

# **Propagation**

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## Propagation of Gordonieae Trees by Hardwood Stem Cuttings

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**Index Words:** *Franklinia alatamaha*, *Gordonia lasianthus*, *Schima wallichii*, *Schima khasiana*, Potassium Salt of Indolebutyric Acid

**Significance to Industry:** Trees in the Theaceae tribe Gordonieae have exceptional ornamental merit and considerable potential for breeding and improvement, but some taxa can be difficult to propagate from stem cuttings. Hardwood stem cuttings of *Franklinia alatamaha*, *Gordonia lasianthus*, *Schima wallichii*, and *Schima khasiana* were successfully rooted with rooting percentages ranging from 23 to 69%. There was no benefit of K-IBA application (2500, 5000, 7500 or 10000 ppm) on rooting percentage for *Schima* spp., and rooting percentages of *F. alatamaha* and *G. lasianthus* were reduced at K-IBA concentrations > 2500 ppm.

**Nature of Work:** The Theaceae tribe Gordonieae contains three genera of trees (6). Two of these, *Franklinia* (Bart. ex Marshall) and *Gordonia* (L.) Ellis, are native to warm temperate and subtropical regions of the New World (including the southeastern U.S.) while the third, *Schima* (Reinw. ex Bl.), is restricted to warm temperate to tropical regions of the Old World. All three genera have large white flowers that vary in bloom time from mid-summer to early fall (4,8). Each genus has desirable foliage characteristics including bright red fall foliage of *Franklinia alatamaha* (Bart. ex Marshall) (4), evergreen and sometimes variegated foliage of *Gordonia lasianthus* (L.) Ellis (4,7), and glossy, bright red new foliage of some *Schima* species (5). Gordonieae trees have also been shown to be adaptable to a wide range of environmental conditions. *F. alatamaha* has been reported to be cold-hardy at temperatures as low as -36°F (-38°C) (2) and *Schima* can tolerate soils that are dry (10), wet (5), or infertile (1).

Previous work on vegetative propagation of these genera is very limited, particularly in regard to hardwood stem cuttings. For *F. alatamaha*, Dirr and Heuser (3) recommended that softwood stem cuttings be taken from June to August and treated with a basal dip of 1000 ppm IBA solution. Another study on softwood cutting propagation of *F. alatamaha* indicated that IBA solutions at concentrations lower than 1000 ppm were not as effective and that concentrations higher than 1000 ppm actually inhibited rooting (9). For *G. lasianthus*, Dirr and Heuser (3) indicated that cuttings may be taken in March or from June until August and treated with a 3000 ppm IBA solution. There is no published work on vegetative propagation of *Schima*, but preliminary findings have indicated that *Schima* spp. can be difficult to root from softwood stem cuttings (Ranney, personal observation).

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In this study, terminal, hardwood stem cuttings from *F. alatamaha*, *G. lasianthus*, *S. wallichii*, and *S. khasiana* were collected on February 1, 2008. Cuttings from each taxon were trimmed to 3 to 4 in. (7.5 to 10 cm) in length and the basal 0.4 in. (1 cm) was dipped in either 0, 2500, 5000, 7500 or 10000 ppm of the potassium salt of indolebutyric acid (K-IBA) dissolved in water. The cuttings were then inserted 0.4 in. (1 cm) in plastic flats (15.75 in L x 15.75 in W x 6.0 in D) filled with a rooting substrate of 2 peat:3 perlite (v/v). Stem cuttings were misted intermittently for 8 sec every 10 minutes between 0600 and 1800 HR. The experimental design was a randomized complete block with five K-IBA treatments and 6 replicates, with each replicate consisting of 6 stem cuttings (subsamples) per treatment. Each taxon was considered a separate experiment. After twelve weeks, cuttings were harvested and percent rooting and number of roots were determined. Data were subjected to analysis of variance and regression analysis using SAS, version 9.1.

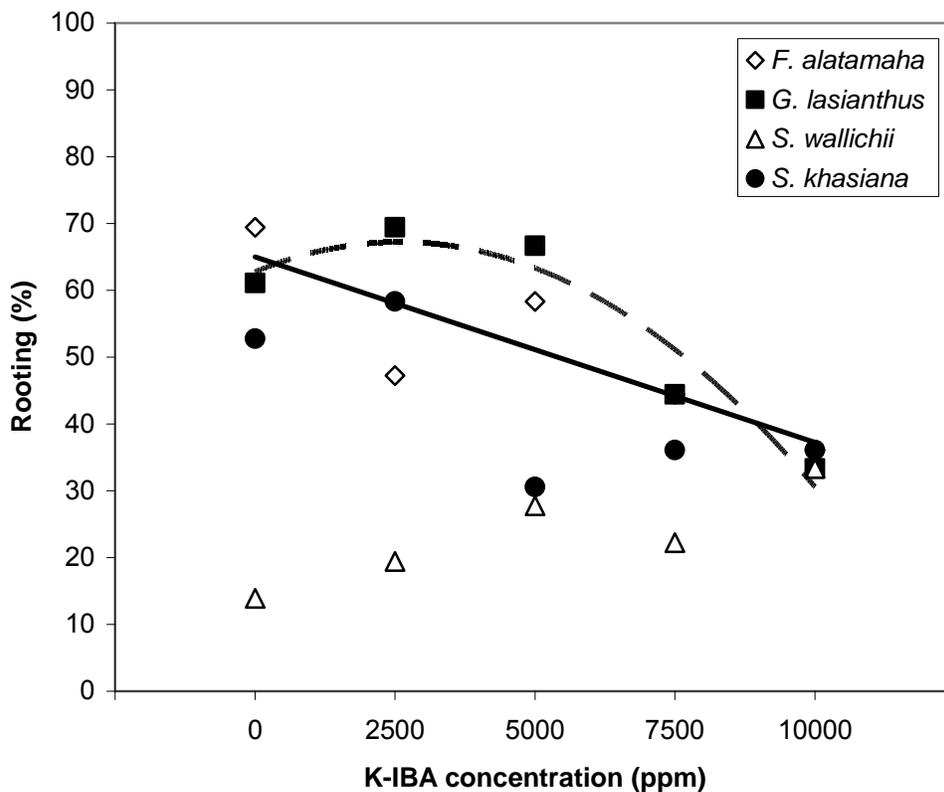
**Results and Discussion:** Rooting percentage was significantly influenced by K-IBA concentration for *F. alatamaha* and *G. lasianthus*, but not for either of the *Schima* species (Fig. 1). In *F. alatamaha*, rooting percentage had a negative linear response with increasing K-IBA concentration (Fig. 1). The highest rooting (~69%) occurred in the untreated cuttings. *G. lasianthus* demonstrated a linear and quadratic response to increasing K-IBA concentrations with a predicted maximum rooting percentage at 2500 ppm K-IBA (~69%). *S. wallichii* and *S. khasiana* had mean rooting percentages of only 23% and 43%, respectively, averaged over all K-IBA concentrations.

Root number was significantly affected by K-IBA concentration only in *S. khasiana* (Fig. 2). Rooted cuttings of *S. khasiana* had a linear and quadratic response to K-IBA concentration with the optimum of 22 roots per cutting occurring at 7500 ppm (Fig. 2). Mean root number did vary between species. *G. lasianthus* had the largest mean root number at 33, followed by *S. khasiana* (mean=15), *F. alatamaha* (mean=9), and *S. wallichii* (mean=4). Research is continuing to evaluate responses to K-IBA in softwood and semihardwood cuttings.

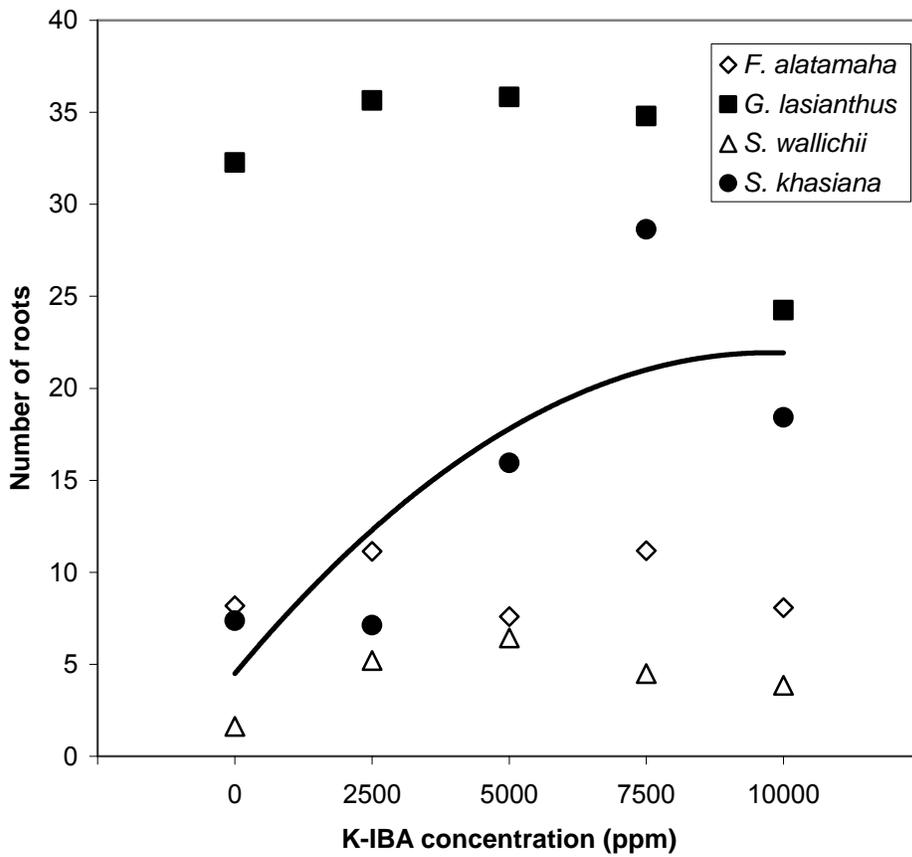
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**Figure 1.** Effect of K-IBA concentration on rooting percentage of hardwood stem cuttings of *F. alatamaha* (solid line— $R^2=0.72$ ,  $P=0.01$ ), *G. lasianthus* (broken line— $R^2=0.93$ ,  $P=0.07$ ), *S. wallichii*, and *S. khasiana*.



**Figure 2.** Effect of K-IBA concentration on number of roots of hardwood stem cuttings of *F. alatamaha*, *G. lasianthus*, *S. wallichii*, and *S. khasiana* (solid line,  $R^2=0.43$ ,  $P< 0.05$ ).

## **Vegetative Propagation of Oconee Azalea (*Rhododendron flammeum*)**

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**Index Words:** Deciduous Azalea, Mound Layering, K-IBA, Timing, Wounding

**Significance to Industry:** The native, Eastern North American deciduous azaleas (*Rhododendron* spp.) possess a plethora of ornamental characteristics in flower form, duration, color, timing, and fragrance. Adaptability and landscape merit are also found within the group. Stem cutting propagation is the principle method for mass production of clonally derived plant material, however, vegetative propagation of many of the deciduous azaleas native to the Eastern United States has been difficult (1). The utility of these plants in the landscape on a commercial scale is dependent upon reliable, productive propagation protocols.

**Nature of Work:** Mound layering is a method of in-field propagation whereby stock plants are hedged severely and emerging shoots are covered with substrate allowing for adventitious root formation. Subsequent roots grow into the surrounding substrate and rooted stems can then be severed from the stock plant. The severe pruning helps to maintain vegetative, juvenile growth that typically has a higher capacity for adventitious root formation (3). Covering the shoots also results in etiolation, which can decrease the light-induced breakdown of endogenous indole acetic acid (IAA) and retard tissue differentiation, resulting in more parenchyma cells with greater potential for root initiation and development (2). Wounding, an application of a rooting hormone, or a combination of both can be used during mounding to increase rooting percentages (4). Mounding is a viable option for propagating difficult-to-root plants and is utilized extensively with temperate fruit trees and *Aesculus* species (5, 6). This technique also lends itself to mechanization in field situations. Upright habit and the ability to produce many new shoots following pruning are characteristics of plants that could be successfully propagated by mound layering (3). The objective of this study was to evaluate the potential of successful propagation of *Rhododendron flammeum* (Michx.) Sarg., Oconee azalea, by mound layering and to determine the effects of timing of mounding, wounding or the application of the potassium salt of indole butyric acid (K-IBA) on rooting percentage, the number of plants produced, and root system quality.

Three gallon plants (12l) were field planted in fall 2005 and pruned to 6 in (15 cm) above the root collar the following March 2006. Plants were then mounded either in mid-March or mid-June. Mounding consisted of covering plants with 18 in (46 cm) of composted pine bark that was held in place by a 24 in (61 cm) diameter cylinder constructed from chicken wire. Prior to mounding in June, stems on each plant were

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either wounded or not wounded and treated or not treated with 5,000 ppm K-IBA using a spray bottle, which represented a 2 × 2 factorial.

All shoots were harvested in March of the subsequent year and evaluated for rooting percentage, number of rooted plants produced per mound, root collar diameter (RCD), relative root score, and root system symmetry. Root system quality was analyzed using the RCD, root score, and root system symmetry measurements. Root collar diameter was measured at the stem to root interface using a caliper. A relative root score was based visually on size of the root ball with small roots systems receiving a 0, intermediate sized root systems receiving a 1, and large root systems receiving a 2 (Fig. 1). Symmetrical root systems had at least two roots 130° apart around the stem (Fig. 1). The experimental design was a randomized complete block design with 5 blocks of 10 plants. The treatments were randomly applied to pairs within the blocks with one set of two being mounded in early March and the other 4 sets of 2 pairs (8 plants total per block) being mounded in June. The experiment was repeated over two years and the data presented represents harvests from 2007 and 2008.

**Results and Discussion:** Mound layering of *Rhododendron flammeum* was successful for both the March and June mounding times. Rooting percentage and the number of rooted plants produced per mound were not significantly different for either mounding time (Table 1.). Additionally, within the June mounding time, neither rooting percentage nor number of rooted plants was affected significantly by wounding, K-IBA application, or their interaction (Table 1.). Regardless of mounding season or treatment, mound layering had a 50% success rate that produced around 6 rooted plants per mound.

Similarly to the rooting percentages and number of rooted plants, there was no effect of timing, wounding, or K-IBA application on root system symmetry (Table 2.). Approximately 60% of the root systems of successfully propagated plants were found to be symmetrical. Significant differences were observed between the March and June mounding times for root collar diameter and relative root scores (Table 2.). March mounding resulted in rooted plants with an average RCD of 5.3 mm and a root score of 1. Plants in the June mounding treatment had a mean RCD of 4.6 mm and a root score of 0.80. Within the June mounding time, RCD was affected by the interaction of wounding and K-IBA treatment. Relative root score was unaffected. Wounding plus K-IBA spray application resulted in a mean RCD of 4.2 mm, which is 0.4 mm less than the overall mean RCD of June mounded plants, and may not be biologically significant because wounding or K-IBA did not affect any other variables measured.

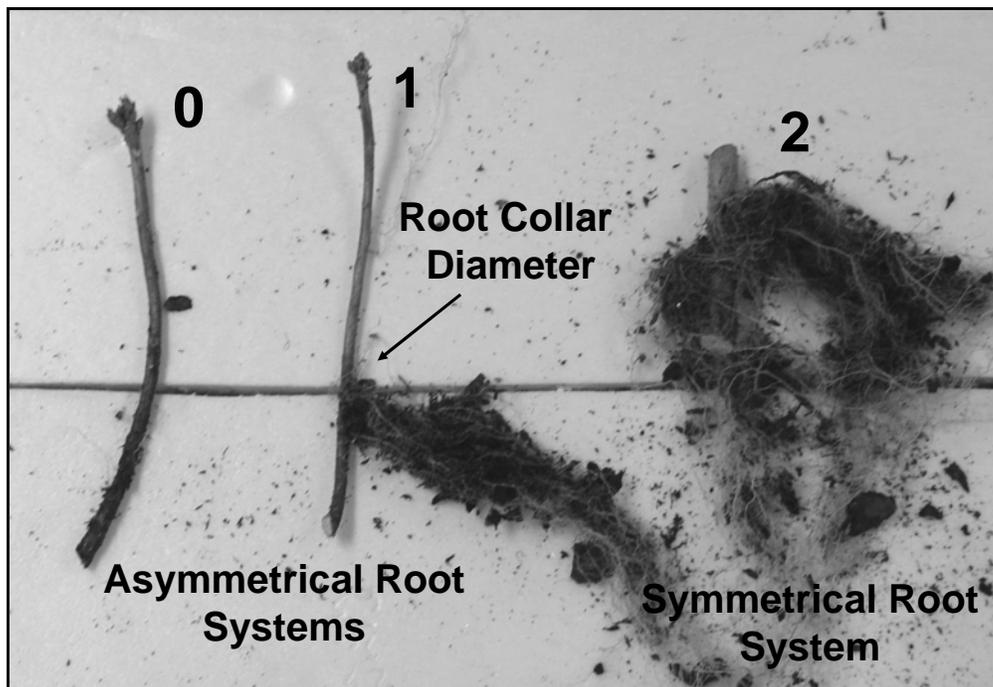
The results of this study indicate mound layering is a practical approach for successful propagation of Oconee azalea. Both mounding times were effective and the use of wounding, K-IBA applications, or both is not necessary to improve rooting percentages, number of rooted plants, or percentage of symmetrical root systems. The earlier March mounding time did allow for slightly larger root collar diameters and visually superior root systems. This effect is probably attributed to a longer root development time throughout the year when compared to the later June mounding time. For the grower,

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this information provides flexible timing in the in-field propagation of *Rhododendron flammeum*. Because mounding in March on a yearly basis might prove stressful for plants, growers can alternate mounding times during production to allow for plants to recover between mounding. Plants could be mounded in March during year one, but be mounded in June during year two.

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**Figure 1.** Root system quality for *Rhododendron flammeum* illustrating relative root score (0, 1, or 2), root collar diameter (RCD), and determination of root system symmetry (symmetrical root systems have two or more roots at least 130 degrees apart).

**Table 1.** Rooting percentage and number of plants produced per mound for March and June mounding treatments of *Rhododendron flammeum*.

Treatment	Rooting %	Number Rooted <sup>1</sup>
March Mounding	48.4±8.3 A	7.3±1.9 A
June Mounding	50.5±4.3 A	5.7±0.7 A
Wounding and K-IBA	60.0±9.1 a	5.6±1.1 a
No Wounding and No K-IBA	44.7±9.3 a	4.9±1.5 a
Wounding and No K-IBA	53.3±9.1 a	7.3±1.6 a
No Wounding and K-IBA	45.4±7.5 a	5.3±1.2 a

<sup>1</sup>Values represent means ± 1 SEM for 10 replications (5 replications × 2 years). Means followed by a different letter, within columns, represent significant differences at  $P<0.05$ . Upper case (A) within a column denotes comparison between March and June mounding, whereas lower case (a) denotes comparisons between wounding and K-IBA treatments during June mounding.

**Table 2.** Root system quality of *Rhododendron flammeum* indicated by root system symmetry, root collar diameter, and relative root score (see Figure 1 for visual descriptions).

Treatment	Symmetry <sup>1</sup>	RCD <sup>2</sup> (mm)	Root Score
March Mounding	0.59±0.05 A	5.3±0.3 A	1.0±0.1 A
June Mounding	0.60±0.03 A	4.6±0.1 B	0.8±0.0 B
Wounding and K-IBA	0.54±0.05 a	4.2±0.2 b	0.8±0.1 a
No Wounding and No K-IBA	0.60±0.05 a	4.7±0.2 ab	0.7±0.1 a
Wounding and No K-IBA	0.66±0.04 a	4.7±0.2 a	0.9±0.1 a
No Wounding and K-IBA	0.58±0.06 a	4.8±0.3 a	0.8±0.1 a

<sup>1</sup>Values represent means ± 1 SEM for 10 replications (5 replications × 2 years). Means followed by a different letter, within columns, represent significant differences at  $P<0.05$ . Upper case (A) within a column denotes comparison between March and June mounding, whereas lower case (a) denotes comparisons between wounding and K-IBA treatments during June mounding.

<sup>2</sup>RCD = Root collar diameter.

## Effect of Preemergence Herbicide Application in Containerized Rootstock on Grafting Success of Various Woody Ornamental Species

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**Index words:** vegetative propagation, plant-production, weed control

**Significance to Industry:** Historically, growers have been reluctant to use preemergence herbicides in containerized rootstocks prior to grafting, because herbicides are thought to affect grafting success. Four common preemergence herbicides were applied to various rootstocks during an 8-9 month production cycle prior to winter bench grafting. Subsequent grafting success was not affected. This information will allow propagators to control weeds during production without reducing grafting success.

**Introduction:** Application of preemergence herbicides to containerized rootstock designated for dormant winter bench grafting is not a common practice for grafters. When rootstocks are brought into a covered structure mid-winter for proper pre-grafting care, weed seeds can germinate and affect grafting efficiency (2). If plants are kept for a second growing season prior to or after grafting, weed populations in containers can become unmanageable. Applying preemergence herbicides during grafting is not reported in Garner (1), Macdonald (2) or in other literature. Therefore, this report investigated the effect of preemergence herbicide application on subsequent grafting success when these herbicides were applied to containerized rootstocks during production.

Two experiments were conducted over two years to study the effect of four common preemergence herbicides on grafting success of 6 common woody ornamental species. Seedlings of *Cercis canadensis* L. redbud, *Acer palmatum* Thunb. Japanese maple, *Ginkgo biloba* L. Ginkgo, *Hamamelis virginiana* L. witch-hazel, *Styphnolobium japonicum* (L.) Schott. (formerly *Sophora japonica* L.) Japanese pagoda tree, and *Ulmus alata* Michx. (winged elm) with root collar diameters approximately 3/16 in (0.48 cm) were obtained in February-March 2006 (Experiment 1) and 2007 (Experiment 2). Plants were potted in either 2 7/8 in (7.1 cm) x 2 7/8 in (7.1 cm) x 5 1/2 in (14.0 cm) (*A. palmatum*, *G. biloba*, *H. virginiana*, *S. japonicum*, and *U. alata*), or 3 5/8 in (9.2 cm) x 3 5/8 in (9.2 cm) x 6 in (15.2 cm) (*C. canadensis* Experiment 1) plastic containers (Anderson Die and Manufacturing Co., Portland, Ore.) or #1 containers (3.79 l) (*C. canadensis* Experiment 2) containing a substrate of composted pine bark amended with 2 lbs/yd<sup>3</sup> (0.69 kg/m<sup>3</sup>) dolomitic limestone and 10 lbs/yd<sup>3</sup> (3.45 kg/m<sup>3</sup>) alfalfa meal. After potting, plants were side-dressed with 0.28 oz (8 g) (or 15 g for a #1 containers) of a commercial controlled-release fertilizer (18:6:8 N,P,K; Nutricote, 5-6-month, Sun-Gro Horticulture, Canada) and placed in a cold frame covered with white polyethylene.

Snapshot 2.5TG (isoxaben + trifluralin) (Dow Agro Sciences LLC, Indianapolis, Indiana) at 5.6 kg ai/ha (5.0 lbs ai/A), Scott's OH2 3G (pendimethalin + oxyfluorfen) (Scotts-Sierra Crop Protection Co. Marysville, Ohio) at 3.4 kg ai/ha (3.0 lbs ai/A), Ronstar (oxadiazon) (Bayer Crop Science Inc Calgary, Alberta) at 4.5 kg ai/ha (4.0 lbs ai/A), or Sureguard (flumioxazin) (Valent U.S.A. Corporation, Walnut Creek, Calif.) at 0.43 kg ai/ha (0.38 lbs ai/A) in Experiment 1 [Broadstar (flumioxazin) (Valent U.S.A. Corporation, Walnut Creek, Calif.) in Experiment 2] were surface applied using either a shaker jar or solo backpack sprayer (Sureguard only). Herbicides were applied eight weeks apart for four applications in Experiment 1 and three applications in Experiment 2. Herbicides were watered in after treatment. All herbicide treatments were compared to nontreated plants. During the growing season plants were under 40% shade cloth and grown according to general nursery practices (4). In November of each year, plants were placed in an over-wintering structure covered with white polyethylene (3 mil). The experimental design was a randomized complete block with a factorial arrangement of treatments in each of seven blocks. Treatments in each block contained three plants per species (7 replications x 5 treatments x 3 plants per species in each treatment = 105 plants per species). Species were considered separate experiments and grown and analyzed separately.

In December of each year, plants were brought into an unshaded greenhouse structure and prepared for grafting (4). Scion wood of *Hamamelis x intermedia* 'Primavera' witch-hazel, *Ginkgo biloba* 'Autumn Gold' Ginkgo (Experiment 1 only), *Acer palmatum* 'Tamuke Yama' Japanese maple, *Ulmus alata* 'Lace Parasol' winged elm, *Styphnolobium japonicum* 'Pendulum' weeping Japanese pagoda tree, and *Cercis canadensis* 'Hearts of Gold' PPAF redbud approximately 4-6 in (10.2-15.2 cm) long and possessing 3-5 nodes was grafted to its respective genera of seedling rootstock in winter using a modified side-veneer graft (4). Grafts were considered successful if leaves on new growth had fully expanded on or after April 10 each year. Data were subjected to analysis of variance (ANOVA) using SAS v 9.1 (3).

**Results and Discussion:** Grafting success in either experiment was not affected significantly by the application of four preemergence herbicides to containerized rootstock (ANOVA not presented) (Table 1). Grafting success was affected significantly by experiment for 'Hearts of Gold' redbud and 'Lace Parasol' winged elm. Mean success was 90% in Experiment 1 and 62.4% in Experiment 2 for 'Hearts of Gold' redbud. For 'Lace Parasol' elm, grafting success was 96% in Experiment 1 and 82.4% in Experiment 2. For either cultivar, there was not a significant treatment by experiment interaction, indicating that overall grafting success was lower in Experiment 2 for these species. Therefore, mean grafting success over both experiments was 76.9% for 'Hearts of Gold' redbud and 88.5% for 'Lace Parasol' elm, regardless of which preemergence herbicide was applied to containerized rootstocks in production prior to grafting (Table 1).

These findings indicate that preemergence herbicides can be used in containerized rootstock to control weed populations prior to grafting. The six cultivars tested represent a major portion of ornamental species bench-grafted in the nursery industry.

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Similarly, the active ingredients tested represent a large portion of the preemergence herbicides applied to containerized nursery stock. By following the methods herein, as well as the label recommendations for the herbicides tested, growers can use preemergence herbicides on an operational scale in rootstocks of the species tested. When implementing preemergence herbicides into any production system, growers are cautioned to test the herbicides on a small scale first prior to widespread use in grafting production.

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**Table 1.** Percent grafting success of six cultivars for two experiments. Rootstocks of cultivars were treated during establishment prior to grafting with nothing (untreated control) or four preemergence herbicides.

Preemergence Herbicide Treatments	Cultivars					
	<i>Acer palmatum</i> 'Tamuke Yama'	<i>Cercis canadensis</i> 'Heart's of Gold'	<i>Ginkgo biloba</i> 'Autumn Gold' Expt. 1 <sup>z</sup>	<i>Hamamelis x intermedia</i> 'Primavera'	<i>Styphnolobium japonicum</i> 'Pendula'	<i>Ulmus alata</i> 'Lace Parasol'
	Grafting Success (%) <sup>y</sup> (SE)					
Untreated Control	92.3 (4.0)	81.6 (6.4)	100 (0)	94.6 (3.8)	94.4 (3.9)	87.5 (5.3)
Snapshot 2.5TG (isoxaben + trifluralin)	92.9 (4.0)	82.3 (5.9)	94.7 (5.3)	95.1 (3.4)	91.7 (4.7)	85.4 (5.6)
OH2 3G (pendimethalin + oxyfluorfen)	92.5 (4.2)	79.5 (6.6)	100 (0)	97.6 (2.4)	92.1 (4.4)	94.9 (3.6)
Ronstar (oxadiazon)	90.2 (4.7)	70.3 (7.6)	100 (0)	94.9 (3.6)	95.0 (3.5)	92.9 (4.0)
Sureguard (flumioxazin) Expt. 1 <sup>w</sup>	NG <sup>x</sup>	85.7 (7.8)	95.2 (4.8)	94.4 (5.6)	NG	NG
Broadstar <sup>y</sup> (flumioxazin) Expt. 2 <sup>v</sup>	76.5 (10.6)	52.6 (11.8)	N/A	100 (0)	80.0 (9.2)	76.2 (9.5)
Mean	90.7 (2.1)	76.9 (3.0)	97.8 (1.5)	95.9 (1.4)	91.7 (2.1)	88.5 (2.4)

<sup>z</sup> *Ginkgo biloba* 'Autumn Gold' was included only in Experiment 1.

<sup>y</sup> There were no significant differences between the untreated control and any preemergence herbicide treatments.

<sup>x</sup> NG, Not grafted after an initial application of Sureguard to rootstocks prior to grafting. Death, stem burn and lesions on rootstocks were noted; as a result cultivars marked NG were not grafted onto their rootstocks in that treatment.

<sup>w</sup> Sureguard, the liquid formulation of flumioxazin, was used only in Experiment 1. (See note <sup>x</sup>)

<sup>v</sup> Broadstar, the granular form of flumioxazin, was used only in Experiment 2.

## Evaluation of the Lightweight Aggregate HydRocks<sup>®</sup> as a Rooting Substrate

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**Index words:** propagation, cuttings, IBA, rooting hormones, *Elaeagnus x ebbingei*, *Forsythia x intermedia*, *Illicium parvifolium*, *Ilex cornuta* 'Dwarf Burford', *Lagerstroemia x Natchez*'.

**Significance to the Industry:** This study evaluated HydRocks<sup>®</sup> as a propagation substrate for bare root liner production of five common woody ornamental species. Results suggest HydRocks<sup>®</sup> performs as well as conventional substrates in most cases across species used in these studies. The ability of HydRocks<sup>®</sup> to be easily removed from root systems makes HydRocks<sup>®</sup> ideal for use as a rooting and bare-rooting substrate.

**Nature of Work:** Producing bare-root liners is an alternative to the expense associated with container-grown liners. The cost of producing bare-root liners is generally less than the cost of containerized liners due to reduced weight and volume during shipping (3). Some Oregon nurseries have converted entire greenhouse floors into in-ground pumice beds where cuttings are directly stuck (2). These cuttings are easily removed from the pumice aggregate at harvest and the pumice is reused for many years without being replaced. Inorganic materials such as monolithic slag and ceramic aggregates with stable particles have been shown to be easily removed from roots after stem cutting propagation, while still producing quality rooted liners (1, 3). Expanded clays are lightweight aggregates formed by firing certain expandable clays in rotary kilns. Expanded clay aggregates could be used in place of pumice in in-ground propagation beds in areas of the country where pumice is not readily available. Like pumice, stable properties of expanded clays would allow reuse for many years without replacement, providing a more sustainable and cost effective approach to bare root production than use of conventional substrates. HydRocks<sup>®</sup> is a lightweight expanded clay aggregate currently marketed for horticulture applications (Big River Industries, Alpharetta, GA). HydRocks<sup>®</sup> is a lightweight porous aggregate formed by calcining clay at temperatures reaching 2000° F. HydRocks<sup>®</sup> is produced from several quarries in the southeast and is generally inert, pH neutral, and has a cation exchange capacity (CEC) of 8 meq/100g. HydRocks<sup>®</sup> used in this study was produced in Livingston, AL. The objective of this study was to evaluate HydRocks<sup>®</sup> as a propagation substrate for bare-root liner production of five common woody ornamental species.

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This study was conducted at the Paterson Greenhouse Complex in Auburn, Ala. On September 23, 2007, cuttings were collected from mature landscape plants in Auburn, Ala., from the following: *Elaeagnus x ebbingei*, *Forsythia x intermedia*, *Ilex cornuta* 'Dwarf Burford', *Illicium parvifolium* and *Lagerstroemia x 'Natchez'*. Semi-hardwood terminal cuttings of *Elaeagnus x ebbingei* were collected and prepared as 4-in. cuttings with a minimum of 4 leaves, wounded, and treated with 1000 ppm indolebutyric acid (IBA) + 500 ppm naphthalene acetic acid (NAA). *Lagerstroemia x 'Natchez'* cuttings were prepared as 3- to 4-in., wounded, subterminal stem cuttings with 3 leaves per cutting and were treated with 1000 ppm IBA + 500 ppm NAA. *Forsythia x intermedia* cuttings were prepared as 3-in., single-node intermediate cuttings and treated with 1000 ppm IBA + 500 ppm NAA. *Illicium parviflorum* and *Ilex cornuta* 'Dwarf Buford' terminal cuttings were prepared as 3- to 4-in. wounded cuttings and treated with 3000 ppm IBA + 1500 ppm NAA. Dip'N Grow<sup>®</sup> (Dip'N Grow Inc., Clackamas, OR) was used for all IBA + NAA formulations. All cuttings were stuck into deep 606 cell packs (12.5 in<sup>3</sup> (205 cm<sup>3</sup>) per cell) filled with the following substrate treatments: pine bark fines (screened at 0.25 in), Fafard 3B (Fafard Inc., Anderson, SC), construction grade sand, perlite, vermiculite and HydRocks<sup>®</sup> (screened to 0.19 in). Cuttings were placed under intermittent mist in a glass greenhouse. Cuttings were harvested on February 12, 2008 (150 days after sticking) and roots were rated on a quality scale of one to five (1 = non-rooted cuttings and 5 = greatest quality). Quality of roots was rated respective to the population of cuttings being rated for each species. Fresh root and shoot weights were recorded and root:shoot ratios were calculated.

The experimental design was a randomized complete block design with six treatments and six blocks. Each pack contained six cuttings (six subsamples) for each treatment. Data were analyzed using generalized linear mixed models [binomial distribution and logit link function for rooting response (presented as percent rooted); Normal distribution and identity link function for all other response variables] with the GLIMMIX procedure (June 2006 release) of SAS (Version 9.1; SAS Institute, Cary, NC). Substrate was included in the model as the fixed factor and block as a random factor. Comparison of least squares means was carried out with a multiple-comparison-adjusted significance level of 0.05 using the simulation-stepdown method.

### Results and Discussion

***Elaeagnus x ebbingei*.** Based upon the rating scale, rooting of cuttings in HydRocks<sup>®</sup> was similar to rooting in vermiculite, Fafard 3B, and perlite; cuttings in pine bark received a lower rating (Table 1). No significance differences occurred in root weight across treatments. Shoot weight of cuttings rooted in HydRocks<sup>®</sup> was similar to all treatments (Table 1).

***Forsythia x intermedia*.** Superior and similar root quality ratings were obtained with *F. x intermedia* cuttings rooted in pine bark, Fafard 3B, vermiculite, and HydRocks<sup>®</sup>, while sand and perlite produced the poorest root quality ratings (Table 1). Fresh root weights of cuttings rooted in Fafard 3B and vermiculite were similar and had high root weights. Cuttings rooted in HydRocks<sup>®</sup> were similar to cuttings in pine bark, sand and perlite in root weight. Cuttings in Fafard 3B, pine bark and vermiculite were all similar in shoot weight (Table 1). The high root and shoot weights of cuttings in Fafard 3B treatments could be attributed to its containing a starter fertilizer charge that was absent from all other treatments.

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***Ilex cornuta* 'Dwarf Burford'**. Root quality was similar for cuttings rooted in pine bark, Fafard 3B, sand, and vermiculite (Table 1). Perlite produced the lowest quality roots of any treatment. No differences among substrate treatments occurred for root dry weights. Cuttings in HydRocks<sup>®</sup> were similar to cuttings in all other treatments in shoot weight (Table 1).

***Illicium parviflorum***. Cuttings rooted in pine bark, Fafard 3B, and vermiculite were similar in rooting and had the highest root quality ratings and root weights among treatments (Table 1). Cuttings rooted in perlite were similar to cuttings rooted in sand and HydRocks<sup>®</sup> in root weight. Excluding pine bark, cuttings in HydRocks<sup>®</sup> were similar to cuttings in all other treatments in root weight. Cuttings in all treatments produced similar results in shoot weight (Table 1).

***Lagerstroemia* x 'Natchez'**. Cuttings rooted in pine bark, vermiculite, and Fafard 3B were similarly high in root quality (Table 1). Cuttings rooted in perlite and sand were low in root quality. Root weight of cuttings in HydRocks<sup>®</sup> was similar among all treatments. (Table 1).

Rooting percentages were high (84-100%) and were similar for all substrate treatments among species. HydRocks<sup>®</sup> rooted as well as conventional substrates in most cases for all species in these studies (Table 1). As a propagation substrate, HydRocks<sup>®</sup> could have greater utility over conventional substrates when ease of bare-rooting is taken into account. Use of HydRocks<sup>®</sup> in propagation has some sustainability advantages. Washing roots with high pressure water as generally used in bare-root liner production is not needed with HydRocks<sup>®</sup>. Since HydRocks<sup>®</sup> does not degrade over time, it could potentially be reused for years. The results of these studies suggest that HydRocks<sup>®</sup> can be used as a successful rooting substrate. More work is needed to determine how fertilizer and water requirements might be modified to improve growth of cuttings rooted in HydRocks<sup>®</sup>.

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Table 1. Rooting response of five woody species rooted in six different substrates.

	Rooting %	Root	Fresh Weight	
		quality rating <sup>Z</sup>	Root (g)	Shoot (g)
<i>Elaeagnus x ebbingei</i>				
Pine bark	89 a <sup>X</sup>	2.7 c <sup>W</sup>	0.60 a	1.83 b
Fafard 3B	97 a	3.6 ab	0.69 a	2.01 ab
Sand	97 a	3.0 bc	0.60 a	2.20 a
Perlite	94 a	3.6 ab	0.63 a	2.17 ab
Vermiculite	97 a	3.8 a	0.64 a	1.88 ab
HydRocks <sup>®V</sup>	94 a	3.9 a	0.85 a	2.14 ab
<i>Forsythia x intermedia</i>				
Pine bark	100 a	3.9 a	1.85 bc	1.5 abc
Fafard 3B	100 a	4.1 a	2.48 a	1.72 a
Sand	100 a	3.0 b	1.61 c	1.24 c
Perlite	100 a	2.4 b	1.74 bc	1.31 bc
Vermiculite	100 a	4.1 a	2.13 ab	1.66 ab
HydRocks <sup>®</sup>	100 a	4.0 a	1.59 c	1.32 bc
<i>Ilex cornuta</i> 'Dwarf Burford'				
Pine bark	97 a	3.7 ab	0.51 a	2.61 ab
Fafard 3B	97 a	4.1 ab	0.54 a	2.52 ab
Sand	97 a	3.6 ab	0.51 a	2.82 a
Perlite	84 a	1.9 c	0.41 a	2.72 ab
Vermiculite	97 a	4.4 a	0.53 a	2.31 b
HydRocks <sup>®</sup>	95 a	3.3 b	0.56 a	2.45 ab
<i>Illicium parviflorum</i>				
Pine bark	99 a	4.0 a	1.95 a	2.92 a
Fafard 3B	97 a	4.3 a	1.73 ab	2.75 a
Sand	97 a	3.1 bc	1.35 bc	2.65 a
Perlite	99 a	2.6 c	1.17 c	2.39 a
Vermiculite	99 a	4.3 a	1.69 ab	2.58 a
HydRocks <sup>®</sup>	99 a	3.3 b	1.45 bc	2.80 a
<i>Lagerstroemia</i> x 'Natchez'				
Pine bark	92 a	4.3 a	1.04 ab	1.77 a
Fafard 3B	94 a	4.1 ab	0.85 ab	1.63 a
Sand	95 a	3.1 cd	0.75 b	1.56 a
Perlite	86 a	2.7 d	0.89 ab	1.32 a
Vermiculite	97 a	4.4 a	1.15 a	1.53 a
HydRocks <sup>®</sup>	97 a	3.5 bc	0.85 ab	1.46 a

<sup>Z</sup>1 = non-rooted and 5 = greatest root quality.

<sup>Y</sup>Means within a column and species that are followed by the same letter are not significantly different at the 0.05 level according to the simulation-stepdown method.

<sup>X</sup>Screened to ≈ 0.19 in.

## Organogenesis from *Hypericum frondosum* leaves

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**Index Words:** Micropropagation, Propagation

**Significance to Industry:** The genus *Hypericum* contains approximately 370 species found throughout the world. Commonly known as St. John's wort, the genus has received considerable attention for its medicinal qualities. In addition, several species, including *Hypericum frondosum*, have significant commercial value to the ornamental horticulture industry. *H. frondosum* 'Sunburst' grows 2-4 ft, has blue-green foliage and golden flowers. It is reasonably drought tolerant and cold hardy to USDA zone 5. The development of improved lines of *H. frondosum* would be beneficial to the ornamental horticulture industry. However, the improvement of ornamental qualities is often limited by the lack of effective plant regeneration systems. In vitro regeneration systems are useful tools in genetic improvement programs to facilitate the recovery of somaclonal variants and to manipulate ploidy levels, which increases ornamental characteristics and expands breeding opportunities.

**Nature of Work:** Despite the diversity of *Hypericum*, the regeneration of plants via organogenesis and somatic embryogenesis procedures have only been reported for limited species with pharmaceutical value. For example, a few studies have described the successful organogenesis and somatic embryogenesis from leaf segments of *H. perforatum* (5). Other studies have described organogenesis systems from seed for *H. perforatum* and *H. heterophyllum* (1,3), from hypocotyl segments for *H. perforatum* (4) and from nodal buds for *H. brasiliense* (2). In the present study, we report on the development of an organogenesis system from leaves for *H. frondosum*.

In all experiments, not fully expanded leaves from *Hypericum frondosum* 'Sunburst' were obtained from glass house grown plants maintained at the Mountain Horticultural Crops Research and Extension Center, Mills River, NC. Leaves were surface sterilized for 17 min in 20% commercial bleach and rinsed 3 times in sterile deionised water (5 mins each wash).

Leaves were then scored on the underside with a scalpel blade and cut into 5 mm (0.2 in) segments and plated on to different media. The callus induction medium contained Murashige and Skooge (MS) basal salts and vitamins supplemented with 3% sucrose. The pH was adjusted to  $5.75 \pm 0.03$  and the media was solidified with 0.8% agar. The effect of growth regulators was tested using benzylamino purine (BAP) (0, 1.25, 2.5, 5.0 and 10  $\mu\text{M}$ ) in combination with 2,4-dichlorophenoxyacetic acid (2,4-D) or Indoleacetic acid (IAA) (0, 0.5, 2.5, 5.0  $\mu\text{M}$ ). Cultures were maintained in the dark at 23 C (73.4 F).

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Ten leaf segments were cultured per plate, replicated 5 times representing a total of 30 observations per treatment. The frequency of callus and shoot production was determined after 8 weeks and data were analyzed using (PROC GLM; SAS version 9.1, SAS Institute., Cary, N.C., 1988).

For rooting induction, medium salt type and strength were tested. Elongated shoots (2.0 cm long) (0.79 in) were isolated and transferred to either full- or half-strength MS, Shenck and Hilderbrandt (SH) or Gamborgs B5 salts supplemented with 5  $\mu\text{M}$  indolebutyric acid (IBA), 3% sucrose and solidified with 0.8 g/l agar. Seven shoots were placed in 180 ml (6.1 oz) baby food jars and cultured at 23 °C (73.4 F) under cool white fluorescent lights (130  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) with a 16 h photoperiod. The experiment was replicated 10 times representing 70 observations per treatment. The frequency of rooting and average number of roots were determined after 4 weeks and rooted plants were transferred to the glass house.

**Results and Discussion:** In vitro shoot regeneration protocols were successfully developed from leaf segments of *Hypericum frondosum* (Table 1). While callus was observed using BAP in combination with 2,4-D and IAA, shoot formation was only observed using a combination of BAP and IAA. In contrast, studies on *H. perforatum* reported that 2,4-D was essential for shoot formation (5). Species specific in vitro regeneration protocols are often observed in woody plants, even for closely related species (4).

The interaction between BAP and IAA significantly affected both callus formation and shoot regeneration ( $P<0.05$ ). In general, callus formation increased with increasing concentrations of BAP and IAA (Table 1). Highest shoot formation was observed with a combination of 10  $\mu\text{M}$  BAP and 2.5  $\mu\text{M}$  IAA. Decreased concentrations of BAP and lower or higher concentrations of IAA resulted in attenuated shoot formation (Table 1). In contrast, 4.5  $\mu\text{M}$  BAP was optimal for shoot regeneration in *H. perforatum* and *H. heterophyllum* (1,5), again highlighting species specific differences.

Elongated shoots obtained from callus produced roots on all media evaluated. However, the basal salt concentration significantly influenced the number of shoots producing roots and the total number of roots. Higher frequencies of root formation were observed on half-strength MS media supplemented with 5  $\mu\text{M}$  IBA (Table 2). Similar results have been observed for *H. perforatum* (5) and *H. canariense* (5). It is likely that the higher ionic strength of the other media combinations may have an inhibitory effect on woody plant species.

In this study we described an efficient protocol for the regeneration of plantlets via callus induced from leaf segments. The results highlight the species specific nature of regeneration protocols. Protocols developed in this study will be used to assist in mutation and ploidy manipulation studies.

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Table 1. Effect of cytokinin and auxin concentration on callus initiation and shoot formation from leaf segments of *H. frondosum*.

BAP ( $\mu\text{M}$ )	IAA ( $\mu\text{M}$ )	Regeneration Response	
		Callus <sup>1</sup>	Shoot
0	0	0a	0a
1.25	0	8.4 $\pm$ 2.3b	0a
2.5	0	38.7 $\pm$ 6.2d	0a
5	0	36.1 $\pm$ 6.1d	0a
10	0	22.3 $\pm$ 4.8c	0a
0	0.5	0a	0a
1.25	0.5	92.5 $\pm$ 3.6f	0a
2.5	0.5	88.4 $\pm$ 3.3f	11.5 $\pm$ 3.3b
5	0.5	100g	7.3 $\pm$ 2.2ab
10	0.5	100g	33.8 $\pm$ 5.6c
0	2.5	63.7 $\pm$ 6.3e	0a
1.25	2.5	97.5 $\pm$ 1.7g	10.0 $\pm$ 3.3b
2.5	2.5	96.3 $\pm$ 1.8g	11.0 $\pm$ 3.0b
5	2.5	100g	26.3 $\pm$ 5.8c
10	2.5	100g	51.25 $\pm$ 5.6d
0	5	14.7 $\pm$ 63.6b	0a
1.25	5	92.2 $\pm$ 2.6fg	0a
2.5	5	93.2 $\pm$ 2.9fg	0a
5	5	92.2 $\pm$ 3.1fg	16.8 $\pm$ 4.2b
10	5	98.3 $\pm$ 1.6fg	0a

<sup>1</sup>Means in each column followed by the same letter are not significantly different at  $P < 0.05$ .

Table 2. Effect of basal salt composition on root formation and the number of roots of *H. frondosum*.

	Rooting Media			
	SH <sup>1,2</sup>	B5	MS	½ MS
Rooting (%)	79.8 ± 5.0ab	69.1 ± 7.5b	68.3 ± 9.6b	88.0 ± 6.6a
Average roots per shoot	1.8 ± 0.15a	1.8 ± 0.15a	3.1 ± 0.34b	4.2 ± 0.38c

<sup>1</sup>Means in each row followed by the same letter are not significantly different at  $P < 0.05$ .

<sup>2</sup>SH – Shenck and Hilderbrandt basal salts ; B5 – Gamborgs B5 basal salts and vitamins; MS – Murashige and Skooge basal salts and vitamins; 1/2MS – half strength Murishige and Skooge basal salts.

## Improving Germination of Lenten rose (*Helleborus orientalis* Lam.)

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**Index Words:** gibberellin, seed dormancy, propagation

**Significance to Industry:** Lenten rose is well suited to dry shade gardens in the southeastern United States but faces significant production difficulties due to poor germination. Higher and more synchronous germination would result in more efficient propagation. Treatment of seeds with 200 ppm, 400 ppm, and 800 ppm GA<sub>3</sub> prior to warm stratification increased germination rates.

**Nature of Work:** *Helleborus* species are drought tolerant, shade garden perennials that offer winter to early-spring flowers in an assortment of colors. Flowers appear as single, semi-double, or double forms and last up to two months. *Helleborus orientalis* and *Helleborus x hybridus*, both known as Lenten rose, are best suited for the southeastern United States (5).

An obstacle to large scale production of *Helleborus* is inefficient propagation methods. Hellebores are mainly propagated by seed (4); however, seed dormancy results in slow germination with low rates thought to be caused by a combination of an underdeveloped embryo and a physiological dormancy (2). Warm followed by cold stratification is traditionally recommended for breaking dormancy in *Helleborus*. Previous studies (3) have indicated that 10 weeks warm stratification at 25C (77F) followed by 4 weeks cold stratification at 4C (39.2F) improved germination rates.

It is well known that gibberellins promote germination by increasing embryo growth potential and weakening the tissue surrounding the embryo (1). This poses the question of whether cold stratification could be reduced or eliminated and germination rates increased using gibberellic acid? The objectives of this experiment were to determine if gibberellic acid (GA<sub>3</sub>) would promote germination in Lenten rose seeds and to determine the best application time and rate.

Seeds of Lenten rose Red Hybrids were purchased from Jelitto Perennial Seed (Schwarmstedt, Germany). Treatments consisted of three GA<sub>3</sub> concentrations at 200 ppm, 400 ppm, or 800 ppm with three application times, prior to warm stratification, between warm and cold stratification, and after cold stratification, and a control receiving no GA<sub>3</sub> treatment. On November 18, 2007, seeds were allowed to imbibe in aerated, distilled water for 8 h at room temperature followed by a surface sterilization in a solution of 15% bleach (6% sodium hypochlorite) and distilled water. Seeds were sown in containers containing germination paper evenly moistened with a solution of 100 ppm

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Blocker 4F Flowable Fungicide (Amvac Chemical Corporation, Los Angeles, CA) and distilled water. Gibberellic acid 90% (Acros Organics, Morris Plains, NJ) was added to distilled water to obtain 200 ppm, 400 ppm, and 800 ppm solutions. Seeds receiving treatments were removed from their containers, placed on germination paper to which 75ml of the appropriate concentration of GA<sub>3</sub> had been added, then returned to their containers after 24 h. All treatments and the control were given 10 weeks of warm stratification at 25C (77F) followed by 4 weeks of cold stratification at 4C (39.2F), after which they were moved to a cooler at 10C (50F) until radicle emergence. Treatments were monitored as needed, approximately every three days, to record the date of radicle emergence, defined as the day the radicle reached 3 mm in length. Radicle emergence was continuously recorded until termination on May 10, 2008.

The data were analyzed as a completely randomized design with GA<sub>3</sub> concentration and application time in a factorial treatment arrangement. Each treatment and the control were replicated 4 times with 20 seeds per replication. The number of days to radicle emergence was analyzed with PROC GLM. The number of radicles emerged out of the total number of seed sown per treatment combination was analyzed with PROC GENMOD using the binomial probability distribution. This was used to calculate percentage emergence. Single degree of freedom group contrasts were used to test differences among selected treatment combinations at  $\alpha = 0.05$ .

**Results and Discussion:** The application of GA<sub>3</sub> to seeds prior to warm stratification (pre-warm) resulted in 42% to 58% higher germination percentages versus post warm and post cold applications, respectively. Germination percentages were similar among the three GA<sub>3</sub> concentrations when applied pre warm. Seeds receiving GA<sub>3</sub> either post warm or post cold had similar germination percentages regardless of GA<sub>3</sub> concentration (Table 1). There was also no difference in germination percentages among the three GA<sub>3</sub> concentrations when applied pre warm, post warm, or post cold. Germination percentages at all three GA<sub>3</sub> concentrations were higher than the control when applied pre warm and post warm.

GA<sub>3</sub> applied at 200 ppm and 800 ppm resulted in differences in pre warm, post warm, and post cold for days to germination, but at 400 ppm GA<sub>3</sub>, only the pre warm application was different from post warm and post cold applications. Days to germination of all three GA<sub>3</sub> concentrations was equal to the control when applied pre warm, post warm, or post cold. However, the fewest number of days to germination were found at 200 ppm, 400 ppm, and 800 ppm GA<sub>3</sub> when applied pre warm. This experiment demonstrates that GA<sub>3</sub> treatment can be used to increase germination percentage of Lenten rose and 200 ppm GA<sub>3</sub> is effective and economical.

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**Table 1.** Effects of GA<sub>3</sub> concentration and application timing on the germination of *Helleborus orientalis* seeds.

GA <sub>3</sub> concentration (ppm)	Application time	Percent Germination	Days to germination
–	Control	20	136
200	pre warm	55	131
200	post warm	35	136
200	post cold	25	145
400	pre warm	65	133
400	post warm	30	142
400	post cold	25	140
800	pre warm	60	129
800	post warm	40	141
800	post cold	25	148
		Significance <sup>z</sup>	
GA <sub>3</sub> at 200 ppm applied pre warm vs. post warm and post cold		***	***
GA <sub>3</sub> at 200 ppm applied post warm vs. post cold		NS	**
GA <sub>3</sub> at 400 ppm applied pre warm vs. post warm and post cold		***	***
GA <sub>3</sub> at 400 ppm applied post warm vs. post cold		NS	NS
GA <sub>3</sub> at 800 ppm applied pre warm vs. post warm and post cold		***	***
GA <sub>3</sub> at 800 ppm applied post warm vs. post cold		NS	*
GA <sub>3</sub> at 400 ppm applied pre warm vs. 200 ppm and 800 ppm		NS	NS
GA <sub>3</sub> at 400 ppm applied post warm vs. 200 ppm and 800 ppm		NS	*
GA <sub>3</sub> at 400 ppm applied post cold vs. 200 ppm and 800 ppm		NS	*
Pre warm application of all GA <sub>3</sub> concentrations vs. control		***	NS
Post warm application of all GA <sub>3</sub> concentrations vs. control		*	NS
Post cold application of all GA <sub>3</sub> concentrations vs. control		NS	NS

<sup>z</sup>Non-significant (NS) or significant at  $\alpha = 0.05$ (\*), 0.01(\*\*), or 0.001 (\*\*\*) using orthogonal contrasts.

**Effects of cold stratification and chemical treatments on germination of dormant seeds of prickly-pear cactus (*Opuntia megacantha* Salm-Dyck cv. Naranjona)**

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**Index Words:** Sexual reproduction, nopal, plant growth regulators.

**Significance to Industry:** Prickly pear cactus (*Opuntia* spp.) is the most important species in the Cactaceae. It is a multi-purpose plant widely spread in many countries through the world (5). In general, prickly pear cactus plants are commonly propagated by asexual techniques including rooting of single or multiple cladodes, rooting small portions of mature cladodes derived from the dissection of tissues comprising two or more areoles, or by using fruits as propagules. Other available asexual methods include apomixis (3), grafting (4), micrografting (2), and tissue culture (1). Seed propagation may also be performed, however, limited information has been published because it is only used to study genetic variability and factors affecting the germination process (6). The nursery industry in general and the fast growing prickly pear industry in particular, may benefit from studies on seed germination. These studies are essential for genetic improvement programs to generate new cultivars, study heredity, provide material for micropropagation and micrografting, and establish ornamental and more productive combinations.

**Nature of Work:** We studied the conditions to manipulate the *Opuntia megacantha* Salm-Dyck cv. *Naranjona* seed germination. In order to start a series of experiments, we collected healthy and mature fruits from a fruit production area located in Pinos, Zacatecas, Mexico. The selected fruits were peeled by hand and macerated to release the seeds, which were transferred to a container with a 1% solution of H<sub>2</sub>SO<sub>4</sub> as a cleaning treatment. After 24 h, the seeds were transferred to a plastic sieve, washed several times with tap water to eliminate pulp, dried at room temperature for 2 days, and stored in paper bags.

In order to check whether the prickly-pear cactus seeds have internal and external dormancy, we performed a series of experiments to determine the effects of chemical and physical treatments on germination. In the first experiment, we evaluated 5 treatments (0, 2, 4, 6, 12 weeks) of cold stratification (4°C). Before the treatments, a group of clean seeds were immersed in a solution of Vitavax [fungicide](10%) for 10 min. Then, the seeds were dried and packed in groups and transferred to a container with humidified perlite and stored in a refrigerator at 4°C according to the stratification treatments. In the second experiment, we evaluated 7 treatments (0, 5, 10, 20, 30, 60, 120 min.) of acid scarification with H<sub>2</sub>SO<sub>4</sub>. In Experiment 3, we evaluated a series of increasing concentrations of gibberellic acid (GA<sub>3</sub>) (0, 50, 250, 500, 2000 ppm) and immersion time

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(24 and 48h) arranged factorially, which gave  $5 \times 2 = 10$  total treatments. To apply the treatments, the cleaned seeds were packed in groups in serran cloth and immersed in a container with the corresponding  $GA_3$  solution. Oxygen to each solution was supplied with a fish pump. Each treatment in all three experiments included 4 replications ( $n = 4$ ), each of which were represented by 50 seeds. The experiments were repeated at least 3 times to confirm the results.

Seeds from all experiments were sown in plastic containers with a medium of Sunshine potting soil (Sun Gro Horticulture, Canada Ltd.), perlite and vermiculite (3:1:1) plus 4.95 g slow release fertilizer, Osmocote 14N-14P<sub>2</sub>O<sub>5</sub>-14K<sub>2</sub>O (Scotts-Sierra Horticultural Products Company, Marysville, OH, USA), per container. Then, the containers were transferred to a glasshouse and grown with maximum photosynthetic photon flux density (PPFD) of 1100  $\mu\text{mol}/\text{m}^2/\text{s}^{-1}$  at plant level, and an average day/night temperature of  $27/20 \pm 2^\circ\text{C}$ . Once a week after germination, 200 mL fertilizer solution was applied to each container (5g Peters 8-45-14 in 1,000 mL water - - Scotts-Sierra Horticultural Products Company, Marysville, OH, USA) and irrigation was supplied as needed for the experiment. Days to start seed germination were determined in all experiments and the total number of germinated seeds and total percentage of germination were evaluated after 60 days of culture. Treatment effects in all experiments were determined by using analysis of variance (ANOVA) and Tukey ( $\alpha = 0.05\%$ ) for means separation (7).

**Results and Discussion:** The beginning of seed germination varied from 8 to 15 days in all three experiments. According to data obtained in Experiment 1, seeds of prickly pear cactus do not have internal or physiological dormancy because germination was not enhanced by increased stratification at  $4^\circ\text{C}$  for 2, 4, 6, and 12 weeks. Seeds from the control treatment had the highest germination value (67%), which was similar to seeds stored for 2 weeks (55.5%). In general, germination values were negatively affected by cold storage time (Table 1).

Data obtained in Experiment 2 strongly suggests that the *Opuntia megacantha* seeds have an external dormancy, because germination increased significantly for seeds exposed for 60 or 120 min to sulfuric acid compared to all other treatments (Table 2).

Gibberellic acid ( $GA_3$ ) concentration, but not immersion time, enhanced seed germination in *Opuntia megacantha*. Germination was enhanced by the intermediate concentrations of 250 and 500 ppm  $GA_3$  (95 and 89.58%, respectively), however these values were similar to the control (88.34%) (Table 3).

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Table 1. Effects of cold stratification on seed germination of *Opuntia megacantha* Salm-Dick, 60 days after the treatment.

<b>Stratification at 4°C (weeks)</b>	<b>Seed Germination (%)<sup>1</sup></b>
0	67.00a
2	55.55ab
4	43.00 b
6	42.50 b
12	25.00 c
MSD	0.1534

<sup>1</sup>Means with different letter are significantly different according to Tukey test ( $\alpha= 0.05$ ).

Table 2. Effects of acid scarification with H<sub>2</sub>SO<sub>4</sub> on seed germination of *Opuntia megacantha* Salm-Dick, 60 days after the treatment.

<b>H<sub>2</sub>SO<sub>4</sub> (min)</b>	<b>Seed Germination (%)<sup>1</sup></b>
60	70.0a
120	64.5a
0	56.0 b
20	52.0 bc
30	50.5 bc
10	49.5 bc
5	47.0 c
MSD	0.1577

<sup>1</sup>Means with different letter are significantly different according to Tukey test ( $\alpha= 0.05$ ).

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Table 3. Effects of gibberellic acid (GA<sub>3</sub>) on seed germination of *Opuntia megacantha* Salm-Dick, 60 days after the treatment.

<b>GA<sub>3</sub> (ppm)</b>	<b>Seed Germination (%)<sup>1</sup></b>
250	95.00a
500	89.58ab
0	88.34ab
50	85.83 b
2,000	82.08 b
MSD	0.0964

<sup>1</sup>Means with different letter are significantly different according to Tukey test ( $\alpha= 0.05$ ).

## Winter Cutting Propagation of Heller's Japanese Holly

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

**Significance to the Industry:** Heller's Japanese holly (*Ilex crenata* 'Helleri') is a popular landscape plant in many areas of the United States due to its dwarf habit, slow rate of growth, and dark green leaves. Plants can be propagated readily by stem cuttings. Use of an auxin treatment is generally recommended for rooting cuttings. Experimental results indicate semi-hardwood, terminal stem cuttings of Heller's Japanese holly can be successfully rooted during winter months without the use of an auxin treatment, permitting elimination of one step in the propagation process. However, use of auxin at a moderate rate can result in larger root systems on the rooted cuttings, which may enhance establishment of liners upon transplanting into larger nursery containers.

**Nature of Work:** Heller's Japanese holly is a popular cultivar for landscape use in full sun or partial shade (1, 6). Plants are readily grown and maintained in the landscape, provided soils are not too dry or too wet (1), and are recommended for landscapes in USDA hardiness zones 5b to 8a (6). Plants are grown primarily for their form and foliage; fruit is rarely produced (6). Heat can be a limiting factor in nursery production of this crop (1). Treatment of cuttings with auxin is typically recommended to promote adventitious root formation (1, 5). Cuttings of other hollies, including dwarf Burford holly (*Ilex cornuta* 'Dwarf Burford'), 'Nigra' inkberry (*Ilex glabra* 'Nigra'), and dwarf yaupon holly (*Ilex vomitoria* 'Nana') can be successfully propagated from winter cuttings without use of an auxin treatment (2, 3, 4).

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

Cutting propagation material of Heller's Japanese holly was collected from healthy plants growing in a residential landscape in Auburn, Alabama for three experiments. Terminal, semi-hardwood, 2.5-inch long stem cuttings were prepared using the previous season's growth (firm but green stems). No leaves were removed from the cuttings. Auxin solutions were prepared by diluting Dip 'N Grow (IBA + NAA; Dip 'N Grow, Inc., Clackamas, OR) with deionized water. In all experiments, cuttings received no auxin

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treatment or a 1-second basal quick-dip to a depth of 0.5 inch in a solution of 2500 ppm IBA + 1250 ppm NAA. Cuttings were inserted to a depth of 0.5 inch into individual pots containing Fafard 3B mix (a blend of peat, perlite, vermiculite, and pine bark; Conrad Fafard, Inc., Agawam, MA) as the rooting substrate.

Expt. 1 was initiated on Jan. 21, Expt. 2 on Feb. 17, and Expt. 3 on Feb. 23 of 2002. Cuttings were placed inside a high-humidity polyethylene-covered enclosure inside a greenhouse at the Patterson Greenhouse Complex at Auburn University. Overhead mist was supplied once daily for 10 seconds at noon to maintain a relative humidity of 95% to 100%. Daily maximum/minimum temperature was  $81 \pm 10\text{F}/64 \pm 5\text{F}$ . A completely randomized design was utilized in all experiments with 30 cuttings per treatment in each experiment. Number of rooted cuttings, number of primary roots emerging from the stem of each rooted cutting, and total length of primary roots on each rooted cutting were determined for all experiments on May 15. Rooting responses of the two treatments were compared using Fisher's Exact Test (percent rooted) and permutation tests (number of roots and total root length) using the MULTTEST procedure of SAS.

**Results and Discussion:** All, or close to all, cuttings rooted in both treatments in all three experiments. Treatment of cuttings with auxin consistently increased number of roots and total root length on rooted cuttings in comparison with nontreated cuttings. Rooting results support prior observation (5) that Heller's Japanese holly may be successfully propagated by stem cuttings late into the winter. Results also indicate, however, cuttings will root acceptably without use of an auxin treatment. Nevertheless, treatment of cuttings with auxin tends to result in production of larger root systems compared with nontreated cuttings. Larger root systems may enhance establishment of liners upon transplanting into larger nursery containers.

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**Table 1.** Root development responses of terminal, semi-hardwood cuttings of *Ilex crenata* 'Helleri' treated with and without auxins [indole-3-butyric acid (IBA) + 1-naphthaleneacetic acid (NAA)] in three experiments initiated during winter<sup>1</sup>. Cuttings were rooted in Fafard 3B substrate<sup>2</sup> in a warm, high-humidity rooting environment inside a greenhouse.

IBA + NAA (ppm)	Rooted (%)	Roots (no.)	Total root length (mm)
Expt. 1			
Nontreated	100	8.7	185
2500 + 1250	100	10.6	284
Significance <sup>3</sup>	1.000	0.013	<0.001
Expt. 2			
Nontreated	100	7.0	232
2500 + 1250	100	11.4	549
Significance	1.000	<0.001	<0.001
Expt. 3			
Nontreated	93	5.0	136
2500 + 1250	100	9.7	329
Significance	0.491	<0.001	<0.001

<sup>1</sup>Expt. 1: initiated Jan. 21; Expt. 2: initiated Feb. 17; Expt. 3: initiated Feb. 23. All experiments were evaluated on May 15.

<sup>2</sup>A blend of peat, perlite, vermiculite, and pine bark.

<sup>3</sup>*P*-values for the differences between means were obtained using Fisher's Exact Test (for percent rooted) and permutation tests (for number of roots and total root length).

## Effect of Salicylic Acid on Somatic Embryogenesis and Plant Regeneration in *Hedychium bousigonianum*

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**Index Words:** ornamental ginger, salicylic acid, somatic embryogenesis.

**Significance to Industry:** *Hedychium* species belong to the Zingiberaceae and consist of about 80 species. They are multipurpose plants which are used as ornamentals, raw material for manufacturing paper, and perfumes. The genus is also used in ethnomedicine due to antibacterial and antifungal activities. In transformation and other genetic improvement schemes, an efficient, rapid, and dependable regeneration system is required. Somatic embryogenesis is a regeneration scheme that is generally preferred over other *in vitro* developmental processes such as organogenesis for plant transformation and other *in vitro* genetic manipulations (6, 7). Somatic embryogenesis in different plant species is affected by various factors including ethylene, a ubiquitous plant hormone with a variety of effects on plant growth and development reported to inhibit *in vitro* plant growth and morphogenesis (3, 2). Because of this, regulation of ethylene perception or biosynthesis appears to be a promising approach for the development of more efficient tissue culture protocols (4). Incorporation of ethylene inhibitors in culture media has resulted in improved *in vitro* regeneration for several plant species (1, 3, 2, 4). Salicylic acid (SA), in particular, has been found to inhibit ethylene biosynthesis (5). The objective of this study was to induce somatic embryogenesis in *Hedychium bousigonianum* Pierre ex Gagnepain and assess the influence of the ethylene inhibitor SA on somatic embryogenesis by modification of a protocol we developed earlier (8).

**Nature of Work:** The experimental design comprised various concentrations of SA [0 (control), 25, 50, 75, 100, 125, 150  $\mu$ M]. There were 20 replicates (plates) per treatment, and the experiment was repeated twice. Data collected included the number of somatic embryos per gram of callus after 30 days in culture.

**Results and Discussion:** Addition of SA to the media had a significant ( $P < 0.05$ ) effect on the number of somatic embryos produced per gram of callus (Fig. 1). All treatments containing SA performed better than the control. The optimum somatic embryo production was reached at 75 and 100  $\mu$ M SA, and a decrease in somatic embryogenesis was observed at higher concentrations (125 and 150  $\mu$ M) (Fig. 1). Somatic embryos and subsequently regenerated plants were successfully obtained 30 days after transfer of embryogenic callus in all treatments (Fig. 2A-D).

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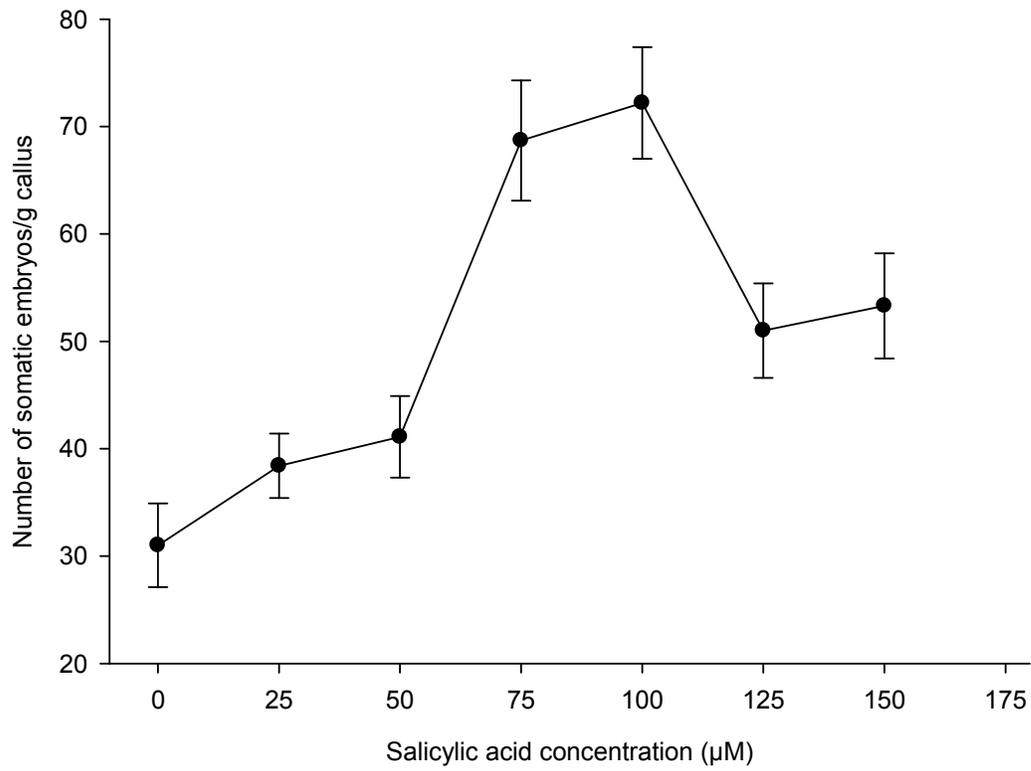
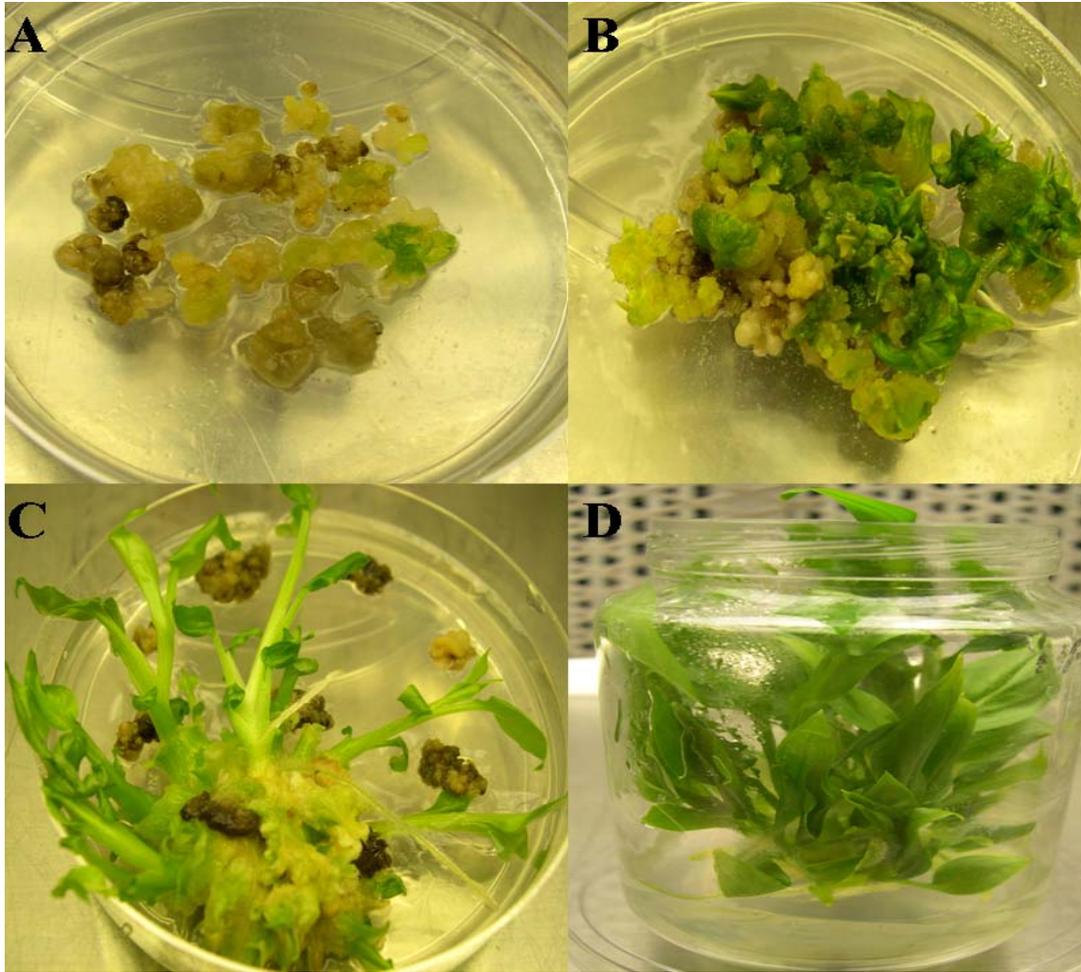


Figure 1. Effect of salicylic acid on somatic embryogenesis in *Hedychium bousigonianum*



**Fig.2.** Somatic embryogenesis and plant regeneration in *Hedychium bousigonianum*. **(A):** Development of embryogenic callus and somatic embryos. **(B):** Somatic embryo development and subsequent plantlet formation 4-5 weeks after transfer to somatic embryo development medium. **(C):** Plantlets with well formed roots were transferred directly to Jiffy pellets for acclimatization. **(D):** Smaller plantlets with or without roots were transferred to vessels for further development before the acclimatization stage.

## Seed Production of *Phlox drummondii*: Weed Control and Fertilization

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**Index Words:** trifluralin, isoxaben

**Significance to Industry:** Snapshot<sup>®</sup> 2.5 TG applied 2 days after transplanting did not provide acceptable control of cool season weeds in Drummond Phlox being grown for seed production. Moreover Snapshot<sup>®</sup> 2.5 TG substantially injured Drummond Phlox even though soil appeared to have settled around the transplants prior to herbicide application. There was preliminary evidence that supplemental fertilization enhanced seed yield in hand weeded plots; seed production was nil in herbicide treated plots. Over 94% of seeds were viable in hand weeded plots, and of those, 94% germinated; fertilization did not affect viability or germination.

**Nature of Work:** Seed production of locally or regionally adapted native wildflowers has increased over the past 10-20 years in response to an ever-growing demand. In the southeastern U.S., this type of seed production is mainly in Florida, with very limited production in Alabama and North Carolina. A major challenge facing this industry is the lack of technical information about weed control and fertilization. We previously reported that supplemental nutrition increases seed yield of two *Coreopsis* species (1, 2) but information is lacking for other species. Weeds are the main pest encountered by seed producers, at least in Florida, especially during establishment of production plots.

Because of its explosively dehiscent seed capsules and indeterminate flowering, seed production of Drummond Phlox (*Phlox drummondii* Hook.) in Florida is carried out on fabric mulch to realize substantial yields. Rows are formed by 2- to 3-inch gaps that are left between parallel strips of landscape fabric. Seed is vacuumed off the fabric over several weeks during the mid-spring to early summer.

Seeds of Drummond Phlox, derived from natural populations in Florida, were sown in a greenhouse on 29 July 2004. Weekly bottom fertilization of seedlings with 100 ppm N (Peter's 15-30-15) began on 11 August. Individual seedlings were transplanted to cell packs (1204 inserts in 1020 trays; 48 cells/tray; Cassco, Montgomery, AL) on 24 August. Plants were not fertilized the week of 13 September to reduce growth rate. On 5 October, seedlings (flowering) were transplanted to field plots, 6 inches on center. Care was taken to firmly pack the backfill soil around the root ball. Each plot within a row consisted of a 10-ft long by 3-inch wide soil strip. Rows were established by leaving a gap between two parallel strips of 6-ft wide landscape fabric. Drip tape (Ro-Drip<sup>®</sup>, 8 mil, 40 gal/hr; Roberts Irrigation Co., San Marcos, CA) was laid under the edge of the fabric immediately adjacent to the row. Plants were irrigated at transplanting, and as needed during

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establishment. Seedlings that died due to disease or uprooting (some seedlings lodged and uprooted during storms) were replaced through 17 November.

Two fertilizer treatments (fertilized; nonfertilized) and two weed management regimes (hand weeded; Snapshot<sup>®</sup> 2.5 TG [isoxaben + trifluralin; Dow AgroSciences, Indianapolis, IN] at 200 lb product/acre [4 lb a.i.]) were evaluated. Weed management regimes were completely randomized within each row, with only one row being fertigated. Weekly fertigation with 415 ppm N (10-30-20; Bloom Booster soluble fertilizer + minors; Southern Agricultural Insecticides, Inc. Palmetto, FL) began on 5 April 2005 and continued through 30 June. Weeds were removed from hand weeded plots on 26 November 2004, 5 January 2005, 5 March 2005, and 28 April 2005; the amount of time needed to weed each plot was recorded. Snapshot<sup>®</sup> 2.5 TG was applied to weed-free plots on 7 October and 6 January. Herbicide treatment began 2 days after transplanting because weed seedlings typically begin emerging in as little as a few days after soil disturbance under field production conditions in north Florida. Weed control was evaluated on 22 November 2004 and 4 March 2005, and plant quality on 4 March 2005. Dispersed seeds were harvested off the landscape fabric every 3 to 4 days from 3 May to 5 July 2005. Dispersed seeds were contained within a plot by 10-ft long wood barriers to which a strip of landscape fabric was attached.

Germination and viability testing were conducted by Mid-West Seed Services, Inc. (Brookings, SD). Seeds from Snapshot<sup>®</sup> 2.5 TG plots were not subjected to testing because there were too few seed for an accurate analysis.

**Results and Discussion:** Weed control 6 weeks after the first application of Snapshot<sup>®</sup> 2.5 TG was slightly better (but not statistically significant) than in hand weeded plots (Table 1). However, about 1 month after the second application, Snapshot<sup>®</sup> 2.5 TG did not provide acceptable weed control. Soil disturbance due to replacing dead or uprooted plants might have contributed to poor weed control in some instances because the herbicide layer was disturbed. Moreover, Snapshot<sup>®</sup> 2.5 TG severely injured most plants. Stunting of Drummond Phlox in Snapshot<sup>®</sup> TG plots was likely a symptom of Drummond Phlox seedling sensitivity to trifluralin, the major component of Snapshot<sup>®</sup> TG. By June, many plants in Snapshot<sup>®</sup> treated plots had died, and those that remained were small. Although soil had settled around the transplants prior to the herbicide application, and care was taken during transplanting to firmly pack soil around the root ball, herbicide apparently reached the root zone and injured the seedlings. Applying Snapshot<sup>®</sup> TG several weeks after transplanting, when seedlings were more developed and had better root systems, might be an option to control cool season weeds. However, a preemergent herbicide program needs to begin soon after transplanting. The ideal herbicide would be applied within a few days after transplanting and before weeds emerge, and should not be a dintroaniline herbicide (class of herbicides to which trifluralin belongs). Moreover, an effective preemergent herbicide could save a substantial amount of labor required for hand weeding the many 1000s of row-feet in a 1-acre fabric seed production field. Over the course of this study, the number of man-hours required for hand weeding only a 100-ft row ranged from 0.4 to 1.8 hr (data not shown).

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The greatest number of seeds were harvested on 24 May, 9 June, and 17 June 2005, regardless of fertilizer treatment); yields in Snapshot<sup>®</sup> treated plots were nil due to phytotoxicity. Although fertilization appeared to enhance yield, substantial plot-to-plot variation precluded detection of a significant fertilizer effect. Variation might have been due partly to genotypic diversity among seedlings. The mean ( $\pm$  SEM) estimated per acre yields (based on 24 6-ft [typical row width] x 300-ft rows per acre ) in fertilized and nonfertilized hand weeded plots were 51.8  $\pm$  14.2 lb and 36  $\pm$  8.1 lb, respectively. Seed viability and germination were not affected by fertilization. Overall means ( $\pm$  SEM) were: viable seed : 94.2  $\pm$  3.3%; germination of viable seed: 94.2  $\pm$  0.0%; abnormal seedlings: 2.9  $\pm$  1.0%.

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Table 1. Effect of Snapshot<sup>®</sup> 2.5 TG on weed control and plant quality of Drummond Phlox in seed production plots. Seedlings were transplanted on 5 October 2004. Snapshot<sup>®</sup> TG was applied to weed-free plots on 7 October 2004 and 6 January 2005. Hand weeded plots were weeded on 26 November 2004, 5 January 2005, 5 March and 28 April 2005.

Date	Hand Weeded		Snapshot <sup>®</sup> 2.5 TG	
	weed control <sup>z</sup>	Phlox quality <sup>y</sup>	weed control	Phlox quality
22 Nov.	76	—	86	—
4 Mar.	79	83	22	21

<sup>z</sup> Weed control was based on percentage of bare soil covered by weeds, with 0 = 100% coverage and 100 = 0% coverage, increments of 10.

<sup>y</sup> Plant quality rated from 0–100, with 0 = all plants in plot were dead to 100 = all plants in plot look normal and healthy, increments of 10.

## Use of the Lightweight Aggregate HydRocks® as a Bare-rooting Substrate in Perennial Offshoot Production

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**Significance to the Industry:** *Ophiopogon japonicus* was potted into the following substrate treatments: 100% aged pine bark, 100% 3/16" HydRocks® (Big River Industries, Alpharetta, GA), 100% Profile™, 100% perlite, 100% sand, 80%:20% (v:v) pine bark:peat moss, and 75%:25% (v:v) 3/16" HydRocks®:sand. Effects of substrate treatments on bib yield and bare rooting time were compared. Results from this study indicate HydRocks® can provide suitable growing conditions for *Ophiopogon japonicus* and reduce harvest time by 50% when compared to pine bark based substrates. Plants grown in the Profile™ substrate were bare rooted 30% faster than those grown in pine bark treatments. Profile™ also produced higher bib numbers than plants grown in pine bark and yielded similarly to plants grown in pine bark:peat moss. Plants grown in HydRocks® substrate were bare rooted 30% faster than Profile™ treatments.

**Nature of Work:** An alternative to the expense associated with container grown liners is producing bare-root liners. Purchase cost of bare root liners is generally less expensive than the cost of "buying in" container liners due to weight and space (4). Some Oregon nurseries have converted entire greenhouse floors into in-ground pumice beds where cuttings are directly stuck (2). The pumice aggregate is easily removed from the cuttings at harvest and the pumice is reused for many years without being replaced. The level of difficulty in removing substrate from roots in bare root production is dependent on the root structure and substrate components. Inorganic materials such as monolithic slag and ceramic aggregates with stable large particle sizes have been shown to be easily removed from roots in cutting propagation, while still producing a quality rooted cutting (1, 3, 4). Expanded clays are lightweight aggregates formed by firing certain clays in rotary kilns. Expanded clays could be used in place of pumice within in-ground propagation beds where pumice supply is not readily available. Like pumice, stable properties of expanded clays would allow re-use for many years without replacement, providing a more sustainable and cost effective approach to bare root liner production. In this study two clay products marketed for horticultural applications were used, Hydrocks® (Big River Industries, Alpharetta, GA) and Profile™ (Profile™ products LLC, Buffalo Grove, IL).

In this experiment HydRocks® (95% of particles  $\approx$  0.24 -0.04 in.), a light, expanded clay aggregate marketed for horticultural applications, and Profile™ (95% of particles  $\approx$  0.04 -0.01 in.), a ceramic clay aggregate, were compared to common propagation materials for ease of bare rooting and plant growth. HydRocks® is a lightweight porous aggregate clay calcined by firing to 2000 °F. Hydrocks® is produced from several

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quarries in the southeast and is generally inert and pH neutral. HydRocks<sup>®</sup> has a cation exchange capacity (CEC) of 8 meq/100g while Profile<sup>™</sup> has a CEC of 33 meq/100g (Fain and Paridon, 2004). HydRocks<sup>®</sup> used in this study was produced in Livingston, AL and Profile<sup>™</sup> was produced in Blue Mountain, MS. Physical properties of the substrates were analyzed using N.C. State Porometers.

The study was conducted at the Paterson Greenhouse Complex in Auburn, AL in 2007. On November 21, 2006, three bare root *Ophiopogon japonicus* bibs were potted into 8 in. wide by 5 in. tall containers. Substrate treatments consisted of 100% aged pine bark, 100% HydRocks<sup>®</sup>, 100% Profile<sup>™</sup>, 100% perlite, 100% sand, 8:2 (v:v) pine bark:peat moss, or 3:1 (v:v) HydRocks<sup>®</sup>:sand. Screen (0.08 in. x 0.08 in.) was placed in the bottom of each container to prevent loss of substrate through container holes. Nine replicates of each treatment were arranged in a randomized complete block design in a double layer polyethylene greenhouse with temperatures averaging 75 °F. Plants were liquid fertilized by hand as needed (20-10-20 Pro Sol). On May 3, 2007, plants were transferred to an outdoor shade structure covered with 40% shade cloth, and top-dressed with 14g of 18-6-12 Polyon (Purcell Technologies, Inc. Sylacauga, AL). One-half inch of overhead irrigation was applied daily.

On October 2, 2007, three workers were randomly assigned three replications from each treatment and instructed to bare root each container as fast as possible by shaking and using pressurized water. Bare rooting time was recorded and bib counts were taken on individual containers. All data were analyzed using the GLM procedure with mean separation by Waller-Duncan K-ratio test (SAS Version 9.1 SAS Institute, Cary, NC).

**Results and Discussion:** In 2007, 45 weeks after potting *Ophiopogon japonicus*, 100% perlite and 100% Profile<sup>™</sup> produced the greatest total bib count per container (37.7) and was similar to pine bark:peat moss (32.6) (Table 1). 100% HydRocks<sup>®</sup> produced a total bib count similar to all other treatments with the exception of Profile<sup>™</sup>. Pine bark:peat moss containers took the longest to bare root of any treatment. Both HydRocks<sup>®</sup> treatments required the least time of all treatments to bare root. HydRocks<sup>®</sup> treatments took about 50% less time to bare root than pine bark:peat moss, 40% less time than 100% pine bark, and 30% less time than Profile<sup>™</sup> per container. No differences were observed between 100% perlite, 100% Profile<sup>™</sup>, HydRocks<sup>®</sup>, HydRocks<sup>®</sup>:sand and 100% sand when time to bare root was calculated on a per bib basis. 100% Pine bark and pine bark:peat moss took 35% longer to bare root per bib than 100% perlite, 100% Profile<sup>™</sup>, 100% sand and both HydRocks<sup>®</sup> treatments. Bib yield from HydRocks<sup>®</sup> was the same as pine bark, but required nearly 50% less time to remove from roots (Table 1). HydRocks<sup>®</sup> containers had the fastest time to bare root of all substrates. During harvest, 100% HydRocks<sup>®</sup> required almost no water in bare-rooting, which made clean up easier.

HydRocks<sup>®</sup> was more easily removed than any of the other substrates and produced total bib counts similar to pine bark but lower than Profile<sup>™</sup> and pine bark:peat moss, which is attributed to the lower water holding capacity of HydRocks<sup>®</sup> (Table 2). HydRocks<sup>®</sup> had 50% less water holding capacity when compared to Profile<sup>™</sup> (Table 2). HydRocks<sup>®</sup> had only 11% less water holding capacity than pine bark but it was observed during the study that HydRocks<sup>®</sup> containers dried out much more quickly than pine bark containers. HydRocks<sup>®</sup> used in this study had much larger particles than Profile<sup>™</sup> (data not shown). HydRocks<sup>®</sup> might have performed better on bib counts if a smaller particle

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size was used to increase water holding capacity. When 25% sand was incorporated into HydRocks<sup>®</sup>, total porosity was decreased and water holding capacity was increased. However, this did not increase yield when compared to plants grown in 100% HydRocks<sup>®</sup> (Table 2). When compared to ranges defined in The Best Management Practices (BMP) Guide for Producing Container Grown Plants (5), HydRocks<sup>®</sup>:sand and 100% sand were the only substrates out of BMP ranges for total porosity (50-85 %) (Table 2). HydRocks<sup>®</sup>, HydRocks<sup>®</sup>:sand and sand were out of range for water holding capacity (46-65%). Profile<sup>™</sup>, sand, and HydRocks<sup>®</sup>:sand were similar and considerably low in air space (Table 2). HydRocks<sup>®</sup> might produce higher yields using cyclic irrigation with increased irrigation frequencies.

Results of these studies suggest that inorganic substrates are more easily dislodged from root systems of *Ophiopogon japonicus* than organic substrates. HydRocks<sup>®</sup> ability to quickly dislodge from roots may offset slightly decreased yields when compared to other inorganic substrates. Sand also produced yields similar to pine bark and provided a moderately easy removal from the root system. Sand, HydRocks<sup>®</sup> and Profile<sup>™</sup> each would provide ease of bare-rooting *Ophiopogon japonicus* while also providing yields comparable or better to that of conventional substrates.

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Table 1. Comparison of substrate effect on time required to bare root and bib yield of container-grown *Ophiopogon japonicus*.

Substrate	Bib number	Time (sec) required to bare root <sup>Z</sup>	
		per container	per bib
1 - Pine bark	25.8 bc <sup>Y</sup>	48.7 b	2.10 ab
2 - 8:2 pine bark:peat moss (v:v)	32.6 ab	61.2 a	2.15 a
3 - Perlite	27.1 bc	39.8 c	1.59 bc
4 - Profile™	37.7 a	44.4 bc	1.49 c
5 - HydRocks®	28.0 bc	30.2 d	1.08 c
6 - 3:1 HydRocks®:Sand (v:v)	22.4 c	29.4 d	1.41 c
7 - Sand	26.4 bc	40.1 c	1.53 c

<sup>Z</sup>Time (in seconds) required to remove plants from container and wash substrate from roots.

<sup>Y</sup>Means within column followed by the same letter are not significantly different (Waller-Duncan K-ratio t test  $p \leq 0.05$ ).

Table 2. Physical properties of 2007 substrate treatments.<sup>Z</sup>

Substrate	Air space <sup>Y</sup>	Container capacity <sup>X</sup>	Total porosity <sup>W</sup>	Bulk density (g/cm <sup>3</sup> ) <sup>V</sup>
Pine bark	38.9 a <sup>U</sup>	34.6 d	73.5 a	0.17 e
8:2 pine bark:peat moss (v:v)	24.2 c	50.4 b	74.7 a	0.18 e
Perlite	17.1 d	48.0 b	65.2 c	0.18 e
Profile (fine)	2.5 e	66.0 a	68.5 b	0.50 d
HydRocks®	31.6 b	30.7 e	62.0 d	0.64 c
3:1 HydRocks®:sand (v:v)	3.5 e	39.3 c	42.9 e	1.11 b
Sand	2.2 e	27.2 f	29.4 f	1.51 a
<i>Recommended range</i> <sup>T</sup>	10-30	46-65	50-85	0.19-0.70

<sup>Z</sup>Analysis performed using the North Carolina State University porometer.

<sup>Y</sup>Air space is volume of water drained from the sample ÷ column of the sample.

<sup>X</sup>Container capacity is (wet weight - oven dry weight) ÷ volume of the sample.

<sup>W</sup>Total porosity is container capacity + air space.

<sup>V</sup>Bulk density after forced-air drying at 221°F for 48 h; g/cm<sup>3</sup> = 62.43 lb/ft<sup>3</sup>.

<sup>U</sup>Means within column followed by the same letter are not significantly different (Waller-Duncan K-ratio t test  $p \leq 0.05$ ).

<sup>T</sup>Recommended ranges as reported in Best Management Practices Guide for Producing Container-Grown Plants (Yeager et al., 2007).