695, PI 441912, and PI 636107) and one genotype that originated from northeastern Senegal, Western Africa. Meristem explants were collected from in vitro grown seedlings and transferred to baby food jars containing M1 or M2 media. The M1 medium consisted of Murashige and Skoog (MS) (11) basal salt mixture supplemented with 20 g/L sucrose, 0.75 g/L MgCl₂, and 2 g/L Gelrite. M2 was a more complex medium composed of MS modified basal medium with B₅ vitamins (3) supplemented with 2 mg/L BA, 0.125 mg/L thidiazuron, 1 mg/L thiamine, 0.2 g/L myo-inositol, 1 g/L casein hydrolysate, 20 g/L sucrose, 0.75 g/L MgCl₂, and 2 g/L Gelrite. The pH was adjusted to 5.8 before autoclaving. All media were sterilized at 121 °C for 15 minutes. Plants were grown in baby food jars in a growth room (22 °C) with 16 hour days under fluorescent lights with a photon flux of 71 μmol m⁻² s⁻¹. Data collected after 60 days included plant height, number of roots and shoots, fresh weight, and a visual quality rating (1 = excellent, 2 = good, and 3 = poor). The experiments were conducted using a completely randomized design and consisted of two types of media, five genotypes, and 10 explants per treatment. The experiment was repeated once. Data were analyzed using the GLM procedure of SAS (version 9.1.3; SAS Institute Inc, Cary, NC).

Results and Discussion: Successful in vitro regeneration of plantlets was obtained using both M1 and M2 media for all five African eggplants evaluated (Table 1). After 60 days in culture, plant height ranged from 3.2 cm to 6.6 cm in M1 medium and 2.2 cm to 4.8 cm in M2 (Table 1). In general, plants grew taller in M1 than in M2. Also, plants formed consistently more roots in M1 than in M2. It is worth noting that two accessions, PI 441912 (Brazil) and PI 636107 (United Kingdom), formed no roots at all after 60 days in M2 even though these two accessions were among those that formed the most roots in M1. Results obtained for shoot number and visual quality evaluation were similar for both media; however, a few explants in M2 showed signs of hyperhydricity or physiological and morphological malformation, even though they tended to outgrow these problems as they matured. In conclusion, *S. aethiopicum* is readily amenable to in vitro culture, and M1 medium is recommended for propagation through meristem or shoot culture. This simple regeneration system could also be useful for genetic transformation of *S. aethiopicum*.

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Table 1. Effect of media type and genotype on plant height, number of roots and shoots, visual quality, and fresh weight on in vitro grown *Solanum aethiopicum* meristems after 60 days.

Accession	Origin	Medium	Height (cm)	Roots (no.)	Shoots (no.)	Visual quality ^z	Fresh weight (g)
PI 194166	Former Serbia & Montenegro	M1	5.6 ab ^y	11.9 a	1.1 bc	1.2 a	0.7 b
PI 374695	India	M1	3.5 c	4.5 b	2.0 ab	1.0 a	0.6 b
PI 441912	Brazil	M1	6.6 a	12.2 a	2.1 a	1.2 a	3.4 a
PI 636107	United Kingdom	M1	3.2 c	7.6 ab	1.0 c	1.1a	0.8 b
-----	Senegal	M1	4.2 bc	6.8 ab	1.1 bc	1.2 a	0.8 b
PI 194166	Former Serbia & Montenegro	M2	3.6 a	0.1 a	1.2 a	1.0 a	0.5 b
PI 374695	India	M2	2.3 b	0.4 a	2.0 a	1.3 a	0.7 b
PI 441912	Brazil	M2	4.8 a	0.0 a	1.9 a	1.4 a	2.0 a
PI 636107	United Kingdom	M2	2.2 b	0.0 a	1.4 a	1.1 a	0.9 b
-----	Senegal	M2	3.9 a	2.0 a	1.7 a	1.0 a	1.5 ab

^zVisual quality was defined as follows: 1 = excellent; 2 = good; 3= poor.

^yMean values within the same column and medium type (M1 or M2) followed by the same letters are not significantly different according to Duncan's multiple range test (P = 0.05). Means are averages of 20 observations.



Fig. 1. A–B: *Solanum aethiopicum* meristems cultured on M1 or M2 media typically produced plantlets with well developed root systems. Multiple shoot production from a single meristem was not uncommon for both media. C–I: *S. aethiopicum* genotypes are known for the diversity found in the color and shape of their leaves, stems, and fruits.

Factors Affecting Early Seedling Development in Whole Pine Tree Substrates

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Index Words: seed propagation, alternative substrates, growing media, phytotoxicity

Significance to Industry: Wood-based substrates can be successfully used in nursery and greenhouse crop production, yet have not been extensively evaluated for seed or cutting propagation. Wood-based materials, such as processed whole pine trees (WPTs), contain organic compounds that can be phytotoxic to sensitive species and inhibit seedling root growth. In our study, seed germination and early root development in sensitive species were inhibited by fresh pine needles, but no such inhibition was observed in aged or fresh WPT. However, a disparity in seedling root development between WPT and a peat-lite (PL) substrate suggests factors other than phytotoxicity are involved.

Nature of Work: Wood-based materials derived from pine trees, such as WPT, can be a viable option for producers looking to offset pine bark or peatmoss usage in container substrates. Wood-based substrates have been evaluated under a variety of production environments using various crops, yet certain issues must be addressed before manufacturers will invest in the commercialization of these products. The most common issues include nitrogen immobilization associated with microbial activity (5), phytotoxicity associated with organic molecules (3, 7), and less than ideal nutrient and water retention properties (1, 4). The negative effects of such factors on plant growth are well documented and can be minimized or prevented during crop production via modified production practices. However, reduced root development of stem cuttings rooted in WPT compared with pine bark (PB) has been observed (9), but the factors involved must be identified in order to develop corrective measures.

Reduced seed germination and seedling development are commonly used as indicators of phytotoxicity, specifically due to heavy metal content, compost maturity, salinity, and growth inhibiting compounds (6, 8). Seed germination and seedling growth tests used for detecting phytotoxicity are quick, simple, and reproducible. Seed germination tests are used for evaluating responses to substrate chemical properties, while seedling

growth tests account for responses due to substrate chemical and physical properties (2, 7). Seeds have nutritional reserves that will support growth for short periods after germination. As a result, unamended substrates can be evaluated, thus minimizing the number of variables involved in plant development. The objective of our research was to identify factors affecting seed germination and seedling development in unamended WPT substrates.

Two studies were performed at the Thad Cochran Southern Horticultural Laboratory in Poplarville, MS using a Phytotoxkit™ and seedling growth test. The Phytotoxkit™ is designed for direct observation and root measurement of germinated seeds in contact with the substrate solution. The Phytotoxkit™ study included three plant species (sorghum, *Sorghum saccharatum*; cress, *Lepidium sativum*; and mustard, *Sinapis alba*). Substrates included a reference soil (RS), aged (WPTA) and fresh (WPTF) whole pine tree, aged (PNA) and fresh (PNF) pine needles, pine bark (PB), peatmoss (PM), and saline pine bark (SPB). Whole pine tree substrates were produced from 2.0- to 2.5-in diameter (at 1 ft above ground) loblolly pine (*Pinus taeda*) trees harvested in Pearl River County, MS. Main stems were chipped on July 29, 2010 (WPTA) or March 14, 2011 (WPTF), and a combination of 9 chipped stems : 1 needles (by weight) was ground with a hammer mill (Model 30; C.S. Bell Co., Tiffin, OH) to pass a 1/4-inch screen. On March 14, 2011, pine needles were collected directly from trees (PNF) or from the ground (PNA) surrounding the same trees and hammer-milled to pass a 3/16-inch (PNA) or 1/2-inch (PNF) screen. Saline pine bark, pine bark soaked in a sodium chloride (NaCl) solution (16 mS/cm for cress or 30 mS/cm for mustard and sorghum), was included to produce a negative effect on seed germination and initial root growth.

Substrates were passed through a 2-mm sieve, and three 95-cm³ samples of each substrate were placed in a container (SVD-250, T.O. Plastics Inc., Clearwater, MN). Samples were bottom-saturated to the upper substrate surface with deionized water for 1 hour (SPB was saturated in NaCl for 10 hours), drained, transferred to individual test plates, and covered with filter paper. Ten seeds of a test species were placed in a single row, a clear plastic cover was placed on each test plate, and test plates (three per substrate) were completely randomized by species and incubated vertically in a dark growth chamber at 75°F for 5 (cress and sorghum) or 6 (mustard) days. Plates were digitally scanned and analyzed using ImageTool software (ImageTool Version 3.0; UTHSA, San Antonio, TX). Germination rate (%) and root length (mm) data were collected at the conclusion of the experiment.

In the second study, a seedling growth test was used to evaluate seedling development in unamended substrates under a simulated production environment. Test plant species included oat (*Avena sativa* 'Jerry'), lettuce (*Lactuca sativa* 'Green Ice'), and tomato (*Solanum lycopersicum* 'Brandywine'). Substrates included WPTA, WPTF, PL (3 peatmoss : 1 perlite : 1 vermiculite by vol.), and PB. Individual cells (41-cm³) were filled with substrate (36 replications), completely randomized into 72-cell propagation trays (36 cells per tray), and saturated. Two seeds of a single test species were sown on the substrate surface and covered with ½ tsp of substrate. Trays were grouped by species

and placed in separate growth chambers at 72°F for oat and lettuce or 77°F for tomato, each receiving a 14-hour light and 10-hour dark photoperiod. At 9 days after sowing, seedlings were thinned to one per cell. At 14 (oat), 25 (tomato), or 33 (lettuce) days after sowing, roots were washed, digitally scanned, and analyzed using WinRhizo software (WinRhizo Version 2007d; Regent Instruments Inc., Canada). Initial substrate pH and soluble salt concentration (data not shown), emergence rate (%), and total root length were collected. Germination/emergence rate, root length, and substrate physical properties (air space, container capacity, total porosity, and bulk density) data were analyzed with analysis of variance using the GLIMMIX procedure of SAS (SAS Version 9.2; SAS Institute, Inc., Cary, NC). Differences between treatment means were evaluated using the Shaffer-Simulated method.

Results and Discussion: Using the Phytotoxkit™, germination rates were lowest in PNF (cress) and SPB (mustard and sorghum) (Table 1). The highest germination rate (97%) was observed for mustard in RS, PB, and WPTA; for sorghum in PNF and WPTA; and for cress in RS. Germination rates were similar between WPTA and WPTF for all three species, whereas significant differences in germination rates between PNA and PNF occurred with cress. Cress root length was statistically similar among all substrates, yet a high level of variability of measurements within substrate could have masked differences between PNF and the other substrates. Mustard root length was significantly greater in PNA compared with PNF. Sorghum root length was greatest overall in RS, while statistically lower in SPB compared with all other substrates.

In the seedling growth test, emergence rate was similar among all substrates for lettuce and oat (Table 2), while tomato emergence rate ranged from 74% (WPTF) to 92% (WPTA). Within each species, total root length was approximately 11 (lettuce), 4.2 (tomato), and 2 (oat) times greater in PL compared to WPTF and WPTA. Aging the whole pine tree material only affected tomato emergence and oat total root length. Air space was statistically different among all substrates (Table 3), but was lowest in PL (5.5%) followed by PB (22.7%). Container capacity was greatest in PL (62.3%), while PB had the greatest bulk density (0.267 g·cm⁻³). Substrate pH for all substrates in both tests ranged from 4.4 (PNA and WPTA) to 5.4 (PB), while substrate soluble salt levels ranged from 76.5 (PM) to 634.5 ppm (PNF).

In both tests, root growth was a more sensitive indicator of phytotoxicity than seed germination. The Phytotoxkit™ did not reveal any significant concerns regarding phytotoxicity resulting from organic compounds present in WPT (aged or fresh), yet PNF can be phytotoxic to sensitive plant species. In the seedling growth test, greater root development occurred in PL, which had the greatest container capacity and lowest air space. All substrates were unamended and had inherently low nutrient content (based on a complete chemical analysis). Although minimal growth occurred during the seedling growth test, water and nutrient availability were undoubtedly a factor in observed differences. Modifying WPT substrate air space and container capacity to mimic that of PL would allow for a more unbiased evaluation. The goal of future research will be to evaluate the relationship among substrate physical properties, water availability, and root development in WPT.

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Table 1. Mean seed germination rate (%) and root length of three plant species after 5 (cress and sorghum) or 6 (mustard) days using a Phytotoxkit™.

Substrate	Germination rate (%)			Root length (mm)		
	Cress	Mustard	Sorghum	Cress	Mustard	Sorghum
Reference soil	97 a ^z	97 a	88 a	56 a	53 bcd	87 a
Pine bark	94 a	97 a	88 a	66 a	89 a	65 ab
Peatmoss	91 a	87 a	94 a	42 a	46 cd	52 b
Saline pine bark ^y	95 a	43 b	79 a	59 a	6e	15 c
Aged pine needles	86 a	93 a	94 a	40 a	62 bc	66 ab
Fresh pine needles	5 b	80 ab	97 a	18 a	41 d	59 ab
Aged whole pine tree ^x	93 a	97 a	97 a	51 a	52 bcd	52 b
Fresh whole pine tree	75 ab	93 a	88 a	40 a	67 b	73 ab

^zMeans followed by different letters within columns indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.

^yPine bark soaked in a saline solution.

^xProcessed whole pine trees (*Pinus taeda*) ground to pass a 1/4-inch screen.

Table 2. Mean seed emergence rate and total root length of three plant species after 14 (oat), 25 (tomato), or 33 (lettuce) days using a seedling growth test.

Substrate ^z	Emergence rate (%)			Total root length (cm)		
	Lettuce	Oat	Tomato	Lettuce	Oat	Tomato
Peat-lite	86 a ^y	88 a	81 ab	208 a	294 a	186 a
Pine bark	92 a	88 a	85 ab	35 b	258 b	67 b
Aged whole pine tree	86 a	89 a	92 a	19 c	135 d	45 c
Fresh whole pine tree	96 a	83 a	74 b	20 c	160 c	43 c

^zPeat-lite (3 peatmoss : 1 perlite : 1 vermiculite); aged and fresh processed whole pine trees (*Pinus taeda*) ground to pass a 1/4-inch screen.

^yMeans followed by different letters within columns indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.

Table 3. Physical properties^z of processed whole pine tree (aged and fresh), pine bark, and peat-lite substrates.

Substrate ^y	Air space -----(% vol)-----	Container capacity	Total porosity	Bulk density (g·cm ⁻³)
Peat-lite	5.5 d ^x	62.3 a	67.7 c	0.209 b
Pine bark	22.7 c	53.5 b	76.3 b	0.267 a
Aged whole pine tree	32.5 b	45.4 c	77.9 b	0.185 b
Fresh whole pine tree	37.6 a	49.9 b	87.6 a	0.196 b

^zData presented as means (n = 3) and obtained using the North Carolina State University porometer method.

^yPeat-lite (3 peatmoss : 1 perlite : 1 vermiculite); aged and fresh processed whole pine trees (*Pinus taeda*) ground to pass a 1/4-inch screen.

^xMeans followed by different letters within columns indicate significant difference at $P < 0.05$ using the Shaffer-Simulated method.