Plant Propagation

Gene Blythe
Section Editor and Moderator

Grafting Fraser fir (*Abies fraseri*): Effect of Grafting Date, Shade, and Irrigation

Haley Hibbert-Frey, John Frampton, Frank A. Blazich, and L. Eric Hinesley

North Carolina State University, Department of Forestry and Environmental Resources
Raleigh, NC 27695-8008

eric_hinesley@ncsu.edu

Index Words: *Abies bornmuelleriana*, Christmas trees, *Phytophthora cinnamomi*, rootstock, scion, vegetative propagation, cleft graft

Significance to Industry: Grafting Fraser fir [*Abies fraseri* (Pursh) Poir.] scions onto rootstocks of Turkish fir (*Abies bornmuelleriana* Mattf.) is a strategy used by some Christmas tree growers in the Southern Appalachian Mountains of North Carolina to reduce losses by phytophthora root rot caused by *Phytophthora cinnamomi* Rands. Results indicated it is prudent to graft Fraser fir in late winter/early spring with freshly collected dormant scion material.

Nature of Work: Fraser fir is one of the most popular Christmas tree species in the United States and is indigenous to isolated mountain tops at elevations between 1370 and 2037 m (4495 and 6683 ft) in southwestern Virginia, western North Carolina, and eastern Tennessee (5). Christmas tree plantations of this species are scattered throughout the southern Appalachian region where Christmas tree sales provide an important economic resource. In 2006, revenue from Christmas tree sales in North Carolina totaled $134 million (6). Fraser fir is grown for its fragrance, soft dark green needles, strong branches, excellent needle retention, and natural Christmas tree shape (2).

Phytophthora cinnamomi, the primary cause of phytophthora root rot, has spread rapidly throughout soils in western North Carolina, causing large economic losses. Once a site is infested, the pathogen is nearly impossible to eradicate. Fraser fir seedlings can die within 2 or 3 weeks from infection (1). Thus, there is a large demand in the region for planting stock that is resistant to, or tolerant of, this pathogen. To ameliorate the impact of this disease, some Christmas tree growers in the region are grafting Fraser fir onto rootstocks of more resistant fir (*Abies* Mill.) species. Grafting onto resistant rootstocks is a widely accepted method of managing phytophthora root rot (4). In a controlled inoculation study (1), momi fir (*A. firma* Sieb. et Zucc.) was the species most resistant to *P. cinnamomi*. Although it is the favored *Abies* rootstock for phytophthora resistance, it is not planted for Christmas tree production because it has undesirable sharp, prickly, light green needles and breaks bud early leaving it susceptible to late frosts. Turkish fir was less resistant than momi fir (1) but has desirable Christmas tree qualities. Furthermore, because momi fir transplants are in short supply most years (as the case for this study), Turkish fir is the next best rootstock choice.

Fraser fir is usually grafted in early spring (April) when the rootstock and scion are dormant, but this is a busy time for growers. The opportunity to graft at other times of the year, e.g., late summer or early fall, would allow Christmas tree growers more flexibility. Therefore, the objectives of this investigation were to (A) compare success and growth of grafting fresh Fraser fir scions onto Turkish fir rootstocks during the traditional time of grafting (April), with other grafting dates; (B) assess the affect of shade and irrigation treatments on graft success and growth; and (C) evaluate grafting (mid-July through mid-October) using dormant Fraser fir scions collected during April and stored at -1°C (30°F). The study compared the traditional time of grafting (April), using a cleft graft, with eight biweekly summer/early fall grafting dates from mid-July through mid-October (3). Shade and irrigation treatments were also superimposed on the drafting dates.

Results and Discussion: Results indicated that to ensure optimal grafting success, grafting should be performed in the late winter/early spring (April) when scions are dormant and the rootstocks are becoming active (3). April graft success was 95% but when grafting fresh scions in summer/fall, graft success decreased from 52% in July to 0% in October. Shade improved summer graft success (52% with, 38% without). Irrigation did not significantly affect graft success or subsequent growth. In a supplemental storage study, grafting of stored scion material in summer/early fall was not successful (less than 1%).

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Grafting Fraser fir (*Abies fraseri*): Effect of Scion Origin (Crown Position and Branch Order)

Haley Hibbert-Frey, John Frampton, Frank A. Blazich, Doug Hundley, and L. Eric Hinesley

North Carolina State University, Department of Forestry and Environmental Resources
Raleigh, NC 27695-8008

eric_hinesley@ncsu.edu

Index Words: cleft graft, orthotropic growth, plagiotropism, topophysis, vegetative propagation, Christmas trees

Significance to Industry: Success and subsequent growth of Fraser fir [*Abies fraseri* (Pursh) Poir.] cleft grafts were studied in relation to origin and type of scion material in the tree crown. Results indicated the origin of the scions regarding crown position and branch order has an influence on subsequent growth of successful grafts and should be considered prior to grafting.

Nature of Work: Fraser fir is one of the most popular Christmas tree species in the United States. It occurs naturally at elevations above 1370 m (4495 ft) on isolated mountain tops in the southern Appalachian Mountains from southwest Virginia through western North Carolina and into eastern Tennessee (16). It is grown commercially as a Christmas tree because of its pleasant fragrance, dark green foliage, natural conical shape and strong branches (5). In addition, it has good postharvest keepability (11). North Carolina has more than 1500 Christmas tree growers and 12,000 ha (29,652 acres) in production (17) with Fraser fir representing more than 95% of production. The farm-gate value of Christmas trees in North Carolina was about $134 million in 2006 (20).

Fraser fir normally is propagated by seed, but there is also interest in grafting it onto rootstocks of other *Abies* Mill. (fir) species with more resistance to phytophthora root rot (*Phytophthora cinnamomi* Rands) (12). Resistant fir species might help reclaim infested land previously abandoned for Christmas tree production. Grafting also provides an opportunity to clonally propagate trees with desirable Christmas tree phenotypes.

During the 1980s, the North Carolina Division of Forest Resources used grafting to establish the first clonal seed orchard of Fraser fir near Crossnore, North Carolina. Subsequently, grafting has been used to establish several other seed orchards and clone banks of Fraser fir. Despite this history, there is little published information for grafting Fraser fir. Traditionally, it is grafted in March or April when stock plants are dormant – a busy time for Christmas tree growers. Efforts to identify alternative grafting dates have been unsuccessful (8).

Origin of scion material within the tree crown might affect graft success. Owing to correlative inhibition and differences in vigor, shoots produced in the same year decrease in length and diameter downward and inward within the crown (13, 15, 21). In contrast, entire branches increase in age, length, and diameter from top to bottom of the tree. In vegetative propagation studies, stem cuttings of higher order often root in greater percentages than those of lower order (4,6), but this generalization is not universal (3). Growth following rooting is usually best for first-order shoots because they initially have greater caliper and larger buds (14, 15). In general, rooting capacity of Fraser fir stem cuttings decreases with age of the ortet (10, 18) and is best for cuttings collected lower in the crown (18). However, Garlo (6) found no significant difference in rooting of second-order stem cuttings from the upper and lower crown of 24-year-old Fraser fir in a seed orchard at Crossnore, North Carolina.

Comparisons such as rooting capacity of stem cuttings from the upper vs. lower crown, and first-order vs. second-order cuttings, relate to topophysis, the effect of the position of the propagule in the source plant (ortet) on the growth and phenotype of progeny (ramets) (7). The same issues, but often less pronounced, are relevant for grafting. Because Christmas tree selections normally are made when trees are young, prior to the transition to the adult growth phase, grafts and rooted cuttings initially are not sexually mature so that all growth is vegetative.

Factors affecting rooting of Fraser fir stem cuttings are well understood (1), whereas factors affecting grafting success have received little attention. Garlo (6) found no consistent relationship between rooting capacity and graft success for cuttings and scions from the upper and lower crown. Traditionally, Fraser fir is grafted using dormant scion material from the upper crown.

Plagiotropism – growth at an oblique angle to vertical – can reduce uniformity of rooted stem cuttings and grafted material and decreases overall shoot growth (19). A plagiotropic plant has no vertical (orthotropic) leader; it continues growing like a branch. *Abies* sp. such as Fraser fir are well known for plagiotropism following rooting of stem cuttings (1). The time for grafts to become orthotropic appears to decrease with increasing rootstock vigor at the time of grafting (2), but it might also be influenced by stock plant age, scion position in the ortet, and the propagation environment. If plagiotropism were similar for shoots from all parts of the crown, it would increase the availability of suitable scion material for grafting. Therefore, the objective of this study was to investigate success and growth of Fraser fir grafts as affected by (A) scion origin in the crown (e.g., upper vs. lower) and (B) branch order (first vs. second). The investigation involved collection of first- and second-order shoots (current year) from five zones in the crown, ranging from top to bottom, which were then grafted using a cleft graft to 5-year-old Fraser fir transplants in April (9).

Results and Discussion: Success rates were similar for first- and second-order scions, whereas budbreak and subsequent growth were best for first-order scions (9). In general, results were best for first order scions taken from the upper crown. Plagiotropism of grafts was similar for all crown zones and shoot types.

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Softwood Cutting Propagation of *Agastache* and *Buddleja* Using IBA and IBA+NAA Solutions

Eugene K. Blythe
Coastal Research and Extension Center, Mississippi State University,
South Mississippi Branch Experiment Station, Poplarville, MS 39470

blythe@pss.msstate.edu

Index Words: auxin, indole-3-butyric acid, naphthaleneacetic acid, vegetative propagation

Significance to Industry: The availability of unrooted cuttings from offshore producers in recent years has made it increasingly possible for growers to propagate a wide assortment of crops without the need to maintain their own stock plants. Softwood cuttings from intensively managed stock plants may or not benefit from a quick-dip in auxin prior to sticking, and may respond differently than cuttings obtained from conventional container-grown or landscape-grown stock plants. Using terminal softwood cuttings obtained from an offshore supplier, this study determined that cuttings of *Agastache* 'Tutti Frutti' rooted best using a quick-dip in 1000 ppm IBA or 1000 ppm IBA + 500 ppm NAA, and cuttings of *Buddleja davidii* 'Attraction' rooted best using a quick-dip in 500 ppm IBA or 500 ppm IBA + 250 ppm NAA.

Nature of Work: Since the 1980s, the availability of unrooted cuttings from offshore producers has made it increasingly possible for growers to propagate a wide assortment of crops without the need to maintain their own stock plants (2). Auxin treatment prior to sticking cuttings can be required for economical rooting of some crops, but are ineffective or unnecessary with others (3).

In many cases, maintenance of healthy stock plants specifically for volume production of unrooted cuttings at offshore production facilities has eliminated the need for routine use of auxins (2). Situations that may warrant the use of an auxin treatment include below-optimum substrate or air temperatures, uneven mist coverage, moderate delays in delivery of cuttings to the grower, reduced light levels during the rooting phase, or varieties that often tend to be slow or uneven in rooting (2).

Auxins are generally applied to herbaceous and softwood cuttings of various crops at concentrations of 500 to 1,500 ppm, and can be applied as powders or as quick-dip solutions. The latter offer the advantages of uniformity, consistency, and ease of use (3). *Agastache* will root from softwood cuttings prepared with at least one node (3); however, recommended concentrations have not previously been published. *Buddleja* cuttings root readily from terminal or single-node softwood cuttings (4). An application of 1,000 to 3,000 ppm IBA (and up to 8,000 ppm IBA) as a solution or powder has been recommended for cuttings of *Buddleja*, especially if semi-hardwood cuttings are being used (1, 3).

The objective of this study was to evaluate the rooting and initial shoot growth of terminal softwood cuttings of *Agastache* 'Tutti Frutti' ('Tutti Frutti' hummingbird mint) and *Buddleja davidii* 'Attraction' ('Attraction' butterfly bush) with and without the use of a quick-dip in alcohol-based solutions of IBA and IBA+NAA. *Agastache* 'Tutti Frutti' is an herbaceous perennial originating from a hybrid between *A. barberi* and *A. mexicana*, with plants growing 3 to 4 feet in height and producing clusters of lavender-pink flowers. *Buddleja davidii* 'Attraction' is a deciduous shrub with purplish-red flowers and a more compact habit than 'Royal Red'.

Auxin solutions were prepared by diluting Dip 'N Grow concentrate (10,000 ppm IBA + 5000 ppm NAA; Astoria-Pacific, Inc., Clackamas, OR) to final concentrations of 1000 ppm IBA + 500 ppm NAA and 500 ppm IBA + 250 ppm NAA, and by diluting Dip 'N Grow Lite concentrate (experimental formulation with 10,000 ppm IBA; Astoria-Pacific, Inc.) to final concentrations of 1000 ppm IBA and 500 ppm IBA. Solutions were prepared with isopropyl alcohol and deionized water to contain 50% alcohol (by volume) in the final product.

Cuttings of *Agastache* 'Tutti Frutti' and *Buddleja davidii* 'Attraction' were donated by Yoder Brothers Inc. (Lancaster, PA) and shipped from Flores del Amanecer S.A. (Cundinamarca, Colombia) on March 10, 2008 and received at the South Mississippi Branch Experiment Station in Poplarville, MS on March 12. Cuttings of *Agastache* were 3 cm in length and cuttings of *Buddleja* were 5 cm in length. Cuttings received a 1-second basal quick-dip in their respective auxin solutions (cuttings in one treatment were not treated with auxin), stuck into 50-cell trays in Fafard 3B substrate (Conrad Fafard, Inc., Agawam, MA) using a completely randomized design, and placed under intermittent mist in a greenhouse. There were 40 cuttings per treatment for a total of 200 cuttings per variety.

After one month, all cuttings had rooted. Cuttings were removed from the plug trays and washed to remove substrate. Root systems were scanned and analyzed using WinRHIZO software (Regent Instruments Inc., Quebec, Canada) and each shoot was measured for length. Data was analyzed using the GLIMMIX procedure of SAS (SAS Institute Inc., Cary, NC) with treatment comparisons made using the Schaffer-Simulated method.

Results and Discussion: Cuttings of *Agastache* 'Tutti Frutti' exhibited the best rooting using the two treatments with the highest concentrations of auxin (1000 ppm IBA and 1000 ppm IBA + 500 ppm NAA, with total root length using these treatments being significantly greater than with nontreated cuttings (Table 1). Cuttings of *Buddleja davidii* 'Attraction' exhibited the best rooting using the two treatments with the lower concentrations of auxin (500 ppm IBA and 500 ppm IBA + 250 ppm NAA, with total root length using these treatments being significantly greater than with nontreated cuttings (Table 1). No indication of shoot growth inhibition was exhibited by either variety.

Based on our results, we conclude that terminal softwood cuttings of *Agastache* 'Tutti Frutti' and *Buddleja davidii* 'Attraction' benefit from the use of an auxin treatment by

producing larger root systems than nontreated cuttings. Larger root systems are generally better able to stabilize the substrate plug for transplanting and have the potential for faster establishment of root systems in growing substrate after transplanting.

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Table 1. Rooting and initial shoot growth on terminal softwood cuttings of *Agastache* 'Tutti Frutti' and *Buddleja davidii* 'Attraction' obtained from an offshore supplier, treated with a basal quick-dip in selected auxin treatments, and rooted under intermittent mist in a greenhouse (n=40).

Variety	Treatment	Total root length (mm)	Shoot length (mm)
Agastache 'Tutti Frutti'	1000 ppm IBA + 500 ppm NAA	581 a[z]	219 a
	1000 ppm IBA	540 ab	209 ab
	500 ppm IBA + 250 ppm NAA	434 cd	208 ab
	500 ppm IBA	482 bc	212 ab
	nontreated	373 d	193 b
Buddleja davidii 'Attraction'	1000 ppm IBA + 500 ppm NAA	614 ab	152 ab
	1000 ppm IBA	651 ab	155 ab
	500 ppm IBA + 250 ppm NAA	670 a	159 a
	500 ppm IBA	690 a	160 a
	nontreated	571 b	136 b

[z]Means followed by the same letter within a variety are not significantly different at the 0.05 level according to the Schaffer-Simulated method.

Improving the Success of Microcutting Establishment from Native Azaleas

K.L. Bowen
Department of Entomology and Plant Pathology
Auburn University, AL 36849

bowenkl@auburn.edu

Significance to Industry: Propagation of desirable plants that may be found in their native settings can often be difficult. Micropropagation of such plants has several benefits including conservation of the original plant and the potential for large scale production. One of the problems that can arise when attempting such micropropagation is fungal contamination. This study demonstrates that fungicide amendment of media used for establishment of viable explants in culture can increase incidence of shoot survival.

Nature of Work: There are sometimes occasions when it is desirable to propagate a unique plant found in a natural setting. Often propagation by cuttings is unsuccessful and source material is limited. Micropropagation, however, allows conservation of the original plant and has the potential for large scale production from limited source materials. Problems can also arise with micropropagation such as fungal contamination of cultures. Fungal contaminants can escape disinfestation or may have colonized tissues without showing deleterious symptoms (5). Because of the potential such contaminants have for preventing the establishment of viable explants, micropropagators are advised to grow source plants in a protected environment such as a greenhouse prior to taking cuttings (4), which may not be feasible.

In the two years preceding this study, success of establishing aseptic plant cultures for micropropagation of deciduous native azaleas (*Rhododendron* spp.) has been severely limited, primarily due to fungal contaminants; bacterial contaminants were rare. This study sought to evaluate the feasibility of using fungicides in propagation media.

Shoots of two southern native azaleas, *Rhododendron flammeum* (n = 3) and *R. canescens* (n = 5), growing on private property, were collected in April 2010. These plants had never been treated with fungicide or other pesticides. Excised shoot tips, 7 to 10 cm (3 to 4 inches) in length, were kept in moistened paper towels at ambient conditions until processing which was done within several hours of collection. Leaves were removed from shoots and shoots were placed in 0.5% sodium hypochlorite solution (bleach) containing 1 ml Tween per liter. Shoots in bleach solution were continually stirred for 15 minutes then rinsed 3 consecutive times in sterile, distilled water. In a laminar flow hood, the basal end was re-cut and the apical tip was trimmed on each shoot. The remaining stem was cut into 1- to 2-cm explant pieces, each having at least one node. In most cases, an individual shoot was cut into three pieces. Pieces of each shoot were placed on the three different media.

The culture medium was similar to that used by Economou and Read (3), except that adenine sulfate (80 ppm) was added. The medium also contained 1 ppm indole acetic acid and 12 ppm 2iP. Medium amendments, in addition to a non-amended control, were fungicides. One amendment was azoxystrobin (at 100 ppm) (Heritage®, 50% active ingredient), the second was benomyl (at 20 ppm) (Benlate, 50%). The pH of each medium was adjusted to pH 5.0 after which sucrose (20 gms per L; 2.7 oz per gal) and agar (6 gms per L; 0.8 oz per gal) were added and dissolved. Media was dispensed into test tubes, in 10 ml aliquots, then autoclaved.

Explants were visually examined every 2 to 4 days for contamination or death (browning then blackening of explants), at which point they were removed from the study. Days of survival and survival incidence were recorded. Generalized linear model analysis was done to determine treatment differences in survival with $P < 0.10$.

Results and Discussion: Some explants were observed to have swelling buds by day 13 on culture media. After day 28, no additional removal of explants due to contamination or browning was needed. Numbers of apparently healthy shoots, especially on non-amended and azoxystrobin-amended media, declined rapidly after 10 days in culture (Fig. 1). Two-thirds of the shoots from *R. flammeum* were apparently free from any fungal colonization or had been completely disinfested, and pieces of these shoots remained healthy even on non-amended media. Similarly, two-fifths of *R. canescens* shoots were apparently free of fungal contaminants. Among shoots from which fungal contaminants developed when a piece was on non-amended media, the proximal piece on benomyl-amended medium stayed healthy (Fig. 1). All shoot pieces of *R. canescens* that were started on the benomyl-amended medium remained healthy. Despite the low number of shoots that were collected, differences in percent survival were found due to fungicide amendment ($P = 0.07$) after 28 days in culture (Table 1).

After 44 days on fungicide-amended media, explants with growth of axillary shoots were placed onto fresh media without fungicide amendment. At the time of this writing, these microshoots are developing normally, with transfers being made every 5 to 6 weeks to fresh media, as observed with previous *Rhododendron* explants by this author.

Azoxystrobin and benomyl are both systemic fungicides used for control of a fairly broad range of fungi. The melting point of benomyl (572°F) (2) is somewhat higher than that of azoxystrobin (240°F) (1). It may be that the azoxystrobin became degraded during autoclaving, which decreased its effectiveness in controlling contamination. However, several of the shoot pieces on azoxystrobin turned brown then blackened without evidence of contamination. Browning of shoots with exposure to azoxystrobin may have been due to the use of too high a concentration of that fungicide in the medium, and further tests may be needed.

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Table 1. Explant survival (%) after 28 days on culture media amended with fungicides or non-amended. Data are means of the proportion of the number of shoots as noted.

Media amendment	*R. canescens*	*R. flammeum*	Total
	---- n=5 ----	---- n=3 ----	--- n=8 ---
None	40 a[x]	67 a	50.0 ab
Azoxystrobin	20 a	0 a	12.5 a
Benomyl	100 a	67 a	88.0 b

[x]Letters following means, when different, indicate significant difference according to generalized mixed model analysis with $P < 0.10$.

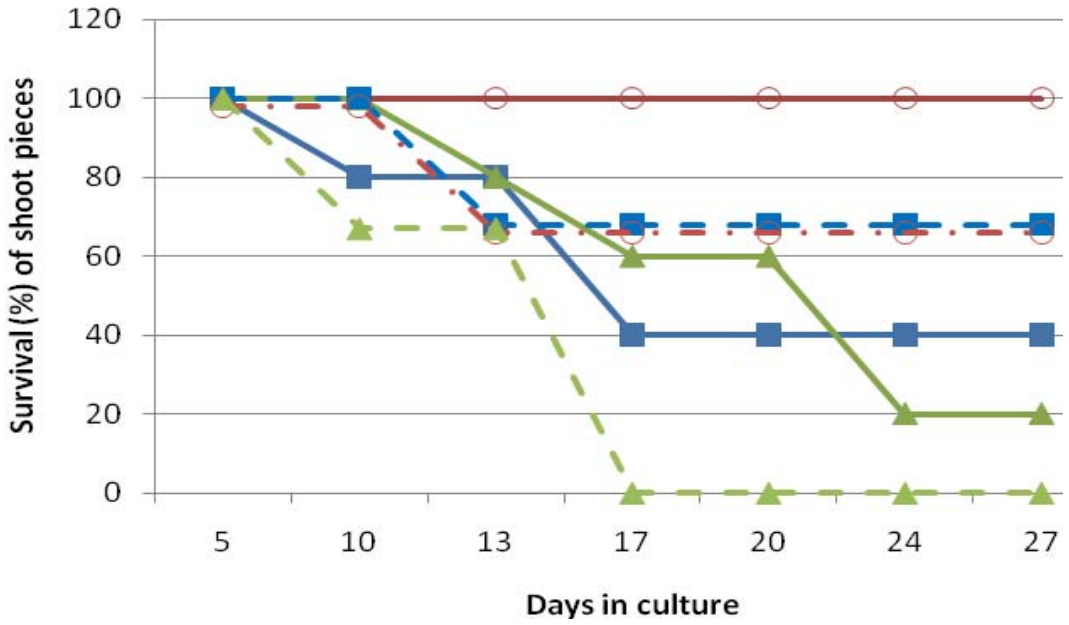

Fig. 1. Percent survival of explants of *R. flammeum* (dashed lines) and *R. canescens* (solid lines) on non-amended (squares), and with media amended with benomyl at 20 ppm (open circles) and azoxystrobin at 100 ppm (triangles) for 27 days after placement on media.

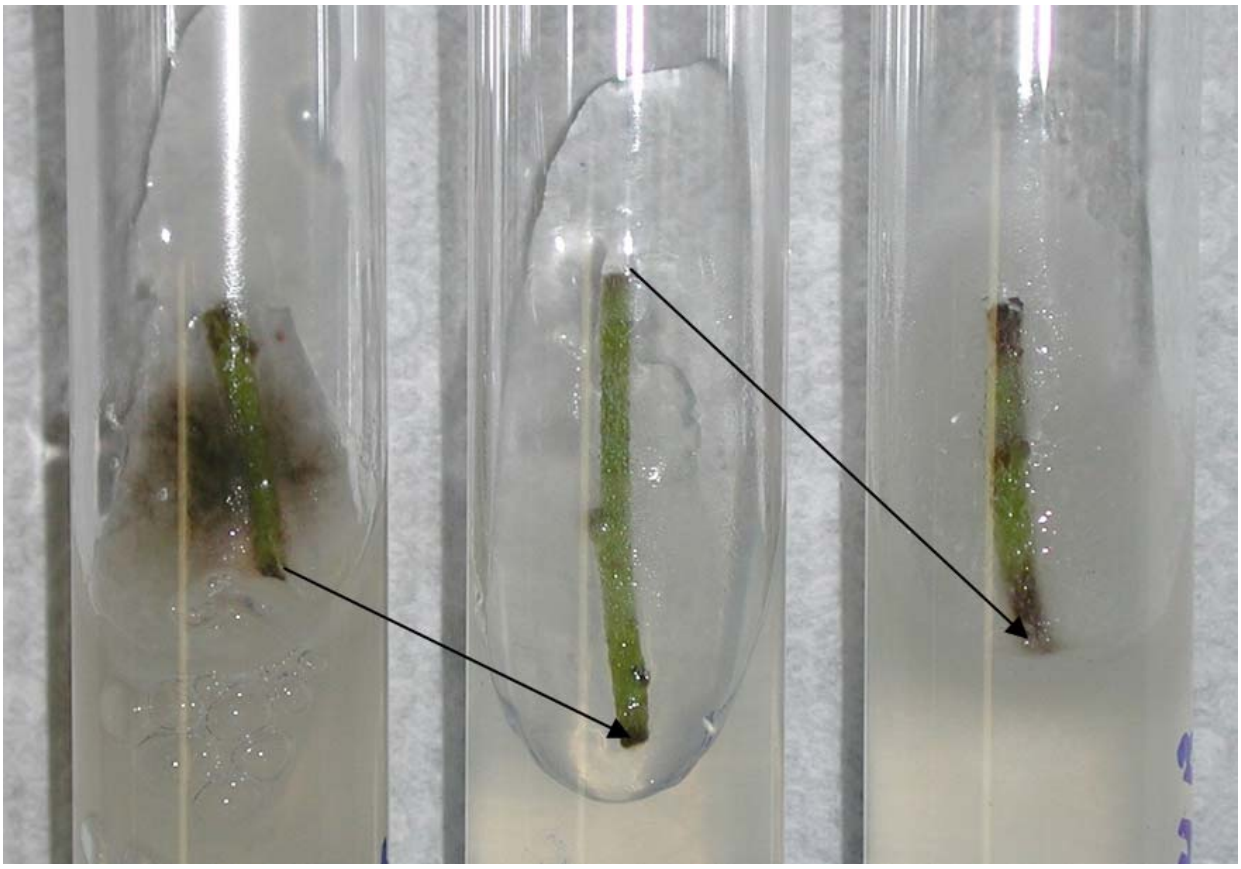

Fig. 2. Shoot pieces of *R. flammeum*, cut from a single shoot and placed sequentially (as noted by arrows), after 5 days on amended media. From left to right, media amendments are azoxystrobin (100 ppm), benomyl (20 ppm), and non-amended control.

Effect of Cell Size on Growth and Physiology of Mexican Fan Palm (*Washingtonia robusta* H. Wendland: Arecaceae) Seedlings

Andrés Adolfo Estrada-Luna[1]; Horacio Claudio Morales Torres[2]; Victor Olalde-Portugal[2]; Esteban Camarena Olague[1]; Carlos Romero González[1]

[1] Escuela de Agronomía. Universidad De La Salle Bajío. Av. Universidad 602, Col. Lomas del Campestre. León, Gto., México. C.P. 37150
[2]CINVESTAV-IPN. Km. 12.5. Libramiento Norte, Carretera Irapuato-León. Irapuato, Gto., México. C.P. 36821

aestradaluna@yahoo.com

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Significance to Industry: The Mexican fan palm (*Washingtonia robusta* H. Wendland), also known as the Mexican Washington palm, is an endemic plant species native to the desert regions in northwest Mexico (1, 4). It is a fast growing palm that can be adapted to different climate conditions, water-limited areas, and different types of soils (1, 3, 4). Because of this and its peculiar morphology and beauty, it has successfully been planted throughout Mexico and many other countries. The Mexican fan palm is a multipurpose plant that is used as an ornamental, to reforest degraded areas, as a natural barrier, and to recover eroded soils (3, 4, 5). Recently, we started a research program with the goal of studying its biology and physiology to determine the optimum conditions for commercial cultivation and preservation. As a result, we have learned how to manipulate seeds to reduce the germination time, increase germination rates, and standardize seedling emergence (2). Currently, we are performing experiments to determine the interaction of this palm with soil microorganisms including mycorrhizal fungi and plant growth promoting rhizobacteria. Data obtained from this study will benefit the nursery industry, propagators, and growers since optimization of factors affecting plant growth and physiology will provide more uniform and healthy material for commercial uses.

Nature of Work: We evaluated the effect of cell size on seedling growth, performance, and physiology in order to determine the optimum size and stage for transplantation of the Mexican fan palm to be used in commercial propagation systems. In order to begin the study, we collected healthy and mature fruits from selected plants at Irapuato, Guanajuato, Mexico. Healthy seeds were cleaned by immersion in a 2% H_2SO_4 solution for 48 hours to facilitate removal of pulp tissue from the seed. Remaining pulp was manually removed by macerating fruits in a sieve and cleaning with several washes with running tap water. Finally, seeds were dried at room temperature for three days and stored in paper bags. Before planting, seeds were subjected to a priming treatment consisting of an immersion in distilled water (with daily changes of fresh water) for 6 days in an incubator at 28 $\pm$ 4° C and 125 rpm of shaking. In a simple experiment, we

evaluated the effect of two cell sizes on seedling growth, performance, and physiology. Treatment one (cell type A) included plastic germination containers (140 mL; 4.5 X 16.5 X 3.0 cm), while treatment two (cell type B) included germination trays with 60 cells (200 mL capacity; 5 X 12 X 2.5 cm dimensions). Before planting the seeds, germination containers were filled with a sterilized substratum composed of sandy-loam top soil, leaf turf, sunshine potting soil Mix 3 (Sun Gro Horticulture, Canada, Ltd), perlite, and vermiculite, (1:2:3:1:1). There were 60 seedlings in each treatment (n= 60).

After sowing the seeds, containers were transferred to a glasshouse and grown with maximum photosynthetic photon flux density (PPFD) of 1,100 μmol/m^2/s^{-1} at plant level and an average of day/night temperature of 27/20 $\pm$ 3° C. Irrigation was supplied as needed with distilled water and fertilization was provided once a week (200 ppm N) after seedling emergence with Peters Professional 20-20-20 (Scotts-Sierra Horticultural Products Co., Marysville, OH, USA). Seedling growth measurements including plant height (cm), total root length, number of secondary roots, average secondary root length, total secondary root length, leaf, root, and total seedling dry mass, root to shoot ratio and shoot to root ratio recorded every 45 days for three times (135-day-old seedlings) after seedling emergence. Gas exchange measurements including net photosynthesis, stomatal conductance (g_s), and stomatal resistance (r_s), were also performed between 9:00 to 12:00 am on the first fully expanded leaf using a LI-6200 Portable Photosynthesis System (LI-COR, Inc., Lincoln, NE).

Treatment effects in the experiment were determined by using analysis of variance (ANOVA) and LSD (α= 0.01 and 0.05) for mean separation (6).

Results and Discussion: Seed germination and emergence varied from 13 to 16 days in both types of containers. After 45 days of culture, seedlings from the two treatments had produced a pair of leaves; however, greater growth was observed on root systems of seedlings growing in cell type A (Table 2). Net photosynthesis and stomatal conductance were also higher in this treatment; however, no statistical differences were detected in net photosynthesis data (Table 1). In contrast to what was observed after 45 days of culture, after 90 days seedlings growing in cell type B recovered and showed better growth than in the other treatment (Table 3 and 4). Overall plant growth was greater; however, net photosynthesis showed lower values (Table 3). A similar tendency was observed after 135 days (Table 2, Figure 1). After one year of culture the plants appeared in good condition; however, the overall growth was drastically reduced compared with plants transplanted to larger containers. We conclude that the best time to transplant the Mexican fan palm is between 45 and 90 days after emergence. After this period, seedling growth slows drastically.

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Table 1. Effect of cell size on net photosynthesis (*A*), stomatal resistance (r_s), and stomatal conductance (g_s) on Mexican fan palm (*Washingtonia robusta*) seedlings 45 days after emergence.

Cell Type	A(μmol/m^2/sec^{-1})	r_s(sec/cm^{-1})	g_s(mol/m^2/sec^{-1})
A	9.769 a	4.810 a	0.317 a
B	9.133 a	3.503 b	0.237 b

Means with the same letter are not significantly different according to Fisher's LSD ($\propto$ = 0.05). n = 10.

Table 2. Effect of cell size on seedling growth of Mexican fan palm (*Washingtonia robusta*) seedlings 45 days after emergence.

Cell Type	Plant Height (cm)	Total Root Length (cm)	Secondary Roots (no.)	Average Secondary Root Length (cm)	Total Secondary Root Length (cm)	Shoot Dry Weight (g)	Root Dry Weight (g)	Plant Dry Weight (g)	Root to Shoot Ratio	Shoot to Root Ratio
A	14.4 a	14.1 a	42 a	5.1 a	207 a	0.14 a	0.10 a	0.24 a	0.74 a	1.42 b
B	16.1 a	11.9 a	32 b	4.0 b	128 b	0.16 a	0.06 b	0.22 a	0.35 b	3.06 a

Means with the same letter are not significantly different according to Fisher's LSD ($\propto$ = 0.01). n = 10.

Table 3. Effect of cell size on net photosynthesis (*A*), stomatal resistance (r_s), and stomatal conductance (g_s) on Mexican fan palm (*Washingtonia robusta*) seedlings 90 days after emergence.

Cell Type	A (µmol/m^2/sec^{-1})	r_s (sec/cm^{-1})	g_s (mol/m^2/sec^{-1})
A	14.21a	1.19 b	0.84 a
B	9.68 b	1.60 a	0.72 b

Means with the same letter are not significantly different according to Fisher's LSD ($\propto$ = 0.05). n = 10.

Table 4. Effect of cell size on seedling growth of Mexican fan palm (*Washingtonia robusta*) seedlings 90 days after emergence.

Cell Type	Plant Height (cm)	Total Root Length (cm)	Secondary Roots (no.)	Secondary Root Length (cm)	Total Secondary Root Length (cm)	Shoot Dry Weight (g)	Root Dry Weight (g)	Plant Dry Weight (g)	Root to Shoot Ratio	Shoot to Root Ratio
A	27.9 b	13.7 a	62 b	5.5 a	348.2 b	0.84 b	0.43 b	1.26 b	0.52 a	1.97 b
B	33.2 a	13.2 a	67 a	5.7 a	382.5 a	1.64 a	0.64 a	2.28 a	0.39 b	2.60 a

Means with the same letter are not significantly different according to Fisher (LSD) test ($\propto$ = 0.01). n = 10.

Table 5. Effect of cell size on net photosynthesis (*A*), stomatal resistance (r_s), and stomatal conductance (g_s) on Mexican fan palm (*Washingtonia robusta*) seedlings 135 days after emergence.

Cell Type	A (µmol/m^2/sec^{-1})	r_s (sec/cm^{-1})	g_s (mol/m^2/sec^{-1})
A	11.41 a	1.09 b	0.74 a
B	9.68 a	1.86 a	0.68 a

Means with the same letter are not significantly different according to Fisher's LSD ($\propto$ = 0.05). n = 10.

Table 6. Effect of cell size on seedling growth of Mexican fan palm (*Washingtonia robusta* H. Wendland) seedlings 135 days after emergence.

Cell Type	Plant Height (cm)	Total Root Length (cm)	Secondary Roots (no.)	Secondary Root Length (cm)	Total Secondary Root Length (cm)	Shoot Dry Weight (g)	Root Dry Weight (g)	Plant Dry Weight (g)	Root to Shoot Ratio	Shoot to Root Ratio
A	30.9 b	14.2 a	70 b	5.5 a	378.2 b	0.96 b	0.48 b	1.45 b	0.50 a	2.00 b
B	35.3 a	13.8 a	72 a	5.9 a	388.7 a	1.95 a	0.74 a	2.69 a	0.38 b	2.64 a

Means with the same letter are not significantly different according to Fisher's LSD ($\propto$ = 0.01). n = 10.

Rooting of Two Woody Ornamental Plants in Eight Propagation Substrates

Celina Gómez and James Robbins
University of Arkansas, Department of Horticulture, Fayetteville, AR 72701

cgomezv@uark.edu

Index words: Parboiled rice hulls, coconut coir, *Lagerstroemia* × 'Natchez', *Forsythia* × *intermedia* Zab.

Significance to the Industry: Parboiled rice hulls (PBH) and coconut coir (CC) were evaluated as substrate alternatives to peat moss (PM) and perlite (PER) in the rooting of semi-hardwood cuttings from two woody ornamentals. Based on rooting percentages and number of roots per cutting, PM was the leading substrate when used alone or when combined with PER. Coconut coir and PBH also appear to be good rooting substrates when combined with PER and PM, respectively.

Nature of work: While peat moss and perlite are considered staple substrates in cutting propagation, coconut coir and rice hulls have been evaluated as potential alternatives. Limited research has been conducted on the use of fresh (6), burnt (7,8), composted (1,2,3), and parboiled (4) rice hulls in propagation. The use of coconut coir as a rooting substrate has also shown to increase rooting of several woody ornamentals (5).

Cutting wood from terminal shoots of 'Natchez' crapemyrtle (*Lagerstroemia* 'Natchez') and border forsythia (*Forsythia* ×*intermedia* Zab.) was collected on 26 May 2009 and 5 May 2010 from stock plants grown in full sunlight. Cutting wood was wrapped in moist paper towels and held in a cooler until cuttings were prepared. On 27 May 2009 and 6 May 2010 cuttings were prepared by stripping basal leaves and trimming the cuttings to a finished length of 8-11 cm (4-5 nodes) and 8-10 cm (3-4 nodes), respectively, for forsythia and crapemyrtle. Prior to sticking the cuttings, the basal 3.5 cm of the cuttings was dipped in Schultz TakeRoot Rooting Hormone talc (Schultz Co., St. Louis, MO; 0.1% indole-3-butyric acid) and inserted into one of eight propagation substrates.

Substrates were horticultural coarse-grade perlite (PER; Scotts Miracle Grow, Marysville, OH), peat moss (PM; Majestic Earth, Agawam, MA), coconut coir (CC; AgroCoir, Agrococo, Laguna Niguel, CA; initial EC = 0.5 mmhos/cm) and parboiled rice hulls (PBH; Riceland Foods, Stuttgart, AR). Substrates were used individually or in combination (1:1 by volume) as listed in Table 1. Cuttings were stuck in 38-cell plastic trays (5.5 cm top ID and 3.8 cm bottom ID and 5.8 cm height) to a depth of 4 cm. Trays were placed under intermittent mist in a poly covered greenhouse with 50% black shade cloth. The greenhouse temperature was maintained between 70° F and 90° F. The mist cycle was controlled by an electronic leaf (Phytotronics, Inc., Earth City, MO) with an average cycle of 15 sec/6 min during 24 hours.

Rooting results for crapemyrtle and forsythia were evaluated on 29 July 2009 and 13 July 2010 (62 days after sticking). Rooting substrate was carefully removed from the cuttings. Rooting performance was assessed by measuring the length of the longest root (RL) per cutting and the number of roots per cutting. Roots were excised from the cutting using a razor blade and weighed; root fresh weight (RFW) was also used to evaluate rooting performance. Moreover, cutting mortality was monitored during the rooting period. Substrate pH was determined in substrates used at planting and at harvest using the saturated paste method.

The treatment design for the rooting responses was an 8 × 2 × 2 factorial with eight substrates, two species, and two years. The experimental design was a completely randomized design with nine cutting replications per treatment combination (n = 9). Data were subjected to ANOVA and means were separated by Tukey's HSD. All data were analyzed with JMP 8 (SAS Institute, Inc., Cary, NC).

Results and Discussion: During the first year of the experiment, substrate pH resulted in a general decrease during the rooting period (Table 1); the exceptions to this were PER and PER:PM in which substrate pH increased. Results for the second year were quite surprising in that, in general, pH increased during the rooting period.

For all substrates, mean number of roots per cutting were significantly greater with forsythia than crapemyrtle (Table 2). For forsythia, all PM-based substrates yielded a greater number of roots per cutting compared to substrates with only PER or PBH. Rooting substrate had no effect on the number of roots per cutting for crapemyrtle. Cuttings from PER:PM substrate yielded significantly longer roots (17.4 cm) than those grown in only PER, or in PBH- or CC-based substrates (≤ 13.8 cm).

For each substrate, results for RFW were the same for both years (Table 3). However, differences among substrates for each year suggested that in 2009 all PM-based substrates yielded a greater root mass than substrate with only PER. In 2010, substrate with PER:PM yielded significantly greater root mass (1293 g) compared to those grown in only PER, PBH- or CC- based substrates (≤ 638 g). Results from the species by substrate interaction also suggested that substrate with PER:PM yielded greater RFW than when PER was used alone, regardless of plant species. Other differences in RFW between species are most likely related to the previously mentioned smaller number of roots per cuttings in crapemyrtle compared to forsythia.

Based on the rooting percentage and the number of roots per cutting under these rooting conditions, PM continues to be a very good rooting substrate. This is somewhat surprising considering the low substrate pH (approximately 3.5). Based on these same criteria, PER:PM and CC:PER also appear to be good rooting substrates under these rooting conditions. Parboiled rice hulls appear to be a suitable rooting substrate when combined with peat moss.

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Table 1. Initial and final pH substrates evaluated on the rooting of forsythia and crapemyrtle cuttings.[z]

Substrate[y]	2009			2010		
	Initial	Final		Initial	Final	
		Crapemyrtle	Forsythia		Crapemyrtle	Forsythia
PER	6.1	7.1	7.1	5.4	5.4	5.1
PER:PM	3.5	3.7	3.8	3.5	5.0	4.9
PM	3.6	3.4	3.3	3.5	4.5	4.5
PM:PBH	4.4	4.1	4.1	4.1	4.7	4.5
PBH	6.2	6.0	6.1	6.3	5.8	5.8
PBH:CC	6.1	5.3	5.6	5.9	6.0	6.1
CC	6.1	5.7	5.6	5.6	5.6	6.0
CC:PER	5.6	5.3	5.6	5.6	6.1	5.9

[z]Substrate pH was measured using saturated paste method.
[y]Substrates were used individually or in combination (1:1 by volume). PER = Perlite; PM = Peatmoss; PBH = Parboiled rice hulls; CC = Coconut Coir.

Table 2. Effect of rooting substrate on mean number of roots per cutting for forsythia and crapemyrtle averaged over two years (2009 and 2010).

Substrate[z]	Mean no. of roots	
	Forsythia	Crapemyrtle
PER	8 cd[y]	4 e
PER:PM	13 a	5 de
PM	14 a	5 de
PM:PBH	11 ab	5 de
PBH	8 cd	4 e
PBH:CC	9 bc	4 e
CC	10 bc	4 de
CC:PER	11 abc	4 de

[z]Substrates were used individually or in combination (1:1 by volume). PER = Perlite; PM = Peatmoss; PBH = Parboiled rice hulls; CC = Coconut Coir.
[y]Similar letters were not significantly different at $P = 0.05$ using Tukey's HSD test.

Table 3. Effect of rooting substrate on root fresh weight for forsythia and crapemyrtle.

Substrate[z]	Root fresh weight (g)			
	Year[y]		Species[y]	
	2009	2010	Crapemyrtle	Forsythia
PER	449 d[y]	446 d	451 d	464 d
PER:PM	993 ab	1293 a	915 bc	1371 a
PM	996 ab	876 abcd	833 bcd	1038 ab
PM:PBH	948 abc	486 cd	510 cd	924 bc
PBH	687 bcd	585 bcd	562 bcd	710 bcd
PBH:CC	680 bcd	517 cd	453 cd	744 bcd
CC	593 bcd	505 cd	382 d	715 bcd
PER:CC	858 bcd	638 bcd	448 d	1048 ab

[z]Substrates were used individually or in combination (1:1 by volume). PER = Perlite; PM = Peatmoss; PBH = Parboiled rice hulls; CC = Coconut Coir.
[y]Similar letters were not significantly different at $P = 0.05$ using Tukey's HSD test.

Direct Seed Germination Methods for Assessing Phytotoxicity of Alternative Substrates

Anthony L. Witcher[1], Eugene K. Blythe[2], Glenn B. Fain[3], Kenneth J. Curry[4], and James M. Spiers[1]

[1]USDA-ARS Southern Horticultural Laboratory, Poplarville, MS 39470
[2]Coastal Research and Extension Center, Mississippi State University, South Mississippi Branch Experiment Station, Poplarville, MS 39470
[3]Auburn University, Department of Horticulture, Auburn, AL 36849
[4]University of Southern Mississippi, Department of Biological Sciences, Hattiesburg, MS 39406

anthony.witcher@ars.usda.gov

Index Words: propagation media, *Pinus taeda*, whole pine tree

Significance to Industry: Whole pine tree (WPT) substrates can be used for horticulture crop propagation and production, although optimum plant growth may require using increased fertilizer rates and/or storing substrates for a period of time before use. Phytotoxicity associated with chemical compounds in pine trees could also affect plant development. This study demonstrated fresh pine needles negatively affected seed germination and initial root growth of sensitive plant species. Detrimental effects were less pronounced between aged and fresh WPT. The methods used in this study could be valuable for quickly assessing potential phytotoxicity of alternative substrates.

Nature of Work: Horticulture crop producers have increasing access to materials not traditionally used as container substrates. Composted materials are used to improve substrate chemical properties and are a source of essential plant nutrients, while wood-based materials can provide improved substrate physical properties and be consistently processed from various tree species (2, 8, 10). Reduced plant growth in wood-based substrates, compared with traditional substrates, has been overcome with increased fertilizer rates (5). Greater plant growth has been reported in aged WPT compared with fresh WPT (4), while less total root length has been reported for stem cuttings rooted in WPT compared with PB (9). A variety of factors have been attributed to reduced plant growth in WPT substrates including nitrogen immobilization, particle size distribution, and reduced cation exchange capacity (3, 11). Phytotoxicity, associated with certain organic compounds found in pine trees, could be another factor contributing to differences in plant development among WPT and traditional substrates (7).

Seed germination and seedling growth tests are used extensively to assess potential phytotoxicity. Such biological tests are less expensive and more practical than chemical analyses and have been adapted for various applications including determining compost maturity and identifying allelochemical activity and possible soil contamination

(1, 6). Ideally, a biological test would involve direct contact between the seed and the substrate or substrate solution, similar to the interaction that would occur in a production environment. The objective of our research was to evaluate methods for identifying potential phytotoxicity in WPT.

Two experiments were performed at the Thad Cochran Southern Horticultural Laboratory in Poplarville, MS. In Experiment 1, a Phytotoxkit™ was used to evaluate seed germination and initial root growth of three test plant species [one monocot: sorghum (*Sorghum saccharatum*) and two dicots: cress (*Lepidium sativum*) and mustard (*Sinapis alba*)] in five treatment substrates and a reference soil (RS). The Phytotoxkit™ is a rapid, reproducible test designed for direct contact of seed with substrate solution, yet allows for direct observation and root measurement of germinated seeds. Treatment substrates included aged (WPTA) and fresh (WPTF) whole pine tree, aged (PNA) and fresh (PNF) pine needles, and saline pine bark (SPB).

Whole pine tree substrates were produced from 8- to 10-inch loblolly pine (*Pinus taeda*) trees harvested and chipped on Sept. 29, 2009 (WPTA) and May 26, 2010 (WPTF) in Macon County, AL, then ground with a Williams Crusher hammer mill (Meteor Mill #40, Williams Patent Crusher and Pulverizer Co., Inc St. Louis, MO) to pass a 3/8-inch screen. Pine needles were collected from a 12-year-old loblolly pine plantation in Stone County, MS, either directly from trees (PNF) or from the ground (PNA) surrounding the same trees. Pine needles were hammer-milled (model 30; C.S.
Bell Co., Tiffin, OH) to pass a 3/16-inch (PNA) or 1/4-inch (PNF) screen. Saline pine bark, pine bark soaked in a sodium chloride (NaCl) solution (30 mS/cm for mustard or 16 mS/cm for cress and sorghum), was included to produce a negative effect on seed germination and initial root growth.

Substrates were passed through a 2-mm sieve to eliminate coarse particles. Three 95-ml samples (loosely filled) were collected in a coffee-filter-lined container (T.O. Plastics SVD-250) for each substrate, bottom-saturated to the upper substrate surface with deionized water (NaCl solution used for SPB) for 1 hour, drained and transferred to individual test plates, and covered with filter paper onto which 10 seeds of a test species were placed in a single row. A clear plastic cover was placed on each test plate, then test plates were incubated vertically in a dark growth chamber at 77°F for 4 (cress) or 5 (mustard and sorghum) days. Data collected included germination percentage and root length (mm), and percent inhibition of germination and root growth was calculated for each substrate compared with RS [percent inhibition = (A – B / A)*100; A = mean germination or root length in RS; B = mean germination or root length in test substrate].

In Experiment 2, a traditional seedling growth test was used to evaluate seedling root growth of two test plant species [lettuce (*Lactuca sativa*) and tomato (*Solanum lycopersicon*)] in four substrates. Substrates included WPTA, WPTF, a peat-lite (PL) substrate (3:1:1 peatmoss:perlite:vermiculite), and pine bark (PB). Individual cells (cut from 72-cell propagation trays) 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. Trays were grouped by species and placed in separate growth chambers at 77°F with no light until germination occurred, thereafter receiving a 14-hour light and 10-hour dark photoperiod. Seedlings were thinned to 1 per cell at 8 days after sowing. At 35 (tomato) and 39 (lettuce) days after sowing, roots were washed and digitally scanned for analysis using WinRhizo software to obtain total root length. Root length data were analyzed with analysis of variance using the GLIMMIX procedure of SAS (SAS Version 9.1.3; SAS Institute, Inc., Cary, NC). Differences between treatment means were determined using the simulation step-down method.

Results and Discussion: In Experiment 1, cress and sorghum germination percentage was lowest in PNF, while mustard had 100% germination in all substrates except SPB (43%) (Table 1). Compared with RS, PNF inhibited germination by 90% in cress and 18% in sorghum, while PNA inhibited germination by 3.5% for both species. The greatest root length for cress and sorghum occurred in WPTA, yet root length was greatest in WPTF for mustard. Compared with RS in cress, PNF and PNA attained 97% and 10% inhibition of root growth, respectively. Sorghum root growth inhibition was 48% (PNF) and 39% (PNA) compared with RS. Percent root growth inhibition was actually negative in WPTA for cress and sorghum, although it was negative in WPTF for mustard. Initial substrate pH ranged from 4.8 (PNA) to 5.7 (WPTF).

In Experiment 2, total root length was greatest in PL and least in WPTA for tomato and lettuce (Table 2). Tomato total root length was 12% greater in WPTF and PB compared with WPTA. Lettuce total root length was 16% and 8% greater in WPTF and PB, respectively, compared with WPTA. Tomato total root length was 2.5 times greater in PL compared with WPTA, and 4.5 times greater in PL compared with WPTA for lettuce. Initial substrate pH ranged from 4.6 (PL) to 6.1 (WPTA).

The Phytotoxkit™ provided the most sensitive test for phytotoxicity, although the seedling growth test could be used for a more practical assessment of potential phytotoxicity for alternative substrates under typical production practices. In both tests, root growth was a more sensitive indicator of phytotoxicity compared with germination. Obviously, one or more chemical compounds present in pine needles can be phytotoxic to certain plant species during the early stages of root development following germination. Future research will include a more complete chemical analysis of substrate treatments to identify potential phytotoxic compounds, and methods for overcoming such issues will be evaluated.

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Table 1. Mean seed germination percentage and root length of three plant species as indicators of potential phytotoxicity. Percent inhibition of seed germination and root growth in test substrate were compared with a reference soil supplied with the test kit.

Species	Substrate	Germination (%)	Root Length (mm)	Inhibition[z]	
				Germination (%)	Root Growth (%)
Cress	Saline Pine Bark[y]	20	1.9[w] b	79.3	85.0
	Fresh Pine Needles	10	2.1 b	89.7	97.1
	Aged Pine Needles	93	39.2 a	3.5	10.2
	Fresh WPT[x]	90	46.5 a	6.9	4.0
	Aged WPT	97	51.0 a	0.0	-30.6
	Reference Soil	97	42.9 a	-	-
Mustard	Saline Pine Bark	43	1.4 c	56.7	98.0
	Fresh Pine Needles	100	42.0 ab	0.0	22.7
	Aged Pine Needles	100	29.4 b	0.0	44.9
	Fresh WPT	100	61.0 a	0.0	-18.1
	Aged WPT	100	36.3 ab	0.0	21.0
	Reference Soil	100	50.5 ab	-	-
Sorghum	Saline Pine Bark	83	45.1 c	10.7	45.8
	Fresh Pine Needles	77	33.4 c	17.8	48.4
	Aged Pine Needles	90	51.2 bc	3.5	38.9
	Fresh WPT	83	55.5 abc	10.7	41.3
	Aged WPT	93	92.2 a	0.0	-3.2
	Reference Soil	93	88.8 ab	-	-

[z]Percent inhibition = (A – B / A)*100; A = mean germination or root length in reference soil; B = mean germination or root length in test substrate.
[y]Pine bark soaked in a saline solution.
[x]Processed whole pine tree.
[w]Means followed by different letters within columns indicate significant difference at $P < 0.05$ using the simulation step-down method.

Table 2. Mean total root length of lettuce and tomato seedlings.

	Total Root Length (cm)	
Substrate	Lettuce	Tomato
Peat-lite[z]	223.7[y] a	178.7 a
Pine bark	43.3 b	82.2 b
Fresh WPT[x]	46.8 b	85.1 b
Aged WPT	42.5 b	80.7 b

[z]Peat-lite = 3:1:1 peatmoss:perlite:vermiculite
[y]Means followed by different letters within columns indicate significant difference at *P* <0.05 using the simulation step-down method.
[x]Processed whole pine tree