

SECTION 8 PROPAGATION

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Influence of Seedling Size and Fertility on Growth of Container Grown Pecan Rootstocks

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Nature of Work: Louisiana pecans are commonly propagated in nursery production by whip grafting scions of commercial cultivars onto seedlings of native trees. Seedling vigor from nuts of native pecan trees is highly variable often resulting in rootstock lots with widely varying diameters. The efficiency of whip grafting can be influenced by how closely matched the diameters of the scion and rootstock. (1) The purpose of this investigation was to evaluate the effectiveness of selecting seedling trees by size from seedling beds on the graftable size of rootstock the following season.

Nuts from a seedling population of native pecans were planted in 4:1 pine bark:sand media in the summer 1994. Approximately three hundred seedlings were randomly selected by size from this population of about 2000 in April, 1995. The seedlings were divided into three size classes. The three sizes were small (1.6 - 3.8 mm dia), medium (4.1-5.5 mm dia), and large (5.5-11.4 mm dia). Seedlings were potted in four gallon pots containing a pine bark media amended with dolomitic lime and micronutrients. Trunk measurements were taken after potting at the top of each pot. The size classes were subdivided into two groups of 48 plants each. Each subgroup was divided into three groups of sixteen plants each with a different fertilizer rate applied to each subgroup. The subgroups were replicated twice. The levels of fertilization were 8, 18 and 36 grams of 14-14-14 slow release (3-4 mo) formulation. These rates were applied in mid- April, June and September of 1995. Diameter measurements were made 10 May and 11 November 1995. Daily irrigation was by overhead sprinklers. Weed control was by use of a preemergence herbicide and routine hand pulling of escapes.

Results and Discussion: Initial seedling size had a strong influence on the caliper of rootstock after 8 months of growth in the nursery. The group of seedlings with the larger initial diameters averaged 10.5 mm diameters at the end of the growing season compared to 6.2 and 5.1 mm for medium and small size groups, respectively.

Analysis of growth of the three size groups measured by diameter differences at the end of the growing season indicated a significant difference ($P=0.05$) in growth of the larger seedling groups compared to the two other groups. There were no significant differences in growth rate between the medium and small size groups. The higher fertilizer levels (18 and 36 g pots) significantly ($P=0.05$) influenced the seedling growth rate of each size group. The low rate of fertilizer (8 grams/pot) did not significantly increase the diameter above the control (zero) of any size group.

Significance to the Industry: Factors other than cultural practices influence the growth of pecan rootstocks grown in nursery containers. Genetic variability needs to be considered and larger seedlings selected for faster growth. The rate of fertilization will influence the final diameter obtained regardless of size.

Literature Cited

1. Goff, W.D., J.R. McVay, and M.S. Gazaway. 1989. Pecan Production in the Southeast. The Ala. Cooperative Exten. Service. Circular 459.

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Micropropagation of *Stewartia pseudocamellia*

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Nature of Work: *Stewartia pseudocamellia* Maxim. (Japanese stewartia) is a small tree which is highly prized as a landscape plant because of its showy flowers, exfoliating bark, and attractive fall color. Unfortunately, the species is not widely utilized as an ornamental due, in part, to propagation difficulties both by seed and stem cuttings. Therefore, two experiments were conducted to investigate the feasibility of propagating *S. pseudocamellia* by micropropagation (tissue culture).

In the first experiment, single-node explants were excised from shoots of actively growing stock plants on three dates which represented specific stock plant growth stages. Following surface sterilization, explants were placed on agar-solidified Woody Plant Medium (WPM) (1) containing either no growth regulators or *N*-(3-methyl-2-butenyl)-1*H*-purin-6-amine (2iP) at 5.0 or 10.0 ppm (24.6 or 48.2 μ M) or 0.025 or 0.05 ppm (0.11 or 0.23 μ M) *N*-phenyl-*N*-1,2,3-thiadiazol-5-ylurea (TDZ).

In the second experiment, the three distal axillary nodes of each shoot were excised at 4-day intervals for 28 days beginning 52 days after stock plants were potted following cold storage at 7°C (44°F). Explants were surface sterilized and placed on WPM supplemented with 10 ppm (49.2 μ M) 2iP either alone or in combination with 3 ppm (8.6 μ M) gibberellic acid (GA₃).

Results and Discussion: The first experiment revealed that the most frequent budbreak occurred for explants placed on media containing 2iP at either concentration. Explants cultured at the softwood stage had less contamination and greater budbreak than explants taken from more mature stem tissue.

In the second experiment neither GA₃ or node position influenced budbreak frequency or shoot elongation. Days after potting (stock plant growth stage) influenced frequency of budbreak and shoot elongation with the optimal period for explant collection being 56 to 72 days after stock plants were potted. Elongated shoots (one microcutting per explant) were produced on both media. Microcuttings \geq 10 mm (0.4 in) were rooted using *ex vitro* procedures and acclimatized to greenhouse conditions.

Significance to Industry: Results indicate that *S. pseudocamellia* is amenable to micropropagation. However, before the aforementioned protocol can be applicable in a commercial situation, *in vitro* axillary shoot proliferation must be increased.

Literature Cited

1. Lloyd, G. and B. McCown. 1980. Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot tip culture. Proc. Intl. Plant Prop. Soc. 30:421-437.

Propagation of 'Carolina Sapphire' Smooth Arizona Cypress by Stem Cuttings: Effects of Growth Stage, Type of Cutting, and IBA Treatment

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Nature of Work: 'Carolina Sapphire' smooth Arizona cypress [*Cupressus arizonica* var. *glabra* (Sudw.) Little 'Carolina Sapphire'] is a fast growing, attractive evergreen tree with considerable potential for use in the landscape and as a Christmas tree. Although interest and subsequent demand for this cultivar are increasing, supplies are limited due in part to propagation difficulties. Therefore, the following study was conducted to develop a protocol for propagation of 'Carolina Sapphire' smooth Arizona cypress by stem cuttings. Specifically, the influence of timing (growth stage), type of cutting, and indolebutyric acid (IBA) treatment on rooting were investigated.

Stem cuttings consisting of 30 cm (12 in) terminals or distal [terminal 15 cm (6 in)] and proximal [basal 15 cm (6 in)] halves of 30 cm (12 in) terminals were taken on three dates that represented three growth stages (semi-hardwood, hardwood, and softwood). Cuttings were treated with IBA in 50% isopropanol ranging from 0 to 16,000 ppm (1.6%) and placed under intermittent mist.

Results and Discussion: Regardless of cutting type and auxin treatment, cuttings rooted at each growth stage. Overall percent rooting was highest during the hardwood stage (70%), followed by the semi-hardwood stage (44%). Softwood cuttings exhibited the lowest overall rooting (33%). At each growth stage, the response to IBA in terms of percent rooting, root count, and root dry weight, was variable depending on cutting type. Rooting percentages $\geq 70\%$ were attained at the semi-hardwood and hardwood growth stages, for particular treatment combinations. For example, semi-hardwood distal and proximal halves exhibited rooting of 72% and 75%, respectively, following treatment with 16,000 ppm (1.6%) IBA whereas rooting $\geq 83\%$ was observed for hardwood distal halves treated with IBA ranging from 8000 (0.8%) to 16,000 ppm (1.6%).

Significance to Industry: Results indicate that stem cuttings of 'Carolina Sapphire' smooth Arizona cypress can be rooted at several growth stages. However, semi-hardwood and hardwood cuttings appear more amenable to rooting than softwood cuttings. Treatment of cuttings with IBA will stimulate rooting depending on growth stage and the type of cutting.

Propagation of *Quercus myrsinifolia* and *Quercus canbyi* by Stem Cuttings

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Nature of Work: *Quercus myrsinifolia* Bl. (Chinese evergreen oak) is a large, evergreen tree indigenous to Japan, southern China, and Laos (3,4). On the other hand, *Quercus canbyi* Trel. (Canby's oak) is deciduous to semi-evergreen and native to the highlands of the Sierra Madre region of Nuevo Leon in northeastern Mexico and grows as a shrub or small tree (2,5).

Specimens of *Q. myrsinifolia* and *Q. canbyi* growing on the campus of North Carolina State University, Raleigh and at the North Carolina State University Arboretum have performed well. The excellent performance of these species strengthens their potential for use in landscapes of the southern United States.

Traditionally, most species of oak (*Quercus* L.) have been propagated by seed. However, since members of this genus are extremely heterozygous, sexual propagation results in great genotypic and phenotypic variability (1). Development of efficient techniques for asexual propagation of oaks would benefit the nursery industry as this would lead to selection and production of particular clones with desirable landscape characteristics.

In a preliminary investigation, stem cuttings of *Q. myrsinifolia* and *Q. canbyi* produced robust root systems under intermittent mist following treatment with indolebutyric acid (IBA). Therefore, the following research was conducted to develop protocols for propagation of these two species by stem cuttings. Specifically, the influence of timing (growth stage) and IBA treatment on rooting were investigated.

Stem cuttings of two clones (clone 1 and 2) of *Q. myrsinifolia* and one clone of *Q. canbyi*, of seedling origin and in the adult growth phase, were taken on various dates representing specific growth stages. Cuttings of clone 1 of *Q. myrsinifolia* were collected at the semi-hardwood, hardwood, or softwood stages in addition to a transitional stage between softwood and semi-hardwood. For clone 2, stem cuttings were taken only at the softwood and transitional softwood/semi-hardwood stages. Cuttings of *Q. canbyi* were collected at the semi-hardwood, hardwood, or softwood stages. Cuttings of both species were treated with 0, 1500 (0.15%), 3000 (0.3%), 6000 (0.6%), or 9000 ppm (0.9%) IBA in 50% isopropanol. All cuttings were placed in a raised greenhouse bench and rooted under intermittent mist.

Results and Discussion: Semi-hardwood or hardwood cuttings of *Q. myrsinifolia* or *Q. canbyi* did not root. Responses of stem cuttings of *Q. myrsinifolia* to IBA treatment varied by growth stage. For softwood cuttings, response to IBA was quadratic with the greatest rooting noted for clones 1 (57%) and 2 (72%) when treated with 1500 and 3000 ppm IBA, respectively. Treatment with IBA had no effect on percent rooting of softwood/semi-hardwood cuttings of clone 1, with rooting ranging from 47% to 58%.

However, a linear decrease in rooting in response to IBA was observed for clone 2 with the greatest rooting occurring for the nontreated cuttings (89%). Softwood cuttings of *Q. canbyi* responded quadratically to IBA treatment, with maximum rooting of 33% noted for cuttings treated with 1500 ppm IBA.

Significance to Industry: Results herein demonstrate that *Q. myrsinifolia* and *Q. canbyi*, when in the adult growth phase, can be propagated by stem cuttings which should allow selection and propagation of trees with desirable physiological and morphological characteristics. Although percent rooting of *Q. canbyi* was low (33%), additional research by the authors has demonstrated that much higher rooting is possible.

Literature Cited

1. Hartmann, H.T., D.E. Kester, and F.T. Davies, Jr. 1990. Plant Propagation: Principles and Practices. 5th ed. Prentice Hall, Englewood Cliffs, N.J.
2. Nixon, K.C. 1993. The genus *Quercus* in Mexico: Origins and distributions, p.447-458. In: T.P. Ramamoorthy, R. Bye, A. Lot, and J. Fa (eds.). Biological Diversity of Mexico. Oxford Univ. Press, New York.
3. Numata, M. 1974. The Flora and Vegetation of Japan. Kodansha Ltd., New York.
4. Ohwi, J. 1984. Flora of Japan. Smithsonian Institution, Washington, D.C.
5. Trelease, W. 1924. The American Oaks. vol. XX. National Academy of Science, Washington, D.C.

Use of Molecular Markers In a Breeding Program for *Cornus florida*

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Nature of Work: Cultivars of flowering dogwood (*Cornus florida* L.) have been developed by nurserymen from either vegetative sports (mutations in buds) or seedlings selected by astute observers for one or more horticulturally pleasing and/or functional attributes. Usually, the genetic basis for these unique qualities remains unknown.

Breeding of dogwood species is practically a nonexistent method for developing new plant materials. Dr. Orton at Rutgers University has produced several hybrids between flowering dogwood and chinese dogwood (*Cornus kousa* Hance), the 'Constellation' series, in a breeding program. Since these two species have very different horticultural and botanical traits and it is relatively easy to identify interspecific hybrids. However, we are unaware of any sustained effort to apply breeding methods intraspecifically to improve the horticultural quality in new cultivars of flowering dogwood. The reasons plantsmen have not tried to breed flowering dogwood are diverse and many, but among these are the lack of diagnostic phenotypic (visible) markers and the absence of knowledge concerning heritability of specific traits (e.g. disease resistance, etc.) for individual cultivars. Lack of both of these hampers identification of progeny resulting from the mating of two similar, but different, cultivars.

When the parentage of seedlings is impossible to determine because of lack of visually discerning characteristics, DNA markers can provide confirmation of hybridization. Recent advances in molecular biology allow identification of successful crosses without prior knowledge of any specific characteristics of either parent. Parent trees can be "DNA fingerprinted"; that is, their unique genetic patterns are recorded on polyacrylamide gels. The fingerprint patterns generated are used to identify individual trees or cultivars that are the parents in the breeding program.

Tree breeding and molecular biology investigations were initiated in 1993 as a concerted effort between scientists at the Tennessee State University Nursery Crops Research Station and the University of Tennessee Agricultural Experiment Station. The ultimate objective was to combine resistance to dogwood anthracnose with desirable horticultural characteristics of established cultivars.

Trees used in the breeding experiments were held in cold storage until native and ornamental populations of flowering dogwood in Knoxville, TN had finished flowering. Hybridization between several combinations of two cultivars was attempted using manual or honey bee-mediated pollination. Young, not fully expanded leaves from each of the parents were collected and total genomic DNA extracted using the method of Yoon et al. (6) as modified by Trigiano et al. (5) or using a Puregene DNA extraction kit. DNA amplification profiles were generated using 15 arbitrary oligonucleotide primers, each consisting of eight base pairs, and the products separated on 4.5% polyacrylamide gels (2). Amplification profiles, especially those with usable (unique) markers, were generated at least three times for each male parent and visualized using a fast and sensitive silver stain (1).

Soft, red fruits were harvested from trees in early October, depulped and the seeds stratified in a moist medium at 36-40°F for about four months. At the end of this period, germinated (radicle showing) as well as ungerminated seeds were placed in individual one inch cells containing a soilless medium and grown in the greenhouse. When the seedlings had at least two nodes, the youngest pair of leaves was harvested and the DNA extracted, amplified and the products stained as with was done with leaves from the parents. Lanes containing putative hybrids were examined for the presence of products unique to the male parent. Hybridization was confirmed by repeated amplifications and analyses of seedling DNA.

Results and Discussion: Under controlled conditions where all extraneous pollen sources were excluded, the resulting fruit and seed sets must be products of fertilization involving either the two parental trees (a cross or hybrid) or only the female tree (self). Fruit was set in both the honey bee-mediated and hand pollination experiments. DNA amplification fingerprints were generated and from two to four unique amplification products (bands) identified and confirmed for each set of parents (Figure 1). Fingerprints from putative hybrid seedlings and both parents were compared. Unique amplification products from the male parent were found in about half of the F₁ seedlings from both the honey bee and hand-mediated pollinations (Figure 1); the other half appeared to be selfs. This is interesting since flowering dogwood was assumed, perhaps wrongly, to be essentially an obligate outcrossing species.

Significance to Industry: These studies represent the first sustained efforts to intraspecifically breed cultivars and wild selections of flowering dogwoods. Methodology for achieving pollination and fertilization have been successfully formulated. Identification of hybrids is possible and certain shortly after germination of seeds from the prior breeding seasons. Hybrids can be increased using axillary bud multiplication in tissue culture (4) to ensure survival and plentiful supply of plants for various experiments and evaluation.

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Literature Cited

1. Bassam, B.J., G. Caetano-Anollés and P. M. Gresshoff. 1991. Fast and sensitive silver staining of DNA polyacrylamide gels. *Anal. Biochem.* 196:80-83.
2. Caetano-Anollés, G., B.J. Bassam and P.M. Gresshoff. 1991. DNA amplification fingerprinting using very short arbitrary primers. *Bio/Technology* 9:553-557.
3. Graham, E.T., R.N. Trigiano and M.T. Windham. 1995. Evaluation of potential applications of Catocin Mountain dogwoods. *Tennessee Agri Science* 175:38-44.
4. Kaveriappa, K. M., L. M. Bell and R. N. Trigiano. 1995. Micro-propagation of flowering dogwood (*Cornus florida*) from seedlings. *Proc. Southern Nurserymens Res. Conf.* 40:263-265.
5. Trigiano, R.N., G. Caetano-Anollés, B.J. Bassam and M.T. Windham. 1995. DNA amplification fingerprinting provides evidence that *Discula destructiva*, the cause of dogwood anthracnose in North America, is an introduced pathogen. *Mycologia* 87:490-500.
6. Yoon, C.-K., D.A. Glawe and P.D. Shaw. 1991. A method for rapid small-scale preparation of fungal DNA. *Mycologia* 83:835-838.

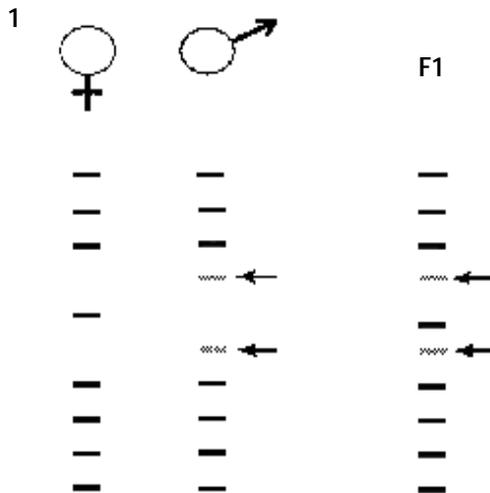


Figure 1. Diagrammatic representation of the use of DNA amplification fingerprinting in determining hybridization. Two amplification products represented as light bands (arrows) found in the fingerprint of the male parent are markers for hybridization. If these light bands (arrows) are present in the seedling (F_1), then the cross was successful and the seedling is a hybrid of the two parents. If the bands were not present, the F_1 pattern would be similar to the female and the seedling considered a self. Reproduced from Graham et al. (3).

Auxin Source Influences Root System Quality of Loblolly Pine Stem Cuttings

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Nature of Work: Development of methods for adventitious rooting of stem cuttings of loblolly pine (*Pinus taeda* L.) for rapid multiplication of superior trees would be of great value to silviculture and horticulture. Treatment of cuttings with various auxins and other growth regulators are beneficial in promoting rooting of loblolly pine (2,4). However, previous research evaluating the effectiveness of liquid formulations of indolebutyric acid (IBA) and phenyl indole-3-thiobutyrate (P-ITB) demonstrated that IBA (1016 to 5080 ppm) or P-ITB (1477 to 5908 ppm) were ineffective in improving rooting percentages of loblolly pine (5). The objective of this research was to evaluate the effectiveness of IBA, Hormodin 3 (8000 ppm IBA in talc), naphthaleneacetic acid (NAA), and Hare's Powder (HP) (4) as potential rooting agents for softwood stem cuttings taken from hedged stock plants of loblolly pine.

One hundred 1-year-old seedlings of loblolly pine representing four families (A, B, C, and D) of 25 plants each were received from Westvaco Corp., Summerville, S.C. on Feb. 25, 1992 and repotted into 11.3 liter (3 gal) containers on March 20, 1992. Initial hedging on April 7, 1992 involved decapitating trees 20 cm (8 in) above the rim of the pot, removing all remaining terminal buds from branches, and leaving half the length of any individual branch intact. Trees were hedged to maintain juvenility as cuttings taken from physiologically adult trees are often difficult or even impossible to root (1, 3). During July 1992, May 1993, July 1993, and June 1994 softwood stem cuttings were taken from the hedged stock plants for rooting. Hardwood cuttings were taken Feb. 1993 and Jan. 1994. These dates coincided with the rehedging of stock plants to a height of 20 cm (8 in). Osmocote 18-6-12 was applied twice annually [24 g (0.8 oz) per pot] in March following hedging for hardwood cuttings and again in June following hedging for softwood cuttings. The basal 2 cm (0.75 in) of 9 cm (3.5 in) long terminal cuttings was inserted into a medium of 1 perlite:1 peat (v/v) in a raised greenhouse bench. Intermittent mist operated 6-8 sec every 5 min from 7 a.m. to 8 p.m. daily. The experimental design was a randomized complete block with six cuttings per treatment and six replications. The present experiment was initiated on June 6-7, 1994 and employed the following treatments: 5 and 10 mM (1016 and 2032 ppm) IBA, 5 and 10 mM (1477 and 2954 ppm) P-ITB, 5 and 10 mM (931, and 1862 ppm) NAA, 39 mM (8000 ppm) IBA-talc, Half- and Full-strength HP, nontreated control, and a 50% isopropanol control (IPA). Auxins were dissolved in 50% IPA and the basal 1 cm (0.45 in) of each cutting was treated with an auxin solution for 1 sec followed by 15 min of air drying prior to insertion into the rooting medium. The basal 1 cm of each cutting treated with talc formulations (IBA-talc and HP) was first dipped in distilled water.

After 14 weeks, the experiment was terminated and the number and length of primary roots 1 mm (0.04 in) recorded. Spatial symmetry of the root system of each rooted cutting was rated as nonsymmetrical or symmetrical. Cuttings with a single root were rated as symmetrical if the root was oriented downward while cuttings having more than one root were rated as symmetrical only if roots were evenly spaced and 50% or more of the roots were oriented downward. Symmetry data are presented as the percentage of rooted cuttings rated as symmetrical. Data were subjected to analysis of variance (ANOVA) procedures and means separated by computing least significant differences (LSD) at $\alpha = 0.05$.

Results and Discussion: Analysis of data for percent rooting revealed treatment to be highly significant with no family treatment interaction. Across all families the nontreated cuttings had the greatest rooting percentage (Table 1). The IPA control, 10 mM (1862 ppm) NAA and both formulations of IBA decreased rooting percentages compared to the nontreated control. The lower rooting percentage of the IPA control may indicate a sensitivity to the carrier, hence the lower rooting percentages noted for the alcohol formulations of auxins. Lower rooting percentages for the IBA-talc and full-strength HP treatments suggests auxin concentration may also be a factor contributing to suppressed rooting percentages. Within each auxin source (P-ITB, NAA, and HP), lower concentrations resulted in higher rooting percentages (Table 1).

Root number data revealed significant family and treatment effects and no family treatment interaction. Analysis of root number data across all treatments revealed that mean root numbers differed among the four families (Table 2). Across all families, mean root number of nontreated cuttings was similar to all treatments except 5 and 10 mM (931 and 1832 ppm) NAA (Table 1). Treatment with NAA resulted in cuttings with a greater mean number of roots than nontreated cuttings (Table 1). NAA also resulted in cuttings with a greater mean number of roots than cuttings rooted with other auxin sources. Although NAA treatment did not improve percent rooting it may prove useful in altering root system morphology by increasing the number of roots per cutting. A similar response occurred with application of IBA or P-ITB where mean root numbers were increased by 0.2 and 0.5 roots per cutting, respectively (5). The ANOVA for mean primary root length (mean length per root) data indicated a significant family effect and no treatment effect or family x treatment interaction. Family A had a greater mean root length than Families B, C, and D (Table 2). Analysis of total primary root length (sum of primary root lengths per cutting) data indicated significant family and treatment effects and no family by treatment interaction. Family A had a greater total root length than families B, C, and D. Across all families, total root length for rooted cuttings treated with 10 mM NAA differed from the controls while total root length for rooted cuttings treated with 5 or 10 mM (931 and 1832 ppm) NAA was greater than total root length for rooted cuttings treated with 10 mM (2954 ppm) P-ITB and full-strength HP. The similarity between root length for individual roots of NAA and nontreated cuttings, combined with an increase in root number per cutting for cuttings treated with NAA, resulted in a greater total root length per cutting for NAA-treated cuttings. Analysis of root system symmetry data (percentage of cuttings rated as symmetrical) indicated a significant treatment effect and no family x treatment interac-

tion. Nontreated cuttings resulted in the least number of symmetrical root systems (Table 1). However, the number of nontreated cuttings with symmetrical root systems did not differ from the IPA control, IBA, or 10 mM (2954 ppm) P-ITB. Treatment with NAA, IBA-talc, and HP resulted in a greater proportion of cuttings with symmetrical root systems than IBA or 10 mM (2954 ppm) P-ITB.

Significance to Industry: Treatment of cuttings with P-ITB, IBA, NAA, or HP was not effective in improving rooting percentages of softwood stem cuttings of loblolly pine. However, NAA increased the number of roots per cutting while application of P-ITB, IBA-talc, or NAA increased the number of cuttings with symmetrical root systems.

Literature Cited

1. Gardner, F.E. 1929. The relationship between tree age and the rooting of cuttings. Proc. Amer. Soc. Hort. Sci. 26:101-104.
2. Greenwood, M.S. and E.S. Nussbaum. 1981. Rooting ability and vegetative growth performance of stem cuttings from one- and five-year-old ortets of loblolly pine. Proc. 16th Southern Forest Tree Improvement Conf. p. 176-183.
3. Hackett, W.P. 1988. Donor plant maturation and adventitious root formation, p.11-60. In:T.D. Davis, B.E. Haissig, and N. Sankhla (eds.). Adventitious root formation in cuttings. Dioscorides Press, Portland, Ore.
4. Hare, R. C. 1974. Chemical and environmental treatments promoting rooting of pine cuttings. Can. J. Forest Res. 4:101-106.
5. Thetford, M. and F. Blazich. 1995. Comparison of IBA and P-ITB for propagation of loblolly pine by stem cuttings. Proc. Southern Nurserymen's Assoc. Res. Conf., 40th Annu. Rpt. p. 269-271.

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Table 1. Effects of rooting treatments on percent rooting, root number, total root length, and root symmetry of softwood stem cuttings from four families of loblolly pine.

Treatment	Concn.		Rooting (%)	Root no.	Total root length (cm)	Root Symmetry ^z (%)
	mM	ppm				
Nontreated	0	0	34.7	1.36	20.95	23.9
Alcohol	0	0	22.2	1.34	22.44	32.5
IBA	5	1016	18.8	1.47	23.00	47.5
IBA	10	2032	25.0	1.89	24.71	47.3
P-ITB	5	1477	30.6	1.68	21.40	49.5
P-ITB	10	2954	25.0	1.54	19.94	28.9
NAA	5	931	32.6	2.23	27.84	52.8
NAA	10	1862	15.3	2.74	28.81	65.3
IBA-talc	39	8000	12.5	1.88	26.73	66.7
Hares powder	Half-strength		31.3	1.52	23.71	61.2
Hares Powder	Full-strength		27.1	1.71	20.38	59.9
	LSD ^y (α=0.05)		11.8	0.62	7.43	25.7

^z Symmetry = percent of rooted cuttings with symmetrical root systems.

^y LSD = Least Significant Difference; means represent an average response across all families.

Table 2. Effects of rooting treatments on root number, mean root length, and total root length of softwood stem cuttings from four families of loblolly pine.

Family	Root No. length (cm)	Mean root length (cm)	Total root
A	2.0	17.3	30.3
B	1.7	13.9	20.5
C	1.8	14.2	22.7
D	1.6	14.3	21.0
LSD ^z (α= 0.05)	0.3	1.0	2.6

^z LSD = Least Significant Difference; means represent an average response across all auxin treatments.

Response of Microshoots of Mountain Andromeda to in Vitro Ericoid Mycorrhizal Inoculation

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Nature of Work: A study was conducted to investigate the effects of selected isolates of ericoid mycorrhizal fungi on in vitro development and growth of plantlets of mountain andromeda (*Pieris floribunda* Pursh.), an underutilized, ornamental shrub indigenous to the Appalachian mountains of the U.S. extending from Virginia southward into Georgia.

On 10 July 1993, actively elongating shoot tips were collected from a mature plant of *Pieris floribunda* cultured in a greenhouse. Shoot tips were decapitated, surface sterilized, and placed in 225 ml (7.6 oz) baby-food jars containing 25 ml (0.9 oz) Woody Plant Medium [(WPM) 4] solidified with 0.8% tissue culture agar and including 200 ppm NaH_2PO_4 , 80 ppm adenine hemi-sulfate, and 4 ppm 6-(g,g,-dimethylallylamino)-purine (2iP). The medium was adjusted to pH 5.2 with 1N KOH prior to autoclaving. Jars were capped with Magenta B-Caps (Magenta Corp., Chicago, Ill.) and the vessels were sealed with Parafilm. Approximately every 2 months thereafter, resultant microshoots were transferred (elongating shoot mass placed on fresh WPM) or subcultured (select shoots decapitated and placed horizontally on WPM for axillary budbreak and continued shoot proliferation) on an alternating basis. Cultures were incubated at 24°C (75°F) with a 16 hr photoperiod provided by cool-white fluorescent lamps [photosynthetic photon flux (PPF); 400-700 nm=50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (4.0 klx)].

On 20 July 1995, microshoots 2.5 cm (1 in) in length were selected, and the basal portions inserted to a depth of 1 cm (0.4 in) into baby-food jars (four shoots per jar) containing 25 ml (0.9 oz) WPM solidified with 0.8% tissue culture agar and including 200 ppm NaH_2PO_4 , 80 ppm adenine hemi-sulfate, and 15 ppm of the potassium (K) salt of 1H-indole-3-butyric acid (K-IBA). Shoots were maintained on this medium for 10 days to stimulate rooting. After 10 days (30 July 1995), shoots were inserted vertically to a 1 cm (0.4 in) depth and placed individually into 7.6 x 10.2 cm (3 x 4 in) Magenta GA-3 culture vessels. Each vessel contained 4.9 in³ (80 cm³) of an autoclaved, sifted medium of 1 peat : 1 vermiculite (v/v) moistened with 40 ml (1.4 oz) liquid WPM including 200 ppm NaH_2PO_4 , 80 ppm adenine hemi-sulfate but with the sucrose content reduced to 5 g (1.6 oz) per liter (0.26 gal). The liquid WPM was adjusted to pH 5.2 with 1 N KOH prior to autoclaving. After placement of each microshoot in a culture vessel, they were inoculated with one of six isolates of ericoid mycorrhizal fungi. Known isolates of ericoid mycorrhizal fungi obtained from the American Type Culture Collection [(ATCC) Rockville, Md.] included: *Hymenoscyphus ericae* (Read) Korf and Kernan (#32985, syn. *Pezizella ericae* Read); *Oidiiodendron griseum* Robak (#60377); and *O. maius* Barron (#66504). Additionally, three unknown ericoid fungal isolates were included in the experiment, two of which were putative isolates of *Hymenoscyphus* hereafter referred to as Dijon A and LPA25. The third unknown isolate was obtained from roots of a wild-collected plant of *Pieris floribunda* and had been identified tenta-

tively as *Oidiodendron* sp. The fungal inoculum consisted of approximately 3 mm³ (0.0002 in³) of aerial hyphae collected from actively growing colonies maintained on malt agar (Difco, Detroit, Mich.). After inoculation, vessels were covered with Magenta GA-3 caps and sealed with Parafilm. There were five replications of each fungal isolate and five noninoculated microshoots (controls). A replication consisted of an individual microshoot per culture vessel. Cultures were maintained at 24°C (75°F) with a 16-hr photoperiod provided by cool-white fluorescent lamps [PPF (400-700 nm)=96 μmol·m⁻²·s⁻¹ (7.4 klx)].

Nine months after inoculation, plantlets were harvested and the following data recorded: degree of root colonization (10 randomly selected root tips per plantlet), total root length, total shoot length, shoot dry weight [dried at 70°C (158°F) for 48 hr], leaf number, and leaf area. Total root and shoot lengths, leaf number, and leaf area were measured with a Monochrome AgVision Root and Leaf Analysis System (Decagon Devices, Inc., Pullman, Wash.). Mycorrhizal infection was determined by staining roots with chlorazol black E according to a technique modified from Brundrett et al. (1), and the roots were examined under a light microscope. Data were subjected to general linear modeling (GLM) procedures (5).

Results and Discussion: Plantlets inoculated with *O. griseum* (OG), *O. maius* (OM) or the C1 isolate (C1) completely colonized all young root tips examined. Of these root tips, all cells examined (within one viewing region at 400x) displayed complete intra- and intercellular colonization (Table 1). Roots of microshoots inoculated with *H. ericae* (HE), the Dijon A isolate (DA) or the LPA25 isolate (LPA) had significantly less colonization (Table 1). The high level of colonization by all isolates of *Oidiodendron* suggests a lack of host specificity among species in this genus. Only C1 was isolated from roots of wild-collected *P. floribunda*, whereas OM was isolated originally from roots of *Rhododendron* (3) and OG was isolated originally from highbush blueberry [*Vaccinium corymbosum* Ait.(2)]. Plantlets inoculated with HE or either of the two related isolates, DA and LPA, showed significantly greater total shoot length, total shoot dry weight, leaf number, and leaf area than either controls or microshoots inoculated with any of the isolates of *Oidiodendron*. Microshoots inoculated with HE, DA or LPA, had significantly greater branching than those inoculated with OG, OM or C1. Interestingly, the greatest total root length occurred in noninoculated (control) plantlets, however, this was not significantly different from microshoots inoculated with DA or LPA. Roots that formed on microshoots inoculated with isolates of *Oidiodendron* all were markedly shorter than roots produced in the presence of any isolates of *Hymenoscyphus* or the noninoculated control.

Significance to Industry: *Pieris floribunda* is a native ornamental shrub that is often underutilized in the landscape. Scarcity of the species in the nursery trade can be attributed to inherent difficulties in asexual propagation (rooting of cuttings). Micropropagation may provide a means to increase availability of this species (6). However, during micropropagation, an effective ericoid mycorrhizal fungus should be introduced to optimize early growth of plantlets. Inoculation by *Hymenoscyphus ericae* or a related isolate has the potential to increase shoot development of plantlets. Ericoid mycorrhizal isolates of *Oidiodendron* are detrimental to root and shoot growth at this stage of plantlet development and should not be used as an *in vitro* inoculum.

Literature Cited

1. Brundrett, M.C., Y. Piché, and R.L. Peterson. 1984. A new method for observing the morphology of vesicular-arbuscular mycorrhizae. *Can. J. Bot.* 62:2128-2134.
2. Couture, M., J.A. Fortin and Y. Dalpe. 1983. *Oidiodendron griseum* Robak: An endophyte of ericoid mycorrhiza in *Vaccinium* spp. *New Phytol.* 95:375-380.
3. Douglas, G.C., M.C. Heslin, and C. Reid. 1989. Isolation of *Oidiodendron maius* from *Rhododendron* and ultrastructural characterization of synthesized mycorrhizas. *Can. J. Bot.* 67:2206-2212.
4. Lloyd, G. and B. McCown. 1980. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. *Proc. Intl. Plant Prop. Soc.* 30:421-437.
5. SAS Institute, Inc. 1985. SAS user's guide: Statistics, version 5 edition. SAS Institute, Inc., Cary, N.C.
6. Starrett, M.C., F.A. Blazich, J.R. Acedo, and S.L. Warren. 1993. Micropropagation of *Pieris floribunda*. *J. Environ. Hort.* 11(4):191-195.

Table 1. Response of rooted microshoots of mountain andromeda to in vitro ericoid mycorrhizal inoculation^z

Fungus	Percent of total root system colonized	Percent of cells colonized per section of root ^y	Total root length (cm)	Total shoot length (cm)	Total shoot dry weight (g)	Branch number	Leaf number	Leaf area (cm ²)
Control	0.0 d ^x	0.0 d	44.4 a	25.9 c	0.099 b	8.0 cd	68.0 c	21.3 b
Oldiodendron								
O. griseum	100.0 a	100.0 a	18.1 c	22.9 c	0.085 b	6.8 de	47.0 cd	9.3 c
O. maius	100.0 a	100.0 a	15.2 c	9.6 d	0.061 b	2.6 f	26.4 d	5.7 c
C1	100.0 a	100.0 a	11.6 c	14.7 cd	0.064 b	3.6 ef	30.0 d	7.0 c
Hymenoscyphus								
H. ericae	10.0 cd	28.6 bc	31.2 b	55.0 ab	0.160 a	11.6 ab	117.4 b	35.1 a
DA	18.0 c	31.6 b	39.6 ab	44.7 b	0.170 a	10.6 bc	104.4 b	36.9 a
LPA	40.0 b	22.4 c	43.6 a	63.7 a	0.184 a	14.2 a	160.2 a	37.8 a
LSD	14.4	8.8	12.3	11.8	0.043	3.4	26.1	7.5

^zObservations are the mean of five replications.^yApproximate number of cells examined per 400x field = 25.^xMean separation within columns by LSD, $P \leq 0.05$.

High Frequency Adventive Regeneration in Daylily Tissue Cultures Stimulated by Thidiazuron (TDZ).

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Nature of Work: Daylilies (*Heemerocallis* spp.) are regarded as the most popular sun loving perennial plants sold in the United States by members of the Perennial Plant Association who also rank the daylily cultivar "Stella de Oro", a clone introduced in 1975, as the number one perennial plant consumed in the U.S. and Canada.. More than 38,000 daylily clones are registered with the American Hemerocallis Society but only a handful of cultivars are available in commercial quantities. Nurseries specializing in daylily production generally offer a few plants of many different cultivars. The popularity of the few clones in the nursery industry suggests that some of the new improved cultivars of daylilies currently available to enthusiasts from speciality nurseries may be a source of superior new products for the nursery industry. Currently, it takes approximately 20 years for an outstanding daylily cultivar to move from the enthusiast market to the mass market.

Tissue culture techniques of plant propagation (micropropagation) have the potential to generate large quantities of desirable clones in a relatively short period of time. Several procedures for micropropagation of daylilies are published. In vitro culture of tissues removed from elongating flower scapes was described by Meyer (5). The method for plant production relies on a two stage procedure. The first stage of culture was designed to produce callus tissue in the primary explant through incubation on a modified Murashige and Skoog basal medium supplemented with 10 mg/liter naphthaleneacetic acid (NAA), 0.1 mg/liter kinetin (K) and 60 g/liter sucrose. The second stage of culture was designed to promote adventitious plantlet regeneration in disorganized callus tissue which was subcultured onto a medium containing 0.5 mg/liter NAA and 0.1 mg/liter K. Plantlets which developed after subculture to the second medium were established ex vitro. Other researchers (1,2,4) found that 2,4 dichlorophenoxyacetic acid (2,4D) can be substituted for NAA.

Three cultivars of daylily ('Dancing Spree,' 'Red Rum,' and 'Porcelain China') were micropropagated according to procedures described by Meyer, 1976; and Krikorian and Kann, 1979. Explants were removed from flower scapes during May and June 1995. Callus developed on the majority of explants in the presence of 0.1 mg/liter K and either 10 mg/liter NAA or 1.0 mg/liter 2,4-D. Plantlets were regenerated after subculture of callus onto media containing 0.1 mg/liter K 0.1 mg/liter NAA. Plants were regenerated from calli of all three clones which developed on either of the initiation media.. However, we noticed that a minimum of six months and several subcultures were generally required before plantlet formation was apparent and approximately a year of in vitro culture was required before daylily tissues were fully acclimated to the aseptic environment.

Thidiazuron (TDZ) is a relatively new, potent, synthetic growth regulator with strong cytokinin like activity when incorporated into tissue culture media. TDZ was reported to rapidly induce high frequency, adventitious shoot development in explants of several species of plants (3). An experiment was initiated to evaluate the effects of TDZ on plantlet production in explants of daylily. Immature scapes were collected from three clones ('McRae Purple', 'Scarlet Orbit', and 'Siloam Double Classic',) in late May 1996. Scapes were surface disinfested by soaking for 20 minutes in 20% dilution of commercial bleach containing a few drops of Tween 20. Plant material was rinsed three times in sterile water. Thin cross sections were cut from the immature scape pieces under aseptic conditions and were transferred at random to test tubes containing 10 ml of one of the 4 media tested. All media consisted of modified MS formulation (5) and were supplemented with 10 mg/liter NAA, and 0.1 mg/liter K or 1 mg /liter NAA combined with 0.022 mg/liter or 0.22 mg/liter or 2.2 mg/liter TDZ. Similar explants from the three clones were transferred to the 4 different media. . Cultures were incubated at 80F under a 12-hr photoperiod provided by cool white fluorescent tubes mounted 12 inches above the shelf holding the culture vessels. Contaminated cultures were removed from the incubation environment and excluded from evaluation. After approximately 6 weeks of incubation cultures were inspected and evaluated to determine the presence of shoot structures and or roots. Data on numbers of shoots were subject to analysis using a Chi squared statistic to test for deviations from independent assortment.

Results and Discussion: The results of the evaluation of explants from scapes of the cultivars McRae Purple and Siloam Double Classic are presented in Table 1. The Chi squared statistics were developed for number of explants which formed at least one shoot or recognizable shoot initial.

Table 1. Effects of growth regulator supplements on morphogenic response in cultured scape tissue of daylily after 6 weeks incubation.

Medium	Number of explants Number with roots		Number with shoots
cv. McRae Purple			
10 NAA 0.1 K	17	2	7
1 NAA 0.02 TDZ	13	5	0
1 NAA 0.2 TDZ	15	4	0
1 NAA 2.2 TDZ	17	12	0
Chi squared (3df) = 9.2 (p < 0.95)			
cv. Siloam Double Classic			
10 NAA 0.1 K	16	0	2
1 NAA 0.02 TDZ	15	7	0
1 NAA 0.2 TDZ	17	4	0
1 NAA 02.2 TDZ	14	5	0
Chi squared (3df) = 7.1 (p > 0.95)			

A significant dependence of numbers of explants forming shoots on growth regulators in the medium was observed in the cultivar 'McRae Purple' but, number of explants forming shoots in cultivar 'Siloam Double Classic' was independent of medium. The medium supplemented with 1.0 mg/liter NAA and 2.2 mg/liter TDZ promoted the greatest frequency of explants with shoots. It appears that TDZ at this concentration significantly influenced morphogenesis in vitro. Roots formed only on the medium containing 10.0 mg/liter NAA and 0.1 mg/liter K in both the responsive cultivars.

Explants of the cultivar 'Scarlet Orbit' failed to produce shoot structures or roots on any of the media during the first six weeks of culture. The majority of explants swelled but became necrotic during the second week of incubation. This observation may have resulted from damage to the explants during the disinfestation treatment which prejudiced further development and differentiation in response to the different culture media.

It appears that incubation of explants of the responsive cultivar MacRae Purple on a medium containing TDZ and NAA offers the opportunity to enhance micropropagation through rapid induction of direct adventitious shoots in the primary explant without production of callus prior to initiation of shoots.

Significance to Industry: More efficient tissue culture procedures for daylily cloning, such as the improvement described herein, will allow specialist propagators to increase the supply of new superior cultivars to the nursery producer. Improved clones usually bring a premium over less desirable clones. Application of this technology to increase the availability of superior daylily cultivars will help satisfy the demand for new well adapted flowering perennial plants for the consumer.

Literature Cited

1. Chen, C.H. and D.J. Holden. 1972. Organogenesis in daylily callus. Proc. S.D. Acad. Sci. 51:141-149.
2. Heuser, C.W. and D.W. Apps. 1976. In vitro plantlet formation from flower petal explants of *Hemerocallis* cv. 'Chipper Cherry'. Can. J. Bot. 54:616-610.
3. Huetteman, C.A. and J.E. Preece. 1993. Thidiazuron: a potent cytokinin for woody plant tissue culture. Plant Cell, Tissue and Organ Culture 33:105-119.
4. Krikorian, A.D. and R.P. Kann. 1979 Micropropagation of daylilies through aseptic culture techniques. *Hemerocallis J.* 33(1):44-61.
5. Meyer, M.M., Jr. 1976. Propagation of daylilies by tissue culture. Hort Science 11:485-487.