

PROPAGATION

Joe Eakes
Section Editor and Moderator

Twenty-eight students competed in the Bryson L. James Student Research Competition and twenty-nine research projects were presented in poster form, which were displayed for review during the SNA Research Conference and Trade Show, this year. Their research is presented in the topical sections which follow and are designated as Student or Poster papers.

How and When Herbaceous Cuttings are Stuck Influences Winter Survival

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Nature of Work: Herbaceous perennials and non-hardy woody species grown for color are frequently purchased as dormant, leafless rooted cuttings or liners for potting into larger containers which are intended for spring and summer sales. Occasionally these liners produce no new top growth yet roots appear to be healthy.

The objective of this study was to investigate whether the time of propagation or the depth of cutting penetration into rooting media has any effect on survival and subsequent growth of plants reported with this problem. Test plants were *Caryopteris divaricata*, *Lantana* 'Miss Huff,' *Monarda* 'Raspberry Wine' and *Phlox paniculata* 'Robert Poore'. Cuttings from container grown stock plants at the Mountain Horticultural Crops Research Station, Fletcher, NC, were stuck in mid June, mid August and early September 1997 except for the Phlox where only June and September cuttings were stuck due to a lack of propagation material in mid August.

Cuttings were treated with 1250 ppm IBA (C-Mone) quick dip and stuck into a 1:1 sphagnum peat:perlite (v/v) rooting medium in 60 cell flats and placed under intermittent mist until rooted. Half of the cuttings were stuck so that a node was at least 0.5 in. beneath the surface of the rooting medium (+) while the other half was stuck so that no buds were beneath the surface of the rooting media (-). The number of cuttings stuck per treatment depended upon availability but no fewer than 18 cuttings per date and location (+,-) treatment were stuck in 3 replicates for any test plant.

Once rooted, all cuttings were transplanted to quart pots in standard MHCRC potting media (8 pine bark/1 sphagnum peat to which 7 lbs dolomitic limestone and 3.0 lbs Esmigran has been added per cu. yd.), topdress fertilized with 0.25 tsp/qt. Wilbro (Polyon) 12-6-6 Nursery Special for the June cuttings or with Peters 20-20-20 at 100 ppm N weekly until October 1 for the summer rooted cuttings. Plants were placed under overhead irrigation at the MHCRC container research facility where they were exposed to ambient temperatures until late November when they were moved to an unheated white plastic covered overwintering structure. On February 2, 1998 all liners were moved to a

heated greenhouse to force vegetative growth. Percent survival was determined on March 13, 1998. Those showing no vegetative growth were determined not to have survived.

Results and Discussion: All cuttings rooted in high (over 90%) numbers and produced mostly vigorous liners. Rooted cuttings with limited vigor were not kept as part of this study.

All cuttings were exposed to twelve sub-freezing (lowest temperature 18 F.) nights before being moved to the overwintering structure. Leaves had been killed and most had abscised. However, stems were not cut back until after new growth appeared in the greenhouse.

Survival of *Lantana* 'Miss Huff' indicates that exposure to the cold temperatures in November was excessive with inadequate numbers surviving to be commercially viable. However, (Table 1) earlier stuck cuttings survived in higher percentages than those stuck in September. From these results it appears to be important to protect 'Miss Huff' from this level of cold. Conclusions for these treatments seem unwarranted when dealing with such low survival.

Survival of *Monarda* 'Raspberry Wine' was excellent. All treatments survived at 100% except the September cuttings without nodes beneath the propagating medium (-). Therefore, placement of cuttings for this cultivar only seems important for late stuck cuttings.

Table 1. Percentage survival for *Lantana* 'Miss Huff' and *Monarda* 'Raspberry Wine' following winter as affected by date and method of propagation.

Date stuck	<u>'Miss Huff'</u>		<u>'Raspberry Wine'</u>	
	-	+	-	+
June 19	35	40	100	100
August 13	20	27	100	100
September 2	0	0	67	100

Caryopteris divaricata survived in commercially acceptable percentages only when cuttings were stuck August 13 or earlier and only when nodes were stuck below the surface of the rooting medium (+). Cuttings stuck in September did not survive in acceptable percentages regardless of node location.

Phlox paniculata 'Robert Poore' survived in commercially acceptable percentages only when nodes were below the surface of the rooting medium (+). Survival percentages were 100% on both propagation dates when nodes were beneath the propagating medium.

Table 2. Percentage survival for *Caryopteris divaricata* and *Phlox paniculata* 'Robert Poore' following winter as affected by date and method of propagation.

Date stuck	<i>Caryopteris divaricata</i>		'Robert Poore'	
	-	+	-	+
June 19	31	86	50	100
August 13	69	100		
September 2	27	43	57	100

Significance to the industry: *Caryopteris divaricata* cuttings should be stuck with nodes beneath the rooting medium and only stuck in mid summer or before. Late summer cuttings did not survive winter in adequate percentages in unheated overwintering structures.

Concern about how and when to propagate *Monarda* 'Raspberry Wine' only exists for late summer cuttings. Late season cuttings should be stuck with nodes beneath the rooting medium.

All *Phlox paniculata* 'Robert Poore' cuttings survived whether stuck in June or early September ONLY if cutting nodes were beneath the rooting medium.

For *Lantana* 'Miss Huff' cold protection appears to be more important than how or when cuttings are rooted.

Propagation of *Magnolia grandiflora* 'Little Gem' by Stem Cuttings

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Nature of Work: One plant that nurseryman are interested in propagating is *Magnolia grandiflora* 'Little Gem.' 'Little Gem' is in high demand in the nursery trade due to its ability to fit into smaller landscapes while retaining the evergreen character and fragrant, creamy white blooms of its species, which are displayed throughout the summer. Several nurseries propagate 'Little Gem' from stem cuttings. However, many propagators are still having inconsistent results. The purpose of this study was to determine the "window" of rooting for 'Little Gem' in the coastal region of South Carolina.

In August 1997, two propagators from Carolina Nurseries and I outlined a long-term study for rooting 'Little Gem.' Beginning in August, we propagated this plant every month through October. There were four constant rooting treatments: 5,000, 7,500, and 10,000 parts per million (PPM) indole-3-butyric acid (IBA); and 7,500 PPM indole-3-butyric acid with potassium-salt (K-IBA). All four treatments also included 2,500 PPM naphthaleneacetic acid (NAA). Two media were used: Carolina Standard (40% bark, 20% peat, 20% sand, and 20% perlite) and experimental mix A, which varied with each experiment. There were two environments: a 3.0-millimeter poly tent structure and no tent. All cuttings were propagated in a poly greenhouse under ambient light and temperatures. Each treatment had twenty replications.

All cuttings were collected and handled in the same manner for each experiment. Cuttings were collected from five-year-old plants, grown in seven-gallon containers under standard nursery conditions. Cuttings were collected early in the morning and placed in a bucket of water in an effort to reduce stress. Cuttings were then stripped to the uppermost four leaves and pruned to approximately 9.0 cm in length. All cuttings were manually double wounded at the basal end using the cutting blade of Felco shears. Next, cuttings were treated with a rooting auxin treatment, inserted in the medium at random, and placed under mist. Rooting was evaluated after eight weeks from initiation.

A mist regime was developed based on previous research papers (Head, 1995; McCracken, 1995), literature references (Dirr, 1990), and personal conversations with other propagators. The mist regime for experiments 1

and 2 (August and September) was eight seconds of mist every thirty-two minutes for the first four weeks. During weeks five through eight, cuttings received eight seconds of mist every sixty-four minutes. The mist was turned off during rainy weather, according to the standard practice of Carolina Nurseries.

August and September's experimental results were unsatisfactory with the highest rooting percent at 60%. Each month, rooting success varied among the auxin treatments; no single auxin treatment appeared superior. Poor rooting may have occurred due to premature leaf drop and cuttings rotting at the basal end. Because of the poor rooting in these experiments, the mist regime and experimental mix A (50% bark, 30% sand, and 20% peat) were adjusted. A more porous medium was developed, which consisted of 65% pine bark and 35% sand, in an effort to reduce moisture buildup in the medium. Mist durations were increased and intervals reduced in an effort to prevent leaf drop.

After two months of poor rooting, we finally had successful rooting with October 12th cuttings. The mist regime consisted of: days 1-10 - four seconds of mist every ten minutes; days 11-21 - six seconds of mist every sixteen minutes; and weeks 3-8 - eight seconds of mist every thirty-two minutes. In addition, the mist was never turned off on rainy days.

Results and Discussion: Our best results came from cuttings treated with 7,500 PPM IBA with 2,500 PPM NAA, which rooted at a success rate of 80% or sixteen out of twenty cuttings. All other treatments rooted at higher percentages than previous experiments, but none exceeded 60%. Cuttings propagated under the tent maintained their leaves and had far less leaf drop than those in the open greenhouse. Cuttings propagated with experimental mix A rooted at higher percentages than the Standard Carolina Mix. All rooted cuttings had an acceptable root system and were ready to be potted up.

We believe these cuttings rooted primarily due to (1) the time of year cuttings were propagated; and (2) the misting schedule. These results also demonstrate that 'Little Gem' may be favorable to rooting under several different environmental conditions.

Significance to Industry: 'Little Gem' is an important and valuable plant of the nursery and landscape industry. Increasing the percentage of rooted cuttings will increase production levels and availability of this plant.

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Influence of Storage Temperatures on Long-Term Seed Viability of Selected Native Ericaceous Species

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Nature of Work: Following harvest of capsules, drying of seeds to moisture contents of 4% to 7%, and seed extraction, seeds of *Kalmia latifolia* L. (mountain laurel), *Leucothoe fontanesiana* (Steud.) Sleum (drooping leucothoe), *Rhododendron carolinianum* Rehd. (Carolina rhododendron), *Rhododendron catawbiense* Michx. (Catawba rhododendron), and *Rhododendron maximum* L. (rosebay rhododendron) were stored for 0, 1, 2, 3, 4, or 5 years at -18° , 4° or 23°C (0° , 39° or 73°F) and then germinated at 25°C (77°F) or an 8/16 hr thermoperiod of $25^{\circ}/15^{\circ}\text{C}$ ($77^{\circ}/59^{\circ}\text{F}$) with daily photoperiods of 0, 1 or 24 hr.

Results and Discussion: Storage at -18° or 4°C (0° or 39°F) were most effective for maintaining seed viability of all species. After 5 years storage at -18° or 4°C (0° or 39°F), viability of *L. fontanesiana*, *R. catawbiense*, and *R. maximum* was relatively unchanged with total germination of 59%, 87%, and 88%, respectively. The same was noted for seeds of *K. latifolia* and *R. carolinianum* with total germination of 77% and 91%, respectively, after storage for 4 years at the same temperatures. Storage at 23°C (73°F) was the least effective for maintaining viability. After storage for 1 year at 23°C (73°F), germination decreased significantly for all species except *R. carolinianum*. By year 3, storage at 23°C (73°F) reduced seed viability of *L. fontanesiana* to essentially zero. The same occurred by year 4 for seeds of *R. catawbiense* and *R. maximum* stored at 23°C (73°F). Viability of *K. latifolia* also decreased under storage at 23°C (73°F) with germination of 14% noted by year 4. Glenn et al., 2 Viability of *R. carolinianum* did not decrease as rapidly as the other species when stored at 23°C (73°F) with total germination of 77% occurring by year 4. Regardless of storage duration, the photoperiod and temperature requirements for maximum germination of all species did not change.

Significance to Industry: Seed viability of *K. latifolia*, *L. fontanesiana*, *R. carolinianum*, *R. catawbiense*, and *R. maximum* can be maintained relatively constant for 4 to 5 years when seeds are dried to moisture contents of 4% to 7% and the seeds stored in sealed containers under freezer [-18°C (0°F)] or refrigerated [4°C (39°F)] conditions. For all species, except *R. carolinianum*, room temperature storage [23°C (73°F)] should be avoided as viability is lost rapidly. Lack of change in seed viability following storage for 4 to 5 years at -18° or 4°C (0° or 39°F)

suggests these storage conditions should permit maintenance of viability for periods greatly exceeding these lengths of time. Results also demonstrated that the photoperiod and temperature requirements for maximum germination of all species remained constant; they did not change with storage duration.

Low Temperature Storage of Micropropagated Hosta Plantlets Under Various Light Qualities

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Nature of Work: Storage systems for micropropagated plantlets offer versatility in managing labor to meet market availability. Storage systems that minimize growth and yet sustain photosynthetic and regrowth potential require temperature and light to be manipulated for obtaining best plantlet quality during and after storage. The objective of this study was to determine the effects of medium composition and light quality on low temperature storage of micropropagated Hosta (*Hosta tokudama* Makeawa 'Newberry Gold') plantlets. Hosta plantlets were subcultured in vitro photoautotrophically (without sugar in medium) or photomixotrophically (with sugar in medium) for 3 weeks in Murashige and Skoog (1962) liquid medium (10 mL per plantlet) supplemented with vitamins (Gamborg, 1970). Four plantlets were cultured on Sorbarod cellulose support plugs (Sorbarod, Baumgartner Papiers SA, Switzerland) in each 375 mL GA-7 vessel (Magenta, Chicago, Ill). Two holes (10 mm in diameter) in opposite sides of the vessel were covered with 0.5 μm membrane filter disks (Milli-Seal, Millipore K.K., Tokyo) to provide approximately 3.2 air exchanges per hour (Kozai et al., 1986). Culture room temperature was $23 \pm 2^\circ\text{C}$, and photosynthetic photon flux (PPF) was $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Light compensation points (when net CO_2 exchange is zero) were determined prior to long term storage so that plantlets could be stored without excessive growth. Plantlets were stored for 4, 8, or 12 weeks at 5°C in darkness or under $7 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (average light compensation point for white and blue light) and $4.5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (average light compensation point for red light). White light was provided by 15 W cool white fluorescent lamps, red light by red light emitting diodes (LED), and blue light by 20 W blue fluorescent lamps (Wilson et al., 1998). After 0, 4, 8, or 12 weeks storage, plantlets were harvested and leaf yellowing and dry mass were recorded. A separate set of plantlets was transferred to soilless medium (Metro Mix 360, The Scotts Co., Marysville, Ohio) in 804 grow packs and placed under mist for 4 weeks followed by an additional 4 weeks in greenhouse. Visual quality of plantlets (based on color and form) in greenhouse was assessed bi-weekly based on a scale from 1 to 5, whereby 1=very poor quality and not marketable or dead, 3=fair quality and marginally marketable, and 5=excellent quality and highly marketable. Data were analyzed by ANOVA and treatment differences separated using LSD at $P=0.05$.

Results and Discussion: Illumination and sucrose during storage were necessary to maintain dry mass of in vitro plantlets (Fig. 1). Plantlets stored without sucrose did not survive 4 weeks in darkness, while those stored with sucrose survived 12 weeks but had reduced leaf dry mass. Plantlet dry mass was similar for different light treatments, although plantlets stored for 4 weeks without sucrose under white light had greater leaf dry mass than did those stored under blue light and plantlets stored for 8 weeks without sucrose under red light had greater leaf dry mass than did those stored under white or blue light. Leaf yellowing increased as storage duration increased and plantlets stored without sucrose had greater leaf yellowing than those stored with sucrose. After 8 weeks storage under red light, plantlets had decreased leaf yellowing as compared to other treatments, regardless of medium composition.

Visual quality of plantlets declined during storage regardless of illumination or medium composition (Fig. 2). However, plantlets stored with sucrose recovered in greenhouse within 4 weeks, regardless of whether illumination was provided during storage. If plantlets were stored without sucrose, illumination was necessary for plant survival, yet visual quality of illuminated plantlets decreased as storage duration increased and plantlets were not marketable after 12 weeks of storage.

Significance to Industry: Micropropagation has worldwide applications for horticulture (i.e., fast production, greater uniformity, phenotypic improvement, disease elimination, facilitated shipping, and full year production). However, conventional micropropagation systems are challenged by high production costs and low profits. It would be highly significant to the nursery industry if quality plantlets could be held, or stored, and maintained for extended time periods in large quantities per unit space. In addition to ensuring availability of seasonal ornamental crops and distributing labor costs, holding techniques of micropropagated plantlets could significantly impact the widening globalization of markets where preservation of quality during extended shipping periods is crucial. Providing sucrose in the medium during storage is more commercially feasible than providing light. Our research shows that providing sucrose to the medium plays a key role in long-term storage of micropropagated *Hosta* plantlets.

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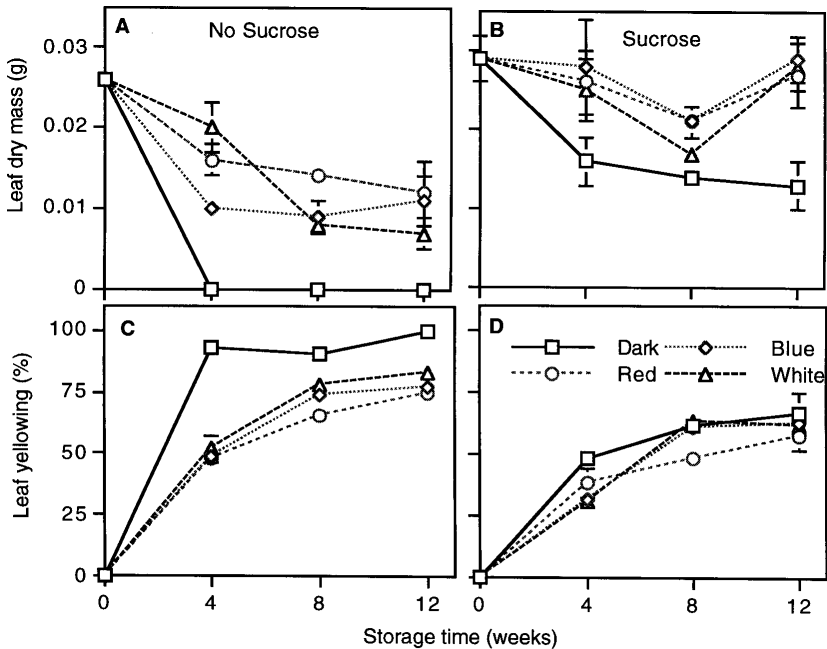


Figure 1. Change in leaf dry mass and leaf yellowing of plantlets grown in medium with or without sucrose during storage in dark or light. Means \pm S.E. are shown.

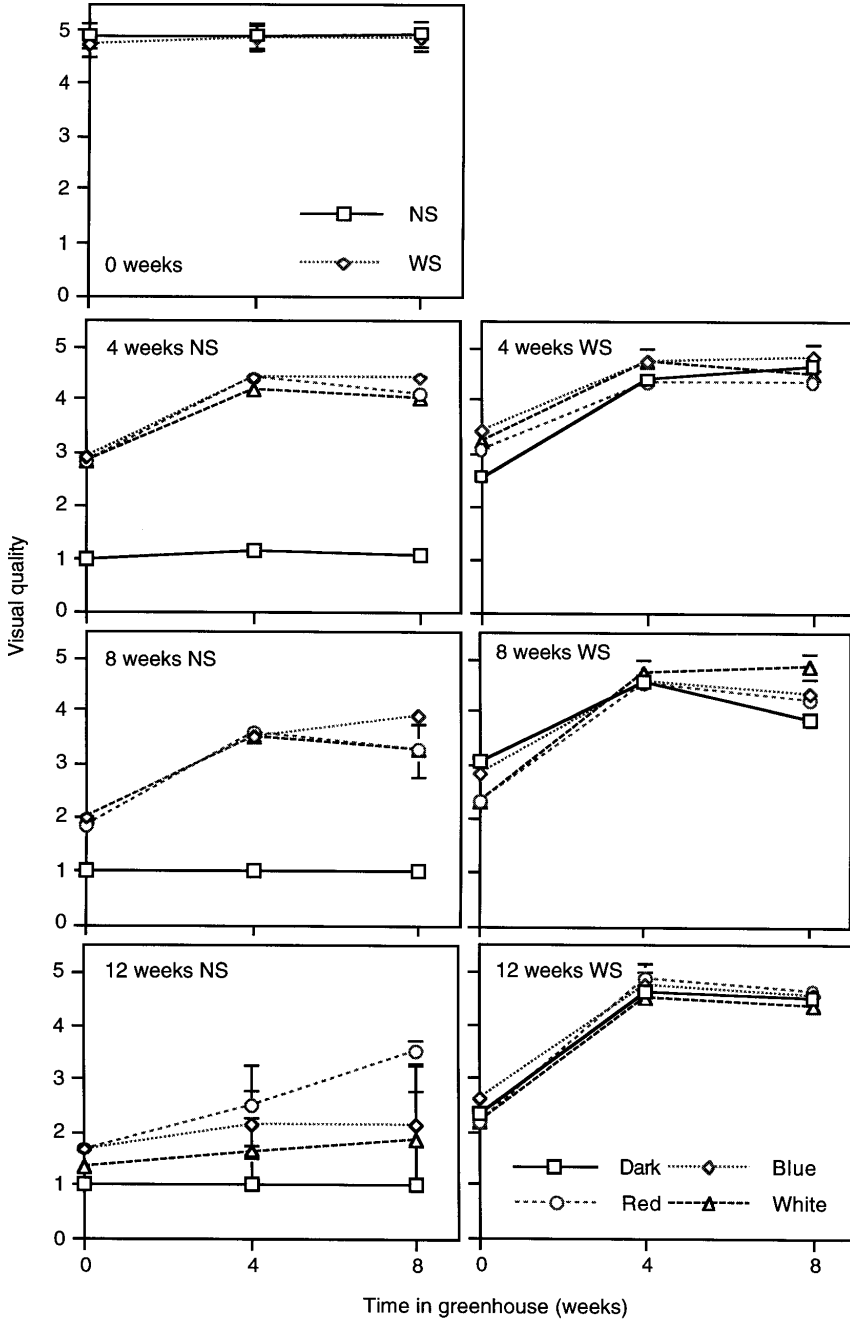


Figure 2. Visual quality of plantlets in greenhouse as affected by storage time, light quality, and medium composition. NS=no sucrose in media, WS=2% sucrose in medium. Means \pm S.E. are shown.

Prospects for Hybridization of *Hydrangea macrophylla* and *H. paniculata*

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Nature of Work: *Hydrangea macrophylla*, or big-leaf hydrangea, is a popular ornamental shrub. Unfortunately its use north of USDA cold-hardiness zone 6b to 7 is limited by the lack of cold-hardiness in flower buds, which are formed on old wood. In the mid-South, lack of flowering in *H. macrophylla* is most often due to late spring freezes that damage shoots and buds that have already broken dormancy. In colder areas of the U.S., and following abnormally cold winters in the South, the entire above-ground part of plants may also be lost.

The most cold hardy *Hydrangea* cultivated in the U.S. is *H. paniculata*, or panicle hydrangea, which is hardy to zone 4. While *H. paniculata* is an attractive and popular shrub, its white to pale pink flowers do not have the visual impact of the deep pink and blue flowers of *H. macrophylla*. Combining the cold-hardiness of *H. paniculata* with the intense flower coloration of *H. macrophylla* should produce an attractive and more widely used ornamental shrub. There has been one report of a hybridization between these two species (1); however, the alleged hybrid was lost during World War I and thus can not be verified. The objective of this study was to determine if *H. macrophylla* and *H. paniculata* will hybridize.

Before making pollinations between *H. macrophylla* and *H. paniculata*, sterile flowers were removed from inflorescences. Anthers were removed from fertile flowers prior to dehiscence, and the inflorescence was covered with a breathable plastic bag to eliminate insect pollination. Freshly dehisced pollen was applied to flowers one to three days after emasculation. The plastic bag was placed back over the inflorescences and allowed to remain on the plant for two weeks after pollination. Reciprocal crosses were made using numerous cultivars of both *H. macrophylla* and *H. paniculata*. Seed were collected when pods were completely dry, and germinated in the greenhouse.

Results and Discussion: Over 2700 crosses were made between *H. macrophylla* and *H. paniculata* during 1997 (Table 1). Approximately 80% of the *H. paniculata* x *H. macrophylla* crosses produced seedpods. However, since most of these pods contained only very small vestigial seed, it appeared that fertilization did not often occur in this hybrid. The two seed that germinated were very small and weak, and died shortly

after germination. These may have been interspecific hybrids; however, since haploidy is occasionally induced by interspecific pollination, it is also possible that they were haploids of *H. paniculata*.

Approximately 40% of the *H. macrophylla* x *H. paniculata* crosses produced seedpods. While most of the seed recovered from these crosses were shrunken, 32 seeds germinated. DNA analysis is needed before the identity of these plants can be determined; however, based on their close resemblance to the maternal species, it appears unlikely that they are interspecific hybrids.

The shrunken seeds that were recovered from the *H. macrophylla* x *H. paniculata* hybridizations are indicative of an interspecific hybrid in which fertilization occurs, but embryo development is disrupted due to endosperm death. Microscopic examination of the developing seeds revealed the presence of embryos, leading support to the theory that fertilization and early seed development occur in this hybrid when *H. macrophylla* is used as the maternal parent. Tissue culture procedures that rescue the developing embryo before it aborts have been very successful in other genera for producing interspecific hybrids (2). We are currently working on developing such a procedure for use with *Hydrangea*.

Significance to Industry: Development of a hydrangea that combines the cold hardiness of *H. paniculata* with the intense flower coloration of *H. macrophylla* will provide the nursery industry with a new summer-flowering shrub with unique characteristics. This study represents the first step in the development of such a plant. While attempts to hybridize the two species have not yet been successful, results of this study indicate that the use of tissue culture may allow us to secure this hybrid. Studies are currently being undertaken to develop an in vitro embryo rescue procedure for use with the *H. macrophylla* x *H. paniculata* hybrid.

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Table 1. Results of hybridization attempts between *H. macrophylla* and *H. paniculata* made during Summer 1997.

Cross	# crosses	#crosses setting	# seeds germinated
<i>H. paniculata</i> x <i>H. macrophylla</i>	951	771	2
<i>H. macrophylla</i> x <i>H. paniculata</i>	1812	730	32

Effect of Penetrating Agents and Quick Dip IBA/NAA on Rooting of 'Nellie R. Stevens' Holly

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Nature of Work: Work with various solvents for rooting hormones and cofactors for improving rooting has been done in the past with some success (1,2,3,4). However, little work has been done evaluating penetrating agents that would hopefully aid rooting hormones enter plant tissues to the site of activity. Currently the only proprietary rooting hormone formulation containing a penetrating agent is Wood's Rooting Compound (5) which contains 20% dimethylformamide (DMF). This work was done to test another organic compound, triethanolamine (TEA), against DMF or tap water as a control, using DIP 'N GROW (DNG) liquid rooting concentrate. DNG contains 10,000 ppm IBA, 5000 ppm NAA, 23% isopropanol, and 45% ethanol according to the MSDS sheet, with the remaining 30.5% presumably water. Fifteen cm (6 in) terminal cuttings of 'Nellie R. Stevens' holly were harvested the morning of 28 Nov 93. Basal leaves were stripped with five or six terminal leaves remaining. Groups of ten cuttings were treated with a 5-s quick dip by inserting stem bases to a depth of 2.5 cm (1 in) into the treatment solution. Treated cuttings were immediately inserted into 12-cm (5 in) deep nursery flats containing a moist medium of 40% Pro-Mix BX/60% perlite. Hormone treatments were dilutions of DNG (3000, 6000, 9000 ppm ai) combined in a factorial arrangement with penetrating agents of 20% TEA or 20% DMF with water as a control for nine treatment combinations. Ten replications were placed on the propagation bench with bottom heat and intermittent mist during daylight hours of six seconds every six minutes. When most cuttings were well-rooted, they were removed from the medium and evaluated on a scale of 1 (little or no rooting) to 5 (heavy rooting). Data were subjected to statistical analysis using the GLM procedure in SAS and significance accepted at the 5% level.

Results and Discussion: Analysis of variance showed each level of rooting hormone to be different from every other level with best rooting at 9000 ppm (3.80). Penetrating agents were different from each other with best rooting in TEA treatments (3.54) followed by DMF (3.29) and controls (2.65). Table 1 shows treatment means of the 100 cuttings in each treatment combination. Results show a marked advantage for using penetrating agents in this experiment, and indicate that TEA may be better than DMF. Also, by using penetrating agents, the level of rooting hormone may be reduced by about a third or more and still

achieve good rooting. We have since used similar quick dips with TEA in successfully rooting the following: *Cephalotaxus harringtonia* 'Fastigiata', x *Cupressocyparis notabilis*, *Ilex x attenuata* 'Alagold', *Ilex x attenuata* 'Blazer', *Ilex x 'Emily Bruner'*, *Ilex x kohneana* 'Lassie', *Myrica cerifera* 'Hiwassee', *Salix eleagnos*, *Pyracantha x 'Rutgers'*, *Viburnum awabuki* 'Chindo', and others. In most cases, but not all (see article on *Cephalotaxus* propagation elsewhere in these Proceedings) rooting has been improved by using TEA as a penetrating agent.

Significance to the Industry: The use of penetrating agents in liquid quick dips offers propagators another tool for achieving good rooting of cuttings. Good quality heavily rooted cuttings may be produced even with lower levels of rooting hormone. TEA may be a somewhat better penetrating agent than DMF, and propagators may want to examine TEA as an enhancement to their propagating procedures for rooting cuttings.

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Table 1. Effect of penetrating agent and hormone level on rooting score of 'Nellie R. Stevens' holly cuttings on a scale of 1 = little or no rooting and 5 = heavy rooting. Values are the mean of 100 cuttings.

penetrating <u>agent</u>	IBA/NAA level		
	<u>3000 ppm</u>	<u>6000 ppm</u>	<u>9000 ppm</u>
control	1.59 f	2.65 e	3.72 b
dimethylformamide	2.62 e	3.46 c	3.78 ab
triethanolamine	3.04 d	3.68 ab	3.91 a

Means followed by the same letter are not significantly different at the 5% level of probability.

Impact of Shade on Propagation of Golden Barberrry

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Nature of Work: This study was conducted to evaluate the influence of shade on propagation of golden barberry. According to Dirr and Heuser, *Berberis thunbergii* species can be readily rooted when IBA solutions of 1000 to 5000 ppm are used (1). Knox and Hamilton (2) have shown that cuttings of *Berberis thunbergii* and *B. thunbergii* 'Atropurpurea' have an increased rooting response when stock plants were grown at less than full sunlight. However, there have been no reports concerning propagation of golden barberry cuttings under different light intensities.

On April 29, 1998, 3-4 inch (7 - 10 cm) long softwood medial stem cuttings of *Berberis* sp. 'Baisel' (Golden Carousel™) were taken from 3 gallon stock plants growing under an open lath house that provided a 50% reduction in ambient light levels. Stock plants were provided by Bailey Nurseries, St. Paul, MN. Cuttings were stuck in open trays lined with 24 cell inserts (vol. 9.0 oz. (267 cm³)) filled with pinebark/sand 6:1 (v:v) substrate amended with 5.0 lbs/yd³ of dolomitic limestone. The experiment was conducted in a standard greenhouse (24 x 36 feet) with treatments based on full sun ambient light conditions consisting of three shade levels. Treatments were: 60% shade (~800 $\mu\text{mol m}^{-2} \text{sec}^{-1}$) provided by a double layer of 4ml polyethylene inflated by air; 70% shade (30% shade cloth ~560 $\mu\text{mol m}^{-2} \text{sec}^{-1}$); and 80% shade (50% shade cloth ~400 $\mu\text{mol m}^{-2} \text{sec}^{-1}$). Nine frames (30 in (76 cm) tall) were constructed of 1 inch (2.5 cm) polyvinylchloride pipe (PVC) on raised benches (8ft x 4ft (2.4x1.2m)). Shade levels were assigned in a completely randomized block design with three frames covered with 30% shade cloth (V.J. Growers Supply, Apopka, FL); three frames covered with 50% shade cloth (V.J. Growers Supply, Apopka, FL); and three frames left uncovered for the 60% treatment. For each treatment, seventy-two stem cuttings were treated with 1250 ppm IBA (Dip 'N Grow, Astoria-Pacific, Inc., Clackamas, OR) for 3-5 seconds, stuck in the media and then placed under intermittent mist with an on/off cycle of 10 sec/10 min from 6am - 7pm CST. Photosynthetically active radiation (PAR) levels during the study were monitored with a quantum sensor (Model LI-190, LI-COR, Lincoln, NE) attached to a LI-COR 6250 Portable Photosynthesis System (data not shown). The study was conducted at the Paterson Greenhouse Complex, Auburn University, Alabama. Rooted liners were harvested 57 days after treatment (DAT) (June 24, 1998). Roots rinsed free of medium were evaluated using a root rating scale (0-

5) with 0 corresponding to plants having no roots and 5 corresponding to plants that were heavily rooted (3). Subjective foliar color ratings were made (data not shown) weekly.

Results and Discussion: Initial signs of root development occurred for all treatments about 20 DAT. After 57 days, root ratings were higher in plants under 70% and 80% shade treatments than 60% shade. Viability was lower and desiccation was greater among plants under the 60% shade level than those occupying either 70% or 80% shade levels (Table 1). Overall foliar color was improved under 70% and 80% shade levels (a more uniform golden hue) compared to cuttings rooted under 60% shade (data not shown) which developed a red hue or began desiccation.

Significance to Industry: This study shows that *Berberis sp.* 'Bailsel' can be successfully propagated from softwood cuttings in early summer with 1250 ppm Dip 'N Grow. Furthermore rooting, leaf retention, and leaf color are improved as shade levels are increased. While various selections of barberry are thought of as tough, full sun plants, this study along with other work (4) suggests that golden barberry selections were actually more suitably grown in part-shade environments.

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Table 1. Effect of shade levels on rooting of Berberis sp. 'Bailsel'^z

Treatment	Root Rating	Viability
60% Shade	2.8 b	0.86 b
70% Shade	4.1 az	0.97a ^z
80% Shade	4.0 a	1.00 a

^zMean separation within column by Duncan=s Multiple Range Test (n=72), considered significant at $P=0.05$.

Effect of Runner Plantlet Size on Propagation of Strawberry

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Nature of Work: Due to ease of rooting, vegetative means are the preferred method of strawberry (*Fragaria x ananassa* Duch.) propagation (1). Propagation of plug plants may offer an advantage to growers over bare-root plants due to quicker field establishment. The well-developed root system and generally high moisture retention of substrate around roots of plug plants reduces the need to irrigate extensively during establishment (8). Strawberry plug plants are generally propagated from plantlets collected from non-rooted daughter plants with 1 to 3 compound leaves. Individual plantlets are rooted in an artificial medium and placed under mist for approximately 7-10 days until the growing medium is held together by the newly-formed roots. Plants are then removed from intermittent mist and receive daily watering, regular fertilization, and fungicidal sprays (8) until a well-developed root system forms - approximately 3 additional weeks under normal growing conditions - after which the plants are field planted. The purpose of this study was to determine if plantlet size used in the propagation phase of plug plant production affected subsequent berry yield.

On 22 Sept., 1992, runner plantlets were taken from 'Chandler' mother plants which had fruited in the spring at the Auburn University Chilton Area Horticultural Substation, Thorsby, AL. Plantlets were placed in refrigerated storage overnight. In 1993 plantlets were collected and stored in the same manner on 26 Aug. Plantlet size classifications (treatments) used in this study were: small plantlets, those with lengths of 2 in (5.1 cm) or less (from base of the crown to tip of longest leaflet) medium plantlets, those greater than 2 in (5.1 cm) in length up to 4 in (10.2 cm); and large plantlets, those greater than 4 in. On 23 Sept., 1992 and 27 Aug., 1993 plantlets were inserted in 60-cell plastic containers with an unamended ProMix BX substrate (Premier Horticultural Products, Red Hill, PA) and placed in a greenhouse under intermittent mist with an on/off cycle of 5 sec/2 min under natural lighting at the Paterson Greenhouse Complex, Auburn, AL. After one week plants were transferred from mist to overhead watering twice daily in the greenhouse until transplanting to field plots. Plants were given weekly fertilization of Peters Special 20-20-20 (O.M. Scotts Co., Maryville, OH) and preventative fungicidal sprays of Captan and Benlate at labeled rates twice weekly.

Plants were transferred to the Chilton Area Horticultural Substation in central Alabama and field planted on 23 Oct., 1992 and 4 Oct., 1993 in a RCBD consisting of 6 replications of 14 plants each for each treatment. Field plots received 50 lbs/acre (56.1 kg/ha) of N, P, and K preplant fertilizer each year. Pre-plant soil tests showed plot pH of 6.0 to 6.5, therefore no soil lime amendments were necessary. Methyl bromide was applied under plastic mulch at 236 lbs/a (265 kg/ha) in 1992, and 150 lbs/a (168 kg/ha) in 1993. Plots received overhead sprinkler irrigation for 7 days to aid in establishment. Plots also received fertilizer applied through the irrigation water weekly at a rate of 1 lb/a (1.1 kg/ha) N and 2 lbs/a (2.2 kg/ha) K₂O per day in 1993 beginning 8 Feb. through 21 May. In 1994 fertilizer was applied through irrigation water weekly at a rate of 1 lb/a (1.1 kg/ha) N and 1 lb/a (1.1 kg/ha) K₂O beginning 7 March through 23 May. Pest control consisted of 4 lbs/a (4.5 kg/ha) Captan, 2 lbs/a (2.2 kg/ha) of Benlate, and 1 fl pt/a (584 ml/ha) of Lannate applied weekly in both years following commercial recommendations. In 1993 no frost protection was necessary. Overhead sprinkler irrigation was applied once in 1994 on 10 March for frost protection. Harvests began 26 April, 1993 and ended 7 June, 1993, and in 1994 began 13 April through 26 May, 1994.

Results and Discussion: Size of plantlets during the propagation of plug plants did not affect total fruit yield (Table 1) or total marketable yield (data not shown). There was no significant interaction in early total yield and early marketable yield. Average berry weight (9.0 - 16.1 g) for either early or late harvest was not different for the three treatments nor was total berry weight for either year (data not shown).

Significance to Industry: Although early British studies reported differences in production due to runner plant size (3,4), the lack of differences in the current study indicate that those findings are not applicable to plug plant production in 'Chandler' in central Alabama. Our results with 'Chandler' strawberry plug production supports other research reporting no influence on yield or berry weight of 'Santa Ana' and 'Sequoia' due to runner order (9), and no influence on fruit production in preliminary findings for 'Chandler' (5). Similar results have been reported for tomato plug production in which plants of the same physiological age produced similar yields even though plants varied in size at transplanting (6).

Small transplants may reduce early yields in some crops (10) and has little effect on others (7), but strawberry plantlet size appears to have little influence on ultimate plant performance since small plantlet size was not found to influence yield. As a result, grading of plantlets during the propagation phase appears to be an unnecessary expense for success-

ful yields from strawberry plug plants. However, establishment of plantlets, survival in the field and vigor of small plantlets appeared to be lower than other treatments from subjective observations. Small strawberry runner plants graded by fresh weight (5 g) have been found to have lower survival than medium (5-10 g) or large (>10 g) (2). Therefore, plug plantlet size influence on survivability, not considered in this study, may be a worthwhile consideration in grading and warrants further investigation.

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Table 1. Effect of plantlet size at time of propagation on yields of 'Chandler' strawberry, 1993 and 1994.

Treatment	1993			1994		
	Early	Late	Total	Early	Late	Total
	Harvest	Harvest	Harvest	Harvest	Harvest	Harvest
large	106.03a ^z	71.95a	177.97a	149.28a	124.82a	274.10a
medium	111.37a	71.37a	182.74a	219.32a	168.72a	388.04a
small	89.76a	65.16a	154.93a	253.73a	197.04a	450.78a

^z Yield per plant (g).

^y Mean separation by Duncan's Multiple Range Test, considered significant at $P=0.05$.

**Improving Adaptability of European Beech
with Stress-tolerant Rootstocks**
(Student)

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Nature of Work: European beech (*Fagus sylvatica*) is grown throughout Europe and the United States for its ornamental characteristics, including majestic form, glossy leaves, and smooth, gray bark. Species, such as *Fagus sylvatica*, native to cooler, more temperate climates often decline when planted in hot, humid environments, including the southeastern United States. Fifteen years of ornamental plant adaptability trials at The JC Raulston Arboretum have suggested the critical limitation is root survival under wet, hot conditions (Raulston, 1995). Strong summer downpours on heavy clay soils result in soil that may remain saturated for several days. Repetition of these conditions in combination with warm temperatures can lead to a gradual decline in health, and eventually death.

Grafting can be used to produce compound plants that combine scions of superior ornamental quality with stress-tolerant rootstocks suitable for the area in which they will be grown. Cultivars of *Fagus sylvatica* are commonly grafted onto seedling rootstock of the same species. Other species of *Fagus* may tolerate conditions of high temperature and poor drainage better than *F. sylvatica*. Very little research, however, has been conducted on potential rootstocks for ornamental plants (Raulston, 1995). Therefore, the objective of this research was to evaluate the response of three species of *Fagus* (*F. sylvatica*, *F. grandifolia*, and *F. orientalis*) to high temperature and root-zone inundation for use as stress-tolerant rootstocks.

The experiment, a 3 x 2 x 3 factorial experiment in a randomized complete block design with 40 replications was conducted in glass greenhouses at the Southeastern Plant Environment Laboratory (Phytotron), Raleigh. Main factors were three *Fagus* species (*F. sylvatica*, *F. grandifolia*, and *F. orientalis*), two temperature regimes, and three levels of flooding (non-flooded, intermittent flooding, and flooded).

Temperature regimes consisted of 9 hr day/ 16 hr night thermoperiods of 30/26 °C (86/79 °F) and 22/18 °C (72/64 °F). Flooding treatments were accomplished by inserting the growing container into an identical container lacking drainage holes resulting in the substrate being covered by about 2 cm (0.8 inch) of water. The flooded treatment (F) lasted 30 days.

Intermittent flooding (I) consisted of a repeating sequence of 6 days flooded and 4 days non-flooded, respectively over a 30 day period. Non-flooded (NF) trees were never flooded.

Two-year old seedlings of *Fagus sylvatica*, *F. grandifolia*, and *F. orientalis* were purchased bareroot in January 1997, and stored at 4 °C (40 °F) until potting. On March 28, 1997, seedlings were potted into 3 quart containers with an 8 pinebark : 1 sand substrate (by vol.) amended on a yd³ basis with 5 lbs dolomitic limestone and 10 lbs of Osmocote 15-9-11 (with minors).

The dormant seedlings were moved to the Phytotron March 29, 1997. Trees were grown for 85 days in the respective temperatures before flooding treatments were initiated June 25 [0 Days After Flooding (DAF)] and terminated July 25 (30 DAF). Ten trees of each species from each temperature regime and flooding treatment combination were harvested on July 28-29. Prior to flooding (June 23-24), 10 plants of each species were harvested from each temperature regime.

At each harvest, trees were separated into leaves, stems, and roots for dry weight measurement. Total plant height, stem diameter at soil level, and leaf number and area were also determined. Net photosynthesis (P_n) and stomatal conductance (g_s) were determined nine times throughout the experiment. Leaf gas exchange was measured with a LI-COR LI-6200 closed portable infrared gas exchange system. An attached leaf was placed in a 0.25 liter (165.4 cm³) cuvette for 30 sec. Measurements commenced immediately after CO₂ concentration decreased. CO₂ concentration ranged from 350 to 390 mg.liter⁻¹ (ppm). Data were subjected to analysis of variance (ANOVA). Mean separations were performed via least significant difference (LSD) procedure at P = 0.05.

The remaining trees were grown in non-flooded conditions for the rest of the experimental period at the designated temperature regimes. At the end of the growing season, trees were moved to an outdoor gravel pad and placed in an overwinter structure. Flooding treatments and measurements will be repeated in 1998 to examine the residual effects of flooding over time.

Results and Discussion: There was a significant species x flooding treatment interaction for most P_n and g_s measurements, whereas the flooding treatment x temperature interaction was not significant. Therefore, the response of each species to flooding is presented separately averaged over temperature (Fig. 1). Each species showed a significant decrease in g_s at 1 DAF (data not presented), whereas P_n was not affected by flooding until 4 DAF for *F. sylvatica* and *F. orientalis*. A

decrease in P_n and g_s are often the first signs of flooding stress (Ranney, 1994). Net photosynthesis of *F. grandifolia* that was flooded (F) was not significantly different from the non-flooded trees until 7 DAF. Highly significant decreases in both net P_n and g_s were evident in flooded and intermittent flooded trees from 7 to 30 DAF in all species. Recovery of P_n from flooding treatments (F, I) differed by species. Thirteen days after flooding had been terminated (43 DAF), *F. grandifolia* that was intermittently flooded had returned to P_n levels in non-flooded trees. Net photosynthesis of flooded *F. grandifolia* had fully recovered 41 days after flooding had been relieved (71 DAF). Neither *F. sylvatica* or *F. orientalis* had recovered from either flooding treatment (F, I) by 41 days after flooding treatments were terminated (71 DAF). In addition, increase in total tree dry weight of *F. grandifolia* was greater than the other *Fagus* species in the 30/26 °C (data not presented).

Significance to Industry: Results herein suggest that of the three *Fagus* species in this study, *F. grandifolia* has the greatest tolerance of flooding and high temperature stress. Given the typical summer conditions in the southeastern United States, *F. grandifolia* may have potential as a superior stress-tolerant rootstock for European beech.

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**Propagation of *Magnolia virginiana*
'Santa Rosa' by Stem Cuttings**
(Student)

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Nature of Work: 'Santa Rosa' magnolia, a selection of the native *Magnolia virginiana* L. (Sweet Bay magnolia) introduced by Woodlanders Nursery, Aiken, S.C. in 1979, is an upright, evergreen tree with an open growth habit. Leaves of 'Santa Rosa' are larger than the species as are the lemon scented flowers (Raulston, personal communication). These attributes along with height growth that approaches 1 to 1.5 m (3 to 4 ft) per year make 'Santa Rosa' a desirable landscape plant. However, production of 'Santa Rosa' magnolia has been hindered due to propagation difficulties associated with rooting stem cuttings.

Propagation of magnolias by stem cuttings is reported to be optimized when cuttings are collected soon after the terminal bud is formed and the wood has hardened (semi-hardwood) (1,2). Likewise, treatment of cuttings with naphthaleneacetic acid (NAA), alone or in combination with indolebutyric acid (IBA) also has improved adventitious rooting (1,2).

In many cases, stem cuttings of 'Santa Rosa' have proven difficult or impossible to root (Allen, Burns, and McCartney, personal communication). Following insertion into a rooting medium, softwood cuttings drop their leaves and soon die, whereas semi-hardwood cuttings retain their leaves, yet fail to root. Propagation of magnolias by hardwood cuttings is not normally practiced. Rooting of 'Santa Rosa' magnolia can vary widely from year to year indicating a narrow window of opportunity may exist for optimum rooting. Therefore, the objectives of this research were to evaluate the influence of stock plant growth stage, auxin form, and auxin concentration on rooting stem cuttings of 'Santa Rosa' magnolia.

Due to a shortage of stock material, softwood and hardwood terminal cuttings were collected from stock plants growing in three separate locations: (1) JC Raulston Arboretum, North Carolina State University, Raleigh, (2) Tar Heel Native Trees, Clayton, N.C., and (3) Gilbert's Nursery, Chesnee, S.C. Softwood, semi-hardwood, and hardwood terminal cuttings were taken in June, November, and February 1997, respectively. All semi-hardwood cuttings were collected from plants at Tar Heel Native Trees.

From the initial cutting material, 10 to 15 cm (4 to 6 in) long terminal cuttings were prepared. Leaves were removed from the lower half of the cuttings and the remaining leaves were cut in half. The basal 1 cm (0.4 in) of the cuttings was then treated for 1 sec with 0.0, 6.15, 12.3, 24.6, or 49.2 mM (0.0%, 0.13%, 0.25%, 0.5%, or 1.0%) IBA in factorial combination with equivalent mM concentrations of NAA (0.0%, 0.11%, 0.23%, 0.46%, or 0.92%) in 50% isopropanol. Hardwood cuttings were not treated with the 6.15 mM concentration. Due to basal stem necrosis on hardwood cuttings at the highest auxin concentration (49.2 mM), softwood and semi-hardwood cuttings were not treated with this concentration.

Following auxin treatment, cuttings were allowed to air dry for 15 min before inserting the basal 5 cm (2 in) into a raised greenhouse bench containing a medium of 2 perlite : 1 peat (v/v) with bottom heat maintained at $24 \pm 2^\circ\text{C}$ ($75^\circ \pm 4^\circ\text{F}$). Intermittent mist operated daily for 5 sec every 6 min during daylight hours. Cuttings were maintained under natural photoperiod and irradiance with day/nights of 24° (5°C ($75^\circ \pm 9^\circ\text{F}$)/ $18 \pm 5^\circ\text{C}$ ($65^\circ \pm 9^\circ\text{F}$)).

For each growth stage the experimental design was a randomized complete block with a factorial arrangement of treatments, 4 IBA concentrations x 4 NAA concentrations x 6 cuttings per treatment x 5 replications. After 14 weeks for each growth stage, cuttings were harvested and data recorded. Data included, percent rooting, number of primary roots ≥ 1 mm (0.04 in), and root lengths. All data except rooting percentages were based on the actual number of cuttings that rooted (at least one primary root). Data were subjected to analysis of variance and regression analysis.

Results and Discussion: The majority of softwood cuttings died within 3 weeks. Overall rooting of softwood cuttings was poor (22%) and was unaffected by auxin treatments (data not presented).

An interaction between IBA and NAA was observed with semi-hardwood cuttings. When NAA was absent from the treatment solutions, rooting increased linearly with increasing IBA concentration (Fig. 1) with a maximum of 83% at 24.6 mM (0.5%) IBA. Addition of increasing concentrations of NAA, never stimulated rooting greater than that of 24.6 mM IBA alone.

Similar to softwood cuttings, hardwood cuttings rooted poorly. At this growth stage, increasing NAA concentrations significantly reduced rooting linearly whereas rooting was unaffected by IBA (Fig. 2). Averaged across IBA concentrations, rooting of 29%, 21%, 16%, and 3% was

observed for hardwood cuttings treated with 0, 12.3, 24.6, or 49.2 mM NAA, respectively. This was unexpected given the previously mentioned reports that NAA increases rooting in species of *Magnolia* (1,2).

Root number and root length were unaffected by auxin treatments. Mean root number for all growth stages and across all treatments varied from 1.9 to 3.2 roots per cutting while mean root length ranged from 7 to 14 cm (2.8 to 5.5 in).

Significance to Industry: Results demonstrate that stem cuttings of *Magnolia virginiana* 'Santa Rosa' can be rooted in high percentages. However, a key factor in successful rooting is the growth stage. The best rooting (83%) was achieved with semi-hardwood cuttings collected once terminal growth ceased (mid-November, Clayton, N.C.) and treated with 24.6 mM (0.5%) IBA in 50% isopropanol for 1 sec. Softwood and hardwood cuttings rooted poorly with the best rooting never exceeding 36% for both growth stages. In contrast to reports that NAA is an effective root-promoting compound for some species of *Magnolia*, our results demonstrated that NAA was of no benefit, and in some cases, inhibited rooting of stem cuttings of 'Santa Rosa' magnolia. Thus, growers should exercise caution when using popular commercial rooting formulations that contain NAA when attempting to root stem cuttings of 'Santa Rosa' magnolia.

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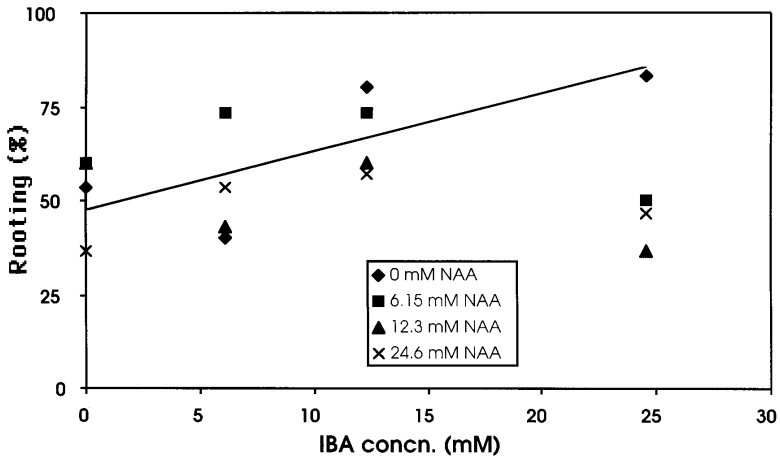


Fig.1. Influence of IBA and/or NAA treatment on rooting semi-hardwood cuttings of 'Santa Rosa' magnolia. Symbols represent means, n=5. Regression line represents predicted response where NAA=0. $LSD_{0.05}=28$

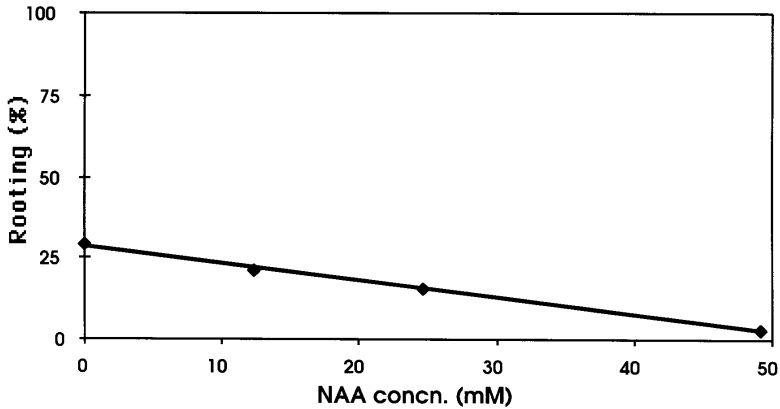


Fig. 2. Influence of NAA treatment on rooting hardwood cuttings of 'Santa Rosa' magnolia. Symbols represent means, n=20. Regression line represents predicted response at a given concentration of NAA. $LSD_{0.05}=12$.

Influence of Liriope Division Size on Subsequent Growth (Student)

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Nature of Work: Liriope, a groundcover with increasing popularity in the United States, is commonly propagated by division. During division, many liriope growers prune both the shoot and root system of each bibb 2 to 3 inches from the crown (1). We observed in a previous experiment that liriope appears to form new roots faster when the shoots are not cut back. This could be the result of either a larger shoot system to support root development or greater root regeneration from a larger root system. However, we have found no published research that examined this aspect of liriope production. The objective of this study is to determine whether cutting back shoots of *Liriope muscari* slows subsequent regeneration of roots and shoots.

Trade gallon containers of *Liriope muscari* 'Big Blue' were divided into single bibbs on June 6, 1997. Bibbs were selected for uniformity of root system; any roots over 4 inches long were cut. Most roots ranged in length from 2 to 4 inches. Bibbs not cut back had an average shoot length of 12.5 inches. After division, each bibb was potted into a 18-inch deep with a 4-inch diameter root chamber (PVC cylinder with acetate over a 7-inch window) in a pinebark : sand medium amended with 12.5 lb of Osmocote 18-6-12, 5 lbs of dolomitic limestone, and 1.5 lb of Micromax / yd³. Root chambers were placed on a tilted bench which directed root growth toward the window. Treatments consisted of: 1) a single unpruned bibb containing 10 or more roots; 2) a single bibb with 10 or more roots and shoots cut to 2 inches; 3) a unpruned single bibb with 5 or less roots; and 4) a single bibb containing 5 or less roots with shoots cut to 2 inches.

Experimental design was a 2X2 factorial with 12 single plant replications in a completely randomized design. Root tips were counted every other day beginning when the first root appeared in the window until 25 root tips appeared. Root were assessed at the end of the study (August 18, 1997) and rated on a 1-5 scale where 1=small root mass, 3=moderate root mass, and 5=large root mass. Number of bibbs per container and shoot and root fresh and dry weights were also collected at the end of the experiment.

Results and Discussion: Plants with shoots not cut developed root systems faster than plants with shoots cut back to 2 inches. For example, unpruned plants with ≥ 10 roots or ≤ 5 roots had 25 new root tips in 31.7 and 36.4 days respectively, while similar treatments with shoots cut took 44.5 and 52.4 days respectively before they had 25 new root tips (Table 1). By developing early root systems, both treatments with the shoots not cut also had larger root systems at the end of the experiment which is shown by the root rating. Unpruned plants with ≥ 10 roots and ≤ 5 roots had root ratings of 3.7 and 3.4, respectively, while plants with similar treatments with shoots cut back had ratings of 2.6 and 1.8, respectively. Plants with shoots not cut also produced more bibbs than plants with shoots cutback. Even though inherent differences existed between not cut (larger) and cut (smaller) plants, shoot fresh and dry weights followed a similar trend to bibb numbers. Overall, the results from the treatment with ≥ 10 roots and shoots not cut were expected; larger plants should generate new growth faster than smaller plants. However, the similarity of that treatment to the treatment with ≤ 5 roots and shoots not cut and the dissimilarity to the treatment with ≥ 10 roots and shoots cut back suggests that shoots play a more important role than roots in the generation of new shoots and roots.

Significance to Industry: Our results show that when liriopae shoots are left intact at time of division, regardless of the root system, they produce more shoots and roots faster than when the shoots are cut back to 2 inches. Growers producing liriopae liners should be able to root and sell a crop more quickly if shoots are not cut at division.

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Table 1. Root rating, bibb number, days until 25 roots tips were visible, and shoot and root fresh and dry weights of liriope.

	<u>>10 roots, shoots uncut</u>	<u>>10 roots, shoots cut</u>	<u><5 roots, shoots uncut</u>	<u><5 roots, shoots cut</u>
Days to 25 root tips	31.7c ^x	44.5b	36.2c	52.4a
Bibb number ^y	6.9a	4.3c	5.8b	2.8d
Root rating ^z	3.7a	2.5b	3.4a	1.8c
Shoot fresh wt.	15.3a	6.5b	16.4a	5.2b
Root fresh wt.	9.1a	3.8b	9.5a	2.5b
Shoot dry wt.	3.3a	1.2b	3.0a	0.9b
Root dry wt.	1.0a	0.3b	0.9a	0.2b

^z Root rating at end of study on a 1-5 scale where 1=small root mass, 3=moderate root mass, and 5=large root mass.

^y Bibb number, shoot and root fresh and dry weight were collected when the experiment was terminated, August 18, 1997.

^x Means were separated using Duncan's Multiple Range Test were P=0.05.

**Effect of Timing and Frequency of Controlled
Pollinations on Seed Set in Flowering Dogwood**
(Poster)

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Nature of Work: Flowering dogwood (*Cornus florida* L.) is subject to several disease and insect problems that could best be controlled by the development of pest-resistant cultivars. Development of improved cultivars through breeding requires controlled pollinations which are usually made by manually apply pollen from one plant onto the stigma of another plant. Flowering dogwood produces inflorescences that contain approximately 30 flowers; however, since these flowers open over a 2-3 week period a single application of pollen is likely to produce only a few seeds. In contrast, daily hand pollinations are not only labor intensive, physical damage may occur to the inflorescence as a result of repeated handling. The purpose of this study was to determine the minimum number of hand-pollinations required to produce the maximum number of seeds in flowering dogwood.

Six hand-pollination treatments and an open-pollinated control were included in this study. Each hand-pollination treatment consisted of five inflorescences on an individual plant, all of which were pollinated with the same pollen source. To eliminate contaminant pollinations, inflorescences were covered with breathable plastic bags both prior to flower opening and following pollination. Since reports (1, 2) have indicated that flowering dogwood is self-sterile, flowers in the hand-pollination treatments were not emasculated. The open-pollinated control consisted of five inflorescences that were marked with tags prior to flower opening, but were neither bagged nor subjected to hand-pollinations. The experiment was repeated 10 times.

The controlled pollinations were pollinated every day (Treatment A), every other day (Treatment B), every third day (Treatment C), every sixth day (Treatment D), and one time only (Treatments E and F). Treatments A, B, C, and D were performed over a 12 day period, with day 1 being the first day in which open flowers were observed in half of the inflorescences in the open-pollinated control (Treatment G). For Treatment E pollinations were made only on day 6, while in Treatment F they were made only on day 12. In all hand-pollination treatments, all open flowers within an inflorescence were pollinated during each pollination event, regardless of whether they had been previously pollinated.

Data were collected on number of seed produced from each group of five inflorescences, and a mean number of seed per inflorescence pollinated was calculated. Data were subjected to analysis of variance procedure. Treatment means were separated by least significant difference (LSD), $P=0.05$.

Results and Discussion: There were significant differences among the pollination treatments in number of seeds produced (Table 1). Daily or every other day pollinations resulted in more seed per inflorescence pollinated than did single applications of pollen. Statistically, there were no differences between the open-pollinated control and any of the hand-pollination treatments.

Table 1. Pollination treatments tested for seed production in flowering dogwood

Treatment	Frequency of pollination	Mean number of seed produced/inflorescence pollinated ¹
A	Every day, up to day 12	3.2 a
B	Every 2 days, up to day 12	3.2 a
C	Every 3 days, up to day 12	2.4 ab
D	Day 6 and day 12	1.9 abc
E	Day 6	0.8 bc
F	Day 12	0.4 c
G	Open-pollinated	1.8 abc

¹Means followed by the same number are not statistically different ($P=0.05$).

While the greatest numbers of seed were produced following daily pollinations, Treatment D, which involved two pollinations, was the most labor efficient. Once an inflorescence has received two pollinations, it appears a greater increase in seed production can be achieved by pollinating additional inflorescences rather than by making additional pollinations on the same inflorescence. This assumes that the number of inflorescences available for pollination is not limited, which may not be the case when dealing with young plants. Further investigations are needed for determining the best timing of these two pollinations.

Significance to Industry: This study indicates that production of seed from hand-pollinations in flowering dogwood can be increased by the use of multiple pollinations. Two pollinations, spaced a few days apart, should result in good seed set with a minimum labor requirement. This information will assist in producing sufficient seed for performing breeding studies in flowering dogwood.

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Propagation of *Cephalotaxus* Cuttings
(Poster)

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Nature of Work: On the Oak Hill Plantation of Berry College, Rome, GA a few ancient specimens of plum yew are growing in the foundation planting of the mansion. We estimated the plants to be at least 75 years old. The largest is about 8 ft tall with about a 10 ft spread and displays a distinctly dimorphic foliage pattern, with spiral arrangement of foliage on upright branches (orthotropic) and two ranked foliage on side branches (plagiotropic). After consulting the literature, we tentatively identified these as *Cephalotaxus harringtonia* ‘Fastigiata’ (1, 2, 3, 6). Dirr (3) mentions this is “not the easiest genus to separate into identifiable species...but from a landscape perspective there is little difference.” ‘Fastigiata’, however, has a distinctive upright growth habit, at least while young, that is similar to a coarse foliaged Hick’s yew, and is said to be the “only garden form which is hardy in cold areas” (2). Tripp and Raulston (6) give the best and most complete treatment of the species in the current literature. Plum yew is heat tolerant (unlike *Taxus*), slow growing, and said to be deer resistant (3), also unlike *Taxus*. A search of the literature led us to agree with Dirr (3,4) that there are no reliable references on propagation. Raulston (5) recommended semihardwood or hardwood cuttings taken anytime from June through April. Dirr and Heuser (4) recommended fall collection of cuttings and 5,000 to 10,000 ppm IBA-talc or solution. They mentioned rooting was slow, taking up to 4 months and that juvenility may also be a factor in rooting success. Impressed with the beauty and durability of the plants at Oak Hill, we endeavored to propagate them in two randomized replicated experiments. The first experiment was with cuttings from the parent plants and the second was with terminal cuttings from the propagules after they had been grown on for two years.

For experiment #1, Mr. John Watkins, horticulturist at Oak Hill and Berry College, collected several hundred cuttings, packaged them and shipped them UPS to Knoxville in late fall of 1995. We trimmed the cuttings to about five inches in length and stripped the basal two inches prior to a five second quick dip of Dip and Grow diluted to hormone levels of 3000, 6000, and 9000 ppm. The Dip and Grow formulation contains 10,000 ppm IBA and 5000 ppm NAA. The other treatment factor was penetrating agents. We used water to dilute Dip and Grow as a control, and added 20% triethanolamine (TEA) or 20% dimethylformamide to the

other dilution series, resulting in a 3x3 factorial combination of treatments. We stuck five cuttings per experimental unit, with four replications in a randomized complete block arrangement of treatments. Media was 40% Pro-Mix BX and 60% perlite, contained in 5 inch deep nursery flats. Flats were placed on a propagation bench with Biotherm bottom heat maintained at 80F. Mist was applied during daylight hours for six seconds every six minutes. Rooting was very slow. Most cuttings were well rooted after nine months, when we counted the number of roots on each cutting and measured the mm spread of the root ball. Data were subjected to analysis of variance using the GLM procedure in SAS. Significance was accepted at the 5% level.

Experiment #2 was similar except that cuttings were taken 23 Oct 97 after the first frost from the plants we grew on from the first experiment. Cuttings were handled as before and the experimental design was the same except we only used one level of Dip and Grow, 9000 ppm diluted with water as a control or using 20% TEA or 20% DMF as penetrating agents. We used 5 plants per experimental unit and had sufficient cuttings to allow 24 replications. The 80F bottom heat was supplied by rubber heating mats and the mist cycle was the same. The media was changed to two parts pine bark, one part Pro-Mix BX and one part perlite. Cuttings were evaluated about nine months later. Roots were counted and root spread measured as above, plus a subjective evaluation rated each cutting on a scale of 1 = poorly rooted to 5 = heavily rooted. Data were subjected to analysis as above.

Results and Discussion: Rooting was extremely slow and variable in both experiments, though eventually a high percentage of cuttings did root. DMF resulted in greater root numbers per cutting than TEA treatment in experiment #1 (18.4 vs 17.2) while controls were intermediate 18.4. The 6000 ppm hormone level had more roots than the 3000ppm level (19.3 vs 16.9) with the 9000 ppm level intermediate (18.5). There was no difference in root spread among the penetrating agent treatments, though the 6000 ppm hormone level had a bigger root ball (24.4 cm or 9.6 in) than those in the 3000 ppm level (21.4 cm or 8.4 in) or the 9000ppm level (22.0 cm or 8.7 in).

In experiment #2 about 85% of cuttings rooted with no differences between the treatments in number of roots per cutting, and the range of values obtained was very similar to experiment #1. There was no effect of penetrating agent on root spread in this experiment, with 17.6, 17.3 and 16.1 cm (6.9, 6.8, and 6.3 in) for TEA, control and DMF respectively. Subjective root ratings were not significant and ranged from 3.1 to 3.3. A correlation analysis showed that our subjective rating was valid, with highly significant Pearson correlation coefficients of .74 for root number

and .85 for root spread. We noted that the basal end of many cuttings appeared to have died back ("burn"), with 48% occurrence in the control, 37% in TEA, and 36% in DMF treatments, but there was no statistical difference.

Significance to Industry: Unlike our excellent results using penetrating agents on rooting 'Nellie R. Stevens' holly (see article elsewhere in this Proceedings), there was little effect on the rooting of *Cephalotaxus*. Using DMF as a penetrating agent resulted in more roots/cutting than TEA in experiment #1 but had no effect in experiment #2. In the holly experiment, TEA was superior. Either DMF or TEA can be used as a penetrating agent for quick dips. *Cephalotaxus* can be successfully rooted in fairly high percentages using methods similar to these experiments, given sufficient time in the propagation bench. It appears that 6000 to 9000 ppm Dip and Grow was the best level of rooting hormone to use.

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Evaluation of *Prunus Cerasus* Germplasm for Cold Resistance

(Poster)

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Nature of Work: An understanding of cold resistance heritability and environmental influence in sour cherry is critical to cultivar development and future germplasm collection. At the whole plant level, resistance to cold is a complex quantitatively inherited character (Cummins and Aldwinkle 1983). Principal Component (PC) analysis has been used successfully to understand the response of complex traits to imposed treatments or evolutionary pressures (Iezzoni and Pritts 1991).

The DTA profile in species which deep supercool indicates two exotherms (Quamme 1985). The first is associated with freezing from heterogeneous nucleation, the high temperature exotherm (HTE). The second exotherm is associated with the tissues that supercool to the homogeneous nucleation temperature, the low temperature exotherm (LTE). Xylem tissue in some species may respond as a heterogeneous population of cells that freeze over a wide temperature range (Wisniewski 1995). The number of LTEs can also vary, Ketchie and Kammereck (1987) observed that only during midwinter was the exotherm peak associated with xylem tissue injury.

The plant material used is displayed in Table 1. A three-way treatment structure, CRD, with four replicates and three sub-samples per treatment was used. The study was conducted from August 1990 to March 1991. Artificial hardening was used according to Sakai (1982). Samples were prepared according to McKenzie and Weiser (1975) and frozen in a converted (Mathers et al. 1991) ultralow Revco freezer. A linear programmed freeze regime was used dropping by 5°C h^{-1} to a series of test temperatures at five-degree intervals from -5 to -50°C . Cross sections of the twigs and vegetative buds were examined under a dissecting microscope (40X) after regrowth and scored numerically (0-4) for occurrence of oxidative browning (Quamme et al. 1982). Quamme et al. (1982) used hand-fitted graphs and defined the temperature of incipient injury as the temperature at which the average injury score did not exceed 1 (a trace of injury). Here incipient injury was defined as the temperature at which injury does not exceed 1.0 using non-linear regression of the 0-4 injury scores (IT). The IT values were determined graphically using PLOT-IT[®] Non-Linear Regression function $Y = B(1)/(1.0+B(2)*\text{EXP}(-B(3)*X))$. The coefficients of B were determined by PLOT-IT[®] and were

different for each curve calculated. Analyses were conducted on the calculated IT means using the SAS© procedure GLM. IT values were rounded to the nearest 0.50C (Cain and Andersen 1976).

In the DTA, four samples per cultivar and seedling were evaluated at each collection date. Linear regression analyses were performed, where the means of the LTE were regressed on the means of IT. The (r^2) values were determined using Microsoft Excel®. Principal component (PC) analysis was performed using the PRINCOMP procedure in SAS©.

The main objective of this study was to compare DTA and visual browning as methods to determine minimum hardiness levels for cherry xylem, phloem-cambium, cortex and vegetative buds. The second objective was to determine if these methods detect differences in these tissues in the cherry germplasm from August to March. The third objective was to determine if the cherry selections differed in their cold resistance. The fourth objective was to use PC analyses to examine the possible association of minimum survival temperatures to geographic origin.

Results and Discussion: There was a significant linear relationship between oxidative browning, measured as IT, and the LTE for the xylem and phloem-cambium tissues from November to March (Fig. 1 and 2). The (Group * X) factor is non-significant indicating the slopes of the two tissues are not significantly different (Table 2). Cortical tissue and vegetative bud injury were not correlated to the stem LTEs.

In acclimation, November, the best correlation of the LTE was to the phloem-cambium (data not shown). In midwinter and deacclimation December to March, the best correlation of the LTE was to the xylem browning (Table 3).

The most susceptible vegetative tissue, evaluated over all months, in 14 of 15 selections, was the phloem-cambium (Table 4). For *P. avium* the lowest survival temperatures for the phloem-cambium tissue occurred in February (Fig. 3) and the most susceptible time for midwinter injury was December and January (Fig. 3). In March, the phloem-cambium deacclimated much more rapidly than the xylem showing a loss in hardiness of 3.8°C averaged over the 15 selections (Table 5).

Proceeding from negative to positive values of PC1, the cultivar and seedling means decrease in hardiness (Fig. 4). The majority of separation of PC1 was due to differences in hardiness values in October, December, January and March. The sweet cherry and Hungarian selections are at the positive end of PC1, while the selections derived from colder regions, Germany and Moldavia are at the negative end.

The two presumed progenitors of sour cherry are situated at the extremes of PC1 (Fig. 4).

Significance to Industry: We observed a significant correlation between the phloem and LTE's in acclimation, and no significant correlation to the xylem until midwinter. The phloem was found to be significantly less hardy than xylem in every month except November and summed over all months was significantly less hardy than the other two woody tissues xylem and cortex. Phloem may be the critical tissue limiting commercial distribution of sour cherry, due to its lack of hardiness during acclimation (September to October) and deacclimation (March). The finding that phloem is important in commercial distribution is supported in the PC analysis conducted on the phloem.

The survival curves developed using the PLOT-IT® Non-Linear regression equation can be used to express hardiness in absolute terms. It is possible to relate absolute hardiness indices to environmental survival (Quamme 1978).

This work is relevant in a sour cherry breeding program but similar cold hardiness evaluations could be conducted in any woody plant with broad geographic distribution. Determination of what tissue is critical to commercial range is essential to grower and breeders. Earlier studies have focused on the xylem and its role in determining geographic distribution. This study is the first to implicate phloem as the tissue critical to commercial range.

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Table 1

Names, abbreviations, presumed origins and type designations of the cultivars and seedlings evaluated.

Name	Abbr	Presumed Origin	Type
<i>Prunus avium</i>			
Emperor Francis	F	Unknown	Southern
Schmidt	S	Unknown	Southern
<i>Prunus cerasus</i>			
Cigany Meggy o.p.	G	Hungary	Southern
Csengodi Csokras o.p.	K	Hungary	Southern
English Morello	E	Germany	Northern
English Morello X Sumadinka	U	Germany X Yugoslavia	Northern
Fructbare von Michurin	V	Moldavia	Northern
Meteor	R	Montmorency X Russian Seedling	Northern
Montmorency	M	France	Northern
Oblacinska o.p.	O	Yugoslavia	Southern
Pandy 114 o.p.	A	Hungary	Southern
Pitic de lasi o.p.	I	Moldavia	Northern
Spaniole X Crisana o.p.	C	Spain X Romania	Southern
Wolynska X Sumadinka	W	Poland X Yugoslavia	Northern
<i>Prunus fruticosa</i> 586-1	P	Unknown	Northern

Table 2. Mean square and F values for GLM analysis of the comparison of regression slopes of xylem (Fig. 1) and phloem (Fig. 2).

Source of variation	df	Mean square	Pr > F
Group	1	1286.51	0.0001
X (LTE)	1	251.48	0.0001
X*Group	1	10.64	0.4030
Error	135	15.11	
Total	138		

Table 3

Correlation coefficient values and significance for xylem incipient injury and twig LTE's for different time periods.

Time	Sample size	R ²	R
Nov-Mar.	69	0.6092	0.7805**
Nov.	14	0.3234	0.5685*
Dec.-Feb.	42	0.6968	0.8347**
Mar.	13	0.9166	0.9574**

Table 4

Comparison of tissue types of 15 cherry crosses/cultivars evaluated over 8 months by exotherm analysis conducted at -5°C per hour and by visual injury of phloem-cambium, xylem and cortical shoot sections and vegetative bud cross-sections frozen to -50°C at -5°C per hour.

Cultivar/Cross	Low Temp. Exotherm ($^{\circ}\text{C}$)		Incipient Temp. ($^{\circ}\text{C}$)		
	Twig ^z	Xylem	Phloem	Cortical	VB
<i>Prunus avium</i>					
Emperor Francis	-19.0 a	-24.0 a	-14.0 a	-20.0 a	-19.5 a
Schmidt	-22.5 a	-24.5 a	-16.5 a	-22.0 a	-20.5 a
<i>Prunus cerasus</i>					
Csengodi Csokas	-27.0 b	-25.5 a	-19.0 a	-22.0 a	-24.0 b
Spaniole X Crisana	-25.0 a	-25.0 a	-21.0 b	-23.5 b	-25.0 b
Pandy 114	-27.5 b	-24.5 a	-20.0 b	-24.0 b	-24.5 b
Pitic de lasi	-29.0 b	-28.0 b	-23.0 c	-25.0 b	-25.0 b
Wolynska X Sumadinka	-29.0 b	-30.0 b	-24.0 c	-28.0 c	-27.0 b
EM X Sumadinka	-27.5 b	-29.0 b	-24.5 d	-25.5 c	-26.0 b
Oblacinska	-30.0 b	-26.5 a	-22.5 c	-24.5 b	-28.5 b
Cigany Meggy	---	-27.0 b	-21.0 b	-24.0 b	-25.0 b
Montmorency	-31.0 b	-29.0 b	-24.0 d	-26.5 c	-23.0 b
Fructbare von Michurin	-31.0 b	-31.5 b	-25.5 d	-27.0 c	-26.0 b
English Morello	-28.0 b	-25.0 a	-23.0 b	-25.0 b	-25.0 b
Meteor	-33.0 b	-31.5 b	-27.0 d	-29.0 c	-28.5 b
<i>P. fruticosa</i> 586-1	---	-27.0 b	-28.0 d	-28.0 c	-26.0 b

^z Means in columns, based on three sub-samples, per four replicates, followed by different letters are significantly different ($P=0.05$) according to Fisher's least significant difference test.

Table 5

Comparison of a February collection of tissue types of 15 cherry cultivar/seedlings evaluated by exotherm analysis conducted at -5°C per hour and by visual injury of phloem-cambium, xylem and cortical shoot sections and vegetative bud (VB) cross-sections frozen to -45°C at -5°C per hour.

Cultivar/Seedling	LTE($^{\circ}\text{C}$)	Incipient Temp. ($^{\circ}\text{C}$)			
	Twig	Xylem	Phloem	Cortical	VB
<i>Prunus avium</i>					
Emperor Francis	-25.0 a ^z	-28.0 a	-25.0 a	-26.0 a	-28.0 a
Schmidt	-27.0 a	-34.0 a	-30.0 a	-32.0 a	-29.0 a
<i>Prunus cerasus</i>					
Csengodi Csokas	-27.5 a	-32.0 a	-20.5 a	-27.0 a	-32.5 a
(Spaniole X Crisana)	-24.5 a	-24.0 a	-26.0 a	-26.5 a	-31.0 a
Pandy 114	-28.0 a	-31.0 a	-29.0 a	-31.5 a	-32.0 a
Pitic de Iasi	-30.5 a	-35.0 a	-34.5 a	-33.0 a	-32.5 a
Wolynska X Sumadinka	-34.0 a	-38.0 a	-33.0 a	-37.0 a	-31.0 a
EM X Sumadinka	-34.5 a	-36.0 a	-31.0 a	-33.5 a	-32.0 a
Oblacinska	-27.5 a	-30.0 a	-26.5 a	-30.5 a	-31.0 a
Cigany Meggy	-32.5 a	-35.5 a	-32.0 a	-32.0 a	-32.0 a
Montmorency	-33.0 a	-37.0 a	-33.0 a	-35.0 a	-31.0 a
Fructbare von Michurin	-30.0 a	-37.0 a	-32.0 a	-35.0 a	-31.0 a
English Morello	-25.0 a	-33.0 a	-24.0 a	-33.0 a	-30.0 a
Meteor	-33.0 a	-37.0 a	-34.0 a	-37.0 a	-32.0 a
<i>P. fruticosa</i> 586-1	-32.0 a	-32.0 a	-30.0 a	-33.0 a	-31.0 a

^z Means in columns, based on three sub-samples, per four replicates, followed by different letters are significantly different ($P=0.05$) according to Fisher's least significant difference test.

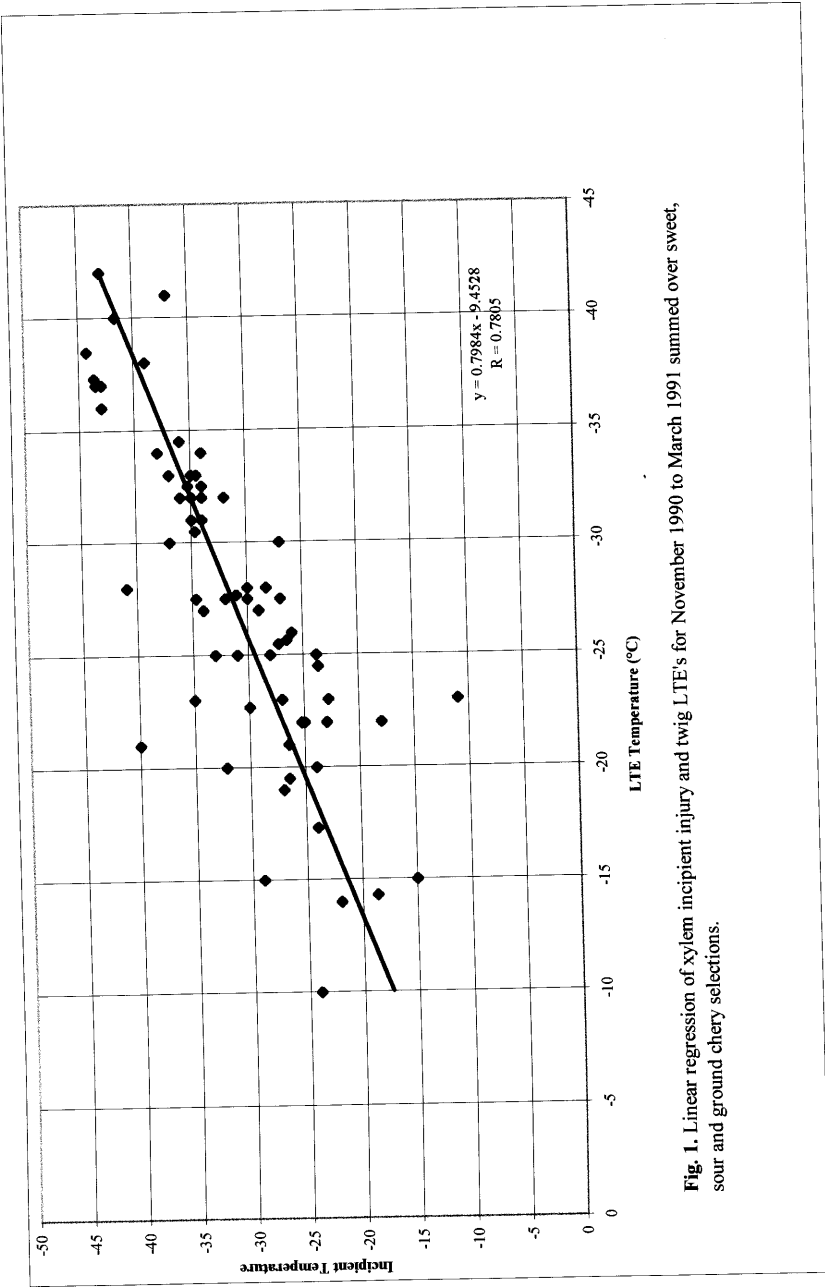


Fig. 1. Linear regression of xylem incipient injury and twig L/TE's for November 1990 to March 1991 summed over sweet, sour and ground cherry selections.

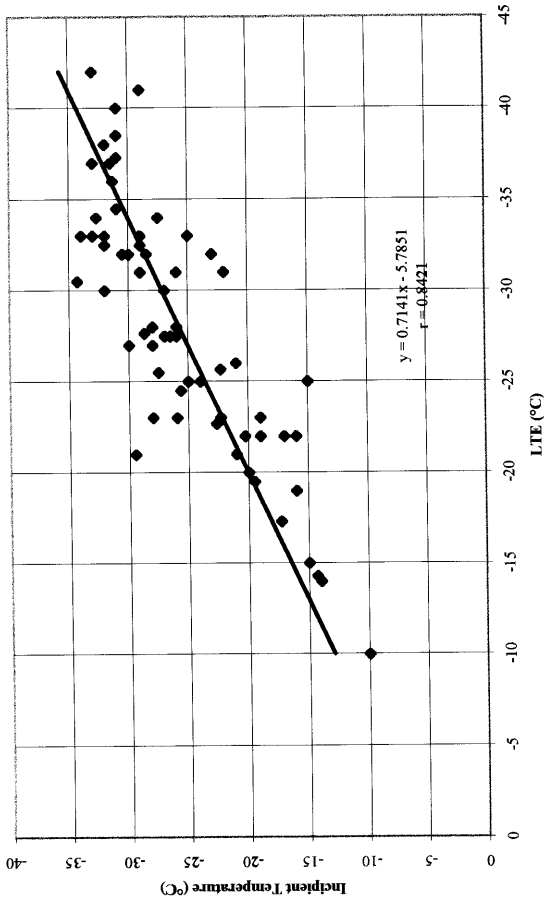


Fig. 2. Linear regression of phloem-cambium incipient injury and the twig LTE's for November 1990 to March 1991 summed over sweet, sour, and ground cherry selections.

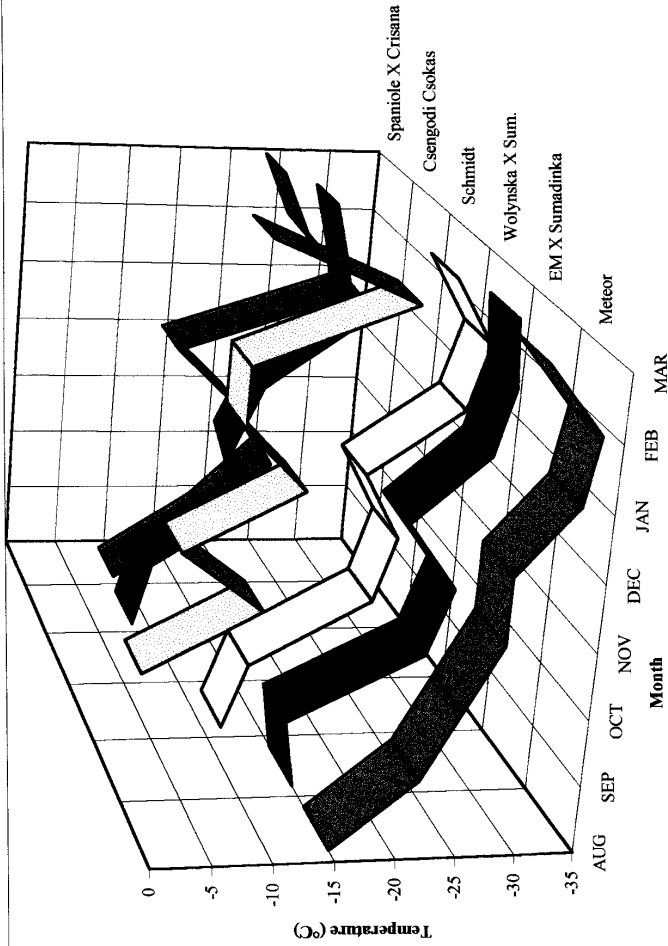


Fig. 3. Cultivar/seedling X month interaction of incipient temperature data of the phloem-cambium tissue, sampled from August 1990 to March 1991.

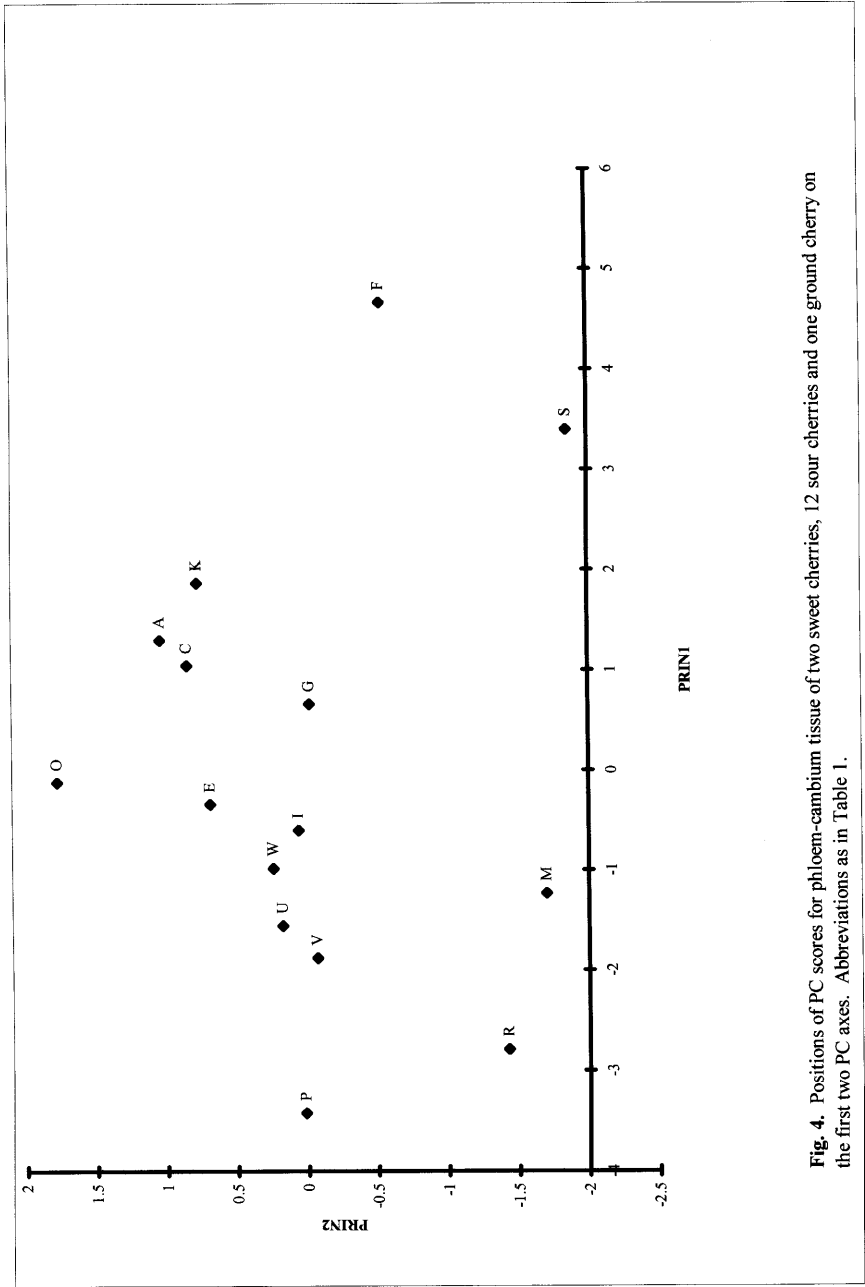


Fig. 4. Positions of PC scores for phloem-cambium tissue of two sweet cherries, 12 sour cherries and one ground cherry on the first two PC axes. Abbreviations as in Table 1.