SECTION 8
PROPAGATION

Dr. Ted Whitwell
Section Editor and Moderator

Micropropagation of Flowering Dogwood (*Cornus florida*) from Seedlings

K. M. Kaveriappa, L. M. Bell and R. N. Trigiano
Tennessee

Nature of Work: Sustained micropropagation (production of whole plants via tissue culture methods or "in vitro") of Flowering dogwood (*Cornus florida* L.) from either mature or juvenile tissues has not been achieved. A few plantlets have been produced using somatic embryogenesis, but these regenerates were never acclimatized and grown in the field (3,4). There are several reports of shoot proliferation from seedling and flowering specimens (1,4). Rooting of microshoots was either not attempted (1) or was not accomplished (4).

Tree species are micropropagated typically from either axillary or apical buds of mature "elite" specimens in which the horticultural characteristics are stable and well-established. Tissue culture propagation from juvenile materials is usually not used because it is nearly impossible to judge the attributes of a mature tree from a seedling — it may or may not be suitable horticulturally. However, there is one notable exception to the above — the ability to clone materials from seedlings is highly desirable in a breeding program since the progeny may represent useful as well as unique genotypes.

A group of research scientists and staff at the University of Tennessee and their counterparts at Tennessee State University have undertaken a breeding program for Flowering dogwood. This program, although in its infancy, has produced seeds from controlled crosses. Furthermore, we have used a DNA fingerprinting technology to ascertain if a seedling truly represents a hybridization between the desired parents as opposed to selfing or contamination from another pollen source (2). The present study reported herein was undertaken to devise methodologies to micropropagate or "clone" individual seedlings of Flowering dogwood.

Soft, red fruits from seven Flowering dogwood trees on the University of Tennessee Agricultural campus were collected in October, depulped and the seeds stored in a moist medium at about 40°F (4°C) for four months. At the end of the chilling period, some seeds had germinated and these, along with ungermimated seeds, were transferred to individual one inch cells containing a soilless medium and grown in the greenhouse. Nodal segments were harvested when seedlings had produced one or two sets of true leaves. Explants were surface disinfested in 20 % commercial bleach and then transferred to various media containing either a cytokinin or a cytokinin-like growth regulator. At least 30 nodal explants were prepared for each medium/ growth regulator combination. All cultures were incubated under relatively low light intensity for 16 h/day at 73°F (23°C) for 40 days. Cultures were subsequently transferred every 4 weeks to fresh medium of the same composition and have been maintained for more than one year. For rooting experiments, microshoots, one inch (2.5 cm) and longer, were first treated for 12 h on auxin containing medium and then transferred to the same medium without auxin and reduced sugar for 2 - 4 weeks. Other microshoots were maintained

on auxin amended medium for the entire 4 week period. Regardless of the initial rooting treatment, microshoots were transferred to medium without auxin for an additional 1 - 2 week period. Rooted microshoots were acclimatized to ambient greenhouse conditions in pots containing soilless medium 8 weeks after the initiation of roots.

Results and Discussion: Axillary buds were easily established in culture regardless of the type of medium or cytokinin — very few seedling nodal cultures became contaminated, which agrees well with the study conducted by Declerck and Korban (1). Generally, both axillary buds on each nodal segment elongated in the first culture cycle of 40 days, thereafter cultures produced between 1.5 and 5.0 microshoots depending on the growth regulator concentration. Specifically, 6-benzyladenine (BA) initially provided superior proliferation rates and quality of microshoots compared to similar concentrations of thidiazuron (TDZ), a compound that has cytokinin-like activity. However, if cultures initiated on TDZ were transferred to BA supplemented medium, they produced similar number and quality of microshoots as cultures that had been only exposed to BA.

About 50 % of the microshoots treated with auxin for 12 h produced an average of less than 2 adventitious roots after 4 weeks whereas, 40 % of the microshoots exposed to 4 weeks of auxin generated between 3 and 5 adventitious roots (see Figure 1). Microshoots rooted with continuous exposure to auxin were more easily acclimatized to greenhouse conditions than those rooted with short exposure to auxin. The number of roots initiated per microshoot may critically influence the ability of the plantlet to be acclimatized.

Significance to Industry: This represents the first successful micropropagation protocol for Flowering dogwood and offers a means to rapidly increase materials generated by breeding programs. Furthermore, the basic protocol may serve as a starting point for developing micropropagation schemes from mature specimens.

Acknowledgement

The authors wish to thank the Indo-US SEC for the Fulbright Award, Tennessee Agricultural Experiment Station for financial support, and USDA grant number 91-34241-5921.

Literature Cited

1. Declerck, V. and S. S. Korban. 1994. Effects of source of macronutrients and plant growth regulator concentrations on shoot proliferation of *Cornus florida*. Plant Cell, Tissue and Organ Culture 38:57-60.

2. Trigiano, R. N. 1994. Molecular techniques are being used to identify cultivars & potential hybrids of dogwood at the University of Tennessee. Tenn. Nurseryman's Newslett. 6(25):22,25.

3. Trigiano, R. N., R. M. Beaty and J. T. Dietrich. 1989. Somatic embryogenesis and plantlet regeneration in *Cornus lorida*. Plant Cell Rpt. 8:270-273.

4. Trigiano, R. N., R. M. Beaty and K. W. Lowe. 1992. Micropropagation of dogwoods (*Cornus* spp.). p. 81-90. IN: Bajaj, Y. P. S. (Ed.) Biotechnology in Agriculture and Forestry, Vol. 20. High-Tech and Micropropagation IV, Springer-Verlag, Berlin.

Figure 1. Microshoot of Flowering dogwood rooted with continuous exposure to auxin for 4 weeks.

Response of Seedlings of Highbush Blueberry to *In Vitro* Ericoid Mycorrhizal Innoculation

Mark C. Starrett, Frank A. Blazich, Larry F. Grand and Steven R. Shafer
North Carolina

Nature of Work: A preliminary study was conducted to investigate the effects of selected isolates of ericoid mycorrhizal fungi on in vitro growth and development of seedlings of highbush blueberry (*Vaccinium corymbosum* L.), an important small fruit crop in the United States.

Seeds of highbush blueberry were surface disinfested, sown on 0.4% water agar in 60 x 15 mm (2.4 x 0.6 in) petri dishes, and the dishes sealed with Parafilm. Dishes were incubated in a controlled environment room maintained at 24°C (75°F) with a 16 hr photoperiod provided by cool-white fluorescent lamps which provided a photosynthetic photon flux [PPF (400-700 nm)] of 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (4.0 klx). Following germination, individual seedlings were transferred to 25 x 150 mm (1.0 x 5.9 in) Pyrex test tubes containing 15 ml (0.5 oz) of 1/4 strength Woody Plant Medium [(WPM) 3] without sucrose and solidified with 0.8% tissue culture agar and containing 50 ppm NaH_2PO_4 and 20 ppm adenine hemi-sulfate. The pH was adjusted to 5.2 with 1N KOH prior to autoclaving. Over the surface of the WPM, approximately 3 mm (0.1 in) of an auto-claved, sifted medium of 1 peat **:** 1 vermiculite (v/v) was applied prior to insertion of the seedlings. At the time of seedling transfer from water agar to the modified WPM, a primary leaf and primary roots were present. After placement of each seedling in a culture tube, the seedling was 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); *Oidiodendron griseum* Robak (#60377); and *O. maius* Barron (#66504). Additionally, three unknown ericoid fungal isolates were included in the experiment and two were putative isolates of *Hymenoscyphus* that are noted as Dijon A and LPA25. The third unknown isolate was obtained from locally collected roots of *Pieris floribunda* (Pursh ex Sims) Benth. and Hook. (mountain andromeda) and has been identified tentatively as *Oidiodendron* sp. The fungal inoculum consisted of approximately 2 mm^3 (0.0001 in^3) of aerial hyphae collected from actively growing colonies maintained on malt agar (Difco-Bacto, Detroit, Mich.). After inoculation, the tubes were covered with Kaput caps and sealed with Parafilm. There were four replications of each fungal isolate and four uninoculated seedlings (controls). A replication consisted of an individual seedling per culture tube. Cultures were maintained at 24°C (75°F) with a 16-hr photoperiod provided by cool-white fluorescent lamps [PPF (400-700nm) = 96 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (7.4 klx) as measured at the tops of the culture tubes]. Four months after inoculation, seedlings were harvested and the following data recorded**:** degree of root colonization, total root length, primary root number, secondary root number, secondary root length, shoot length, shoot fresh weight, shoot dry weight, and leaf number. Shoots were dried at 70°C (158°F) for 48 hr. Mycorrhizal infection was determined by staining roots with chlorazol black E using a technique modified from Brundrett et al. (1), and examined under a light microscope. Data were subjected to analysis of variance procedures (4).

Results and Discussion: *Vaccinium corymbosum* inoculated with *O. griseum* (OG), *O. maius* (OM) or the C1 isolate (C1) had a similar degree of root colonization, which was generally greater than colonization by *H. ericae* (HE), the Dijon A isolate (DA), and the LPA25 isolate (LPA). Seedlings inoculated with HE, DA, or LPA also were similar to each other in extent of root mycorrization, however, the degree of colonization was very low. The high colonization level by OG (62.5%) might be attributable to the isolate having originated on a cultivar of highbush blueberry. Isolates of some ericoid fungi are rather host specific, even to the cultivar level (2). Plants inoculated with HE or either of its two related isolates, DA and LPA, showed significantly greater secondary root number than the controls. Seedlings inoculated with HE, DA, or LPA also demonstrated significantly greater total root length and secondary root length than the control plants. Water uptake can be limited by the extent of the root system. Because ericaceous plants lack root hairs, additional fine root production would be beneficial to future transplanting and plant acclimatization. Plants inoculated with any of the *Oidiodendron* isolates were generally not different from the controls across all root variables examined. In vitro inoculation of *V. corymbosum* seedlings with an *Oidiodendron* isolate would therefore not be beneficial in increasing root growth or development.

Seedlings inoculated with HE, DA, or LPA had similar shoot length. However, only HE inoculated plants were significantly taller than plants inoculated with OG, OM, or C1. All *Hymenoscyphus* type mycorrhizal plants had significantly longer shoots than the control plants. OG, OM, or C1 inoculated seedlings responded similarly to each other in shoot length and although each of their mean shoot lengths was greater than the controls, they were not significantly different from the controls. Interestingly, despite differences in shoot length, there was no difference between any of the inoculated or uninoculated controls in the number of leaves present. This suggests that inoculation affected internode length. Seedlings inoculated with *Hymenoscyphus* isolates were generally greater than the controls in both shoot fresh and dry weights. Plants inoculated with *Oidiodendron* isolates did not exhibit increased fresh or dry weights when compared to controls.

Significance to Industry: The economic importance of blueberries warrants continued investigation into methods which speed new and improved cultivars to the field. Micropropagation procedures which include an ericoid mycorrhizal fungus such as, *H. ericae,* have the potential to enhance clonal production efforts by stimulating in vitro root and shoot development as modelled by the seedling system described herein.

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. Lareau, M.J. 1985. Rooting and establishment of *in vitro* blueberry plantlets in the presence of mycorrhizal fungi, p. 197-201. In: K. Pliszka (ed.). Third Intl. Symp. *Vaccinium* Culture, Warsaw, Poland, 24-28 July 1984. Acta Hort. vol. 165.

3. 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.

4. SAS Institute, Inc. 1985. SAS user's guide:Statistics, version 5 edition. SAS Institute, Inc., Cary, N.C.

Comparison of IBA and P-IPB for Propagation of Loblolly Pine by Stem Cuttings

Mack Thetford and Frank A. Blazich
Florida and North Carolina

Nature of Work: Development of methods for adventitious rooting of stem cuttings of loblolly pine (*Pinus taeda* L.) has been of interest to researchers since the late 1920s (1). Treatments of cuttings with various auxins and other growth regulators have been shown to be beneficial in promoting rooting of loblolly pine (2,4). Therefore, the objective of this research was to evaluate indolebutyric acid (IBA) and phenyl indole-3-thiolobutyrate (P-ITB) as potential rooting agents for softwood stem cuttings taken from hedged stock plants of loblolly pine.

One hundred, one-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 and removing all remaining terminal buds from branches such that half the length of any individual branch remained intact. Trees were hedged to keep them in the juvenile growth phase as cuttings taken from physiologically adult trees are often difficult or even impossible to root (3). During July 1992, and May 1993, softwood stem cuttings were taken from the hedged stock plants for rooting. These dates also coincided with rehedging of stock plants back to a height of 20 cm (8 in) as described previously. Trees were also hedged to a height of 20 cm (8 in) during Feb. 1993. Osmocote 18-6-12 was reapplied [24 g (0.8 oz) per pot] in March and again 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.

Expt. 1. The first study was initiated on July 1-7, 1992 and employed the following treatments: 0, 1016, 2032, 3048, 4063 or 5080 ppm IBA and 1477, 2954, 4431, 5908 or 7385 ppm P-ITB. Both auxins were dissolved in 95% ethanol 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. After 17 weeks, the Thetford and Blazich 2 experiment was terminated and the number and length of primary roots $\geq$ 1 mm (0.04 in) recorded. Each rooted cutting was subjectively rated as commercially acceptable or unacceptable. A commercially acceptable cutting was one rated as having the capacity to grow following potting. Data were subjected to analysis of variance (ANOVA) procedures and means separated by computing least significant differences (LSD) at a = 0.05. Only root number and root length data for commercially acceptable cuttings were subjected to statistical analysis.

Expt. 2. This experiment was initiated on May 31 to June 2, 1993 and was very similar to Expt. 1. However, the treatments differed and consisted of the following: 95% ethanol, 0, 2032, 4064, 6096, 8128 or 10160 ppm IBA and 2954, 5908, 8862, 11816 or 14770 ppm P-ITB. Auxin concentrations were increased in an attempt to identify a range of concentrations which might improve rooting percentages. The experimental design was identical to Expt. 1 with the inclusion of 95% ethanol as an additional control. Sixteen weeks after initiation, the same data as in Expt. 1 were recorded and all data were analyzed as described for Expt. 1.

Results and Discussion: Expt. 1. Analysis for percent rooting revealed family and treatment to be highly significant with no family treatment interaction. Across all treatments, rooting percentages for families B and C were significantly greater than rooting percentage for family A and rooting percentage for family D did not differ from family A (Table 1). Across all families, the nontreated cuttings had the highest rooting (75%) and none of the other treatments were significantly greater (data not presented). The same was noted for the ANOVA of rooting percentages for the individual families. The highest rooting for nontreated cuttings was for family B (93%) which did not differ from families C (72%) and D (77%) whereas family A (67%) had the least percent rooting and differed only from family B (LSD = 24%). Among all auxin treatments (across all families) only rooting percentages for cuttings treated with 1477, 2954, or 4431 ppm P-ITB did not differ from the nontreated control cuttings (data not presented). Thus, P-ITB at a concentration $\geq$ 5908 ppm or IBA (all concn.) were not effective in improving rooting percentages of the four families. The lower rooting percentages for these treatments suggest rooting may be suppressed with IBA application. These results also suggest the inherent differences of family (genotype) may influence rooting percentage to a greater degree than auxin application when rooting softwood cuttings from hedged stock plants. Analysis of root number data revealed a significant family affect and no family treatment interaction. Analysis of root number data across all treatments revealed mean root numbers did differ among the four families (Table 1). While the number of roots did not vary greatly between family, treatment with P-ITB resulted in an increase in root number per cutting compared to nontreated and IBA-treated cuttings (LSD =0.3). Mean root numbers were 2.2, 2.4, and 2.7 for nontreated, IBA- and P-ITB-treated cuttings, respectively . Thus, P-ITB application, while not improving percent rooting, may prove useful in altering root system morphology/ symmetry by increasing the number of roots per cutting. The ANOVA for mean primary root length (mean length per root) and total primary root length (sum of primary root length per cutting) data indicated no rep, family, or treatment effect. Mean primary root length was 14.6 cm (5.7 in) per root and total primary root length was 33.8 cm (13.3 in)per cutting . The increase of root number per cutting for cuttings treated with P-ITB, while altering root system morphology/symmetry, did not result in a change in total root length per cutting.

Expt. 2. Analysis of the rooting percentage data revealed a highly significant family and treatment effect and no significant family x treatment interaction. Rooting percentages of families A, B, C, and D were 26%, 30%, 34%, and 19% respectively (Table 1.). Across all families the best rooting treatments were noted for the nontreated cuttings (48%) and cuttings treated with 95% ethanol (34%). All other treatments resulted in lower rooting percentages (data not presented).This suggests the concentration ranges for IBA and P-ITB were not effective for softwood cuttings. Although auxin treatment did not influence root number, family had a highly significant affect on root number with mean root numbers of 1.9, 1.7, 2.1, and 1.5 for families A, B, C, and D, respectively (Table 1.). No significant differences were noted across families or treatments for root length. Mean root length for all treatment combinations was 13.4 cm (5.3 in) per root and total primary root length was 23.2 cm (9.1 in) per cutting. Similar root length for all family and treatment combinations suggests IBA and P-ITB did not inhibit subsequent root development while decreased percent rooting suggests these auxins may suppress root initiation of softwood cuttings at the concentrations used in these experiments.

Significance to industry: Treatment of cuttings with P-ITB was no more effective than IBA in promoting rooting of softwood stem cuttings of loblolly pine.

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.

Table 1. Rooting percentage and root number of softwood stem cuttings from four families of loblolly pine.

	Family				LSD[z]
	A	B	C	D	(a= 0.05)
Rooting (%)					
Expt. 1	52	75	66	59	**14.0**
Expt. 2	26	30	34	19	**8.0**
Root No.					
Expt. 1	2.5	2.8	2.4	2.1	**0.4**
Expt. 2	1.9	1.7	2.1	1.5	**0.5**

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

Propagation of *Quercus phillyreoides* by Stem Cuttings

Patrick J. McGuigan, Frank A. Blazich and Thomas G. Ranney
North Carolina

Nature of Work: *Quercus phillyreoides* A. Gray (ubame oak) is an attractive, evergreen species indigenous to Japan and China. It grows as a shrub or small tree reaching a height of 6 to 9 m (20 to 30 ft) (3). Although relatively unknown in the United States, it appears *Q. phillyreoides* has great potential for use in landscapes in the southern United States in addition to being well suited for urban situations. Its adaptability to southern landscapes has been supported by the excellent performance of two clones of *Q. phillyreoides* growing in the North Carolina State University Arboretum, Raleigh, N.C.

Since a preliminary investigation indicated that stem cuttings of *Q. phillyreoides* can be rooted, the following research was conducted to develop a protocol for propagation of the species by stem cuttings. Specifically, the influence of timing (growth stage) and indolebutyric acid (IBA) treatment on rooting were investigated.

Stem cuttings of two clones (clones 1 and 2) of seedling origin of *Quercus phillyreoides* in the adult growth phase were taken on four dates that represented four growth stages (semi-hardwood, hardwood, softwood, and transitional growth between softwood and semi-hardwood). Semi-hardwood and hardwood cuttings were treated with 0, 3000 (0.3%), 6000 (0.6%), or 9000 ppm (0.9%) IBA in 50% isopropanol. Softwood and softwood/semi-hardwood cuttings received the same treatments in addition to 50% isopropanol, 3000 ppm (0.3%) or 8000 ppm (0.8%) IBA in talc. All cuttings were placed in a raised greenhouse bench containing a medium of 1 peat : 1 perlite (v/v) and rooted under intermittent mist for 12 weeks.

Results and Discussion: Greatest rooting for both clones was achieved with softwood cuttings with 97% and 56% rooting for clones 1 and 2, respectively, treated with 8000 ppm (0.8%) IBA in talc. Six weeks later when cuttings were in a softwood/semi-hardwood condition, rooting of clone 1 was still comparable to softwood cuttings whereas negligible rooting was noted for cuttings of clone 2. For both clones, rooting of semi-hardwood cuttings was poor which was the same for hardwood cuttings of clone 2. Moderate rooting of 58% was noted for hardwood cuttings of clone 1. Auxin treatments generally increased root number. As mean root number increased mean root length decreased. Greater overwinter survival was observed for rooted softwood cuttings which produced a flush of new growth following rooting in comparison to softwood/semi-hardwood cuttings which did not flush following rooting.

Significance to Industry: The traditional method of propagating most species of oak (*Quercus* L. spp.) is by seed. However, sexual propagation of oaks results in great phenotypic and genotypic variability (1,2). Results herein demonstrate that *Q. phillyreoides*, 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.

Literature Cited

1. Flemer, W., III. 1962. The vegetative propagation of oaks. Proc. Intl. Plant Prop Soc. 12:168-172

2. 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.

3. Ohwi, J. 1984. Flora of Japan. Smithsonian Institution, Washington, D.C.

Influence of Hedged Stock Plant Nitrogen Fertilization on Rooting Stem Cuttings of Loblolly Pine

D. Bradley Rowe, Frank A. Blazich and Farrell C. Wise
North Carolina

Nature of Work: With few exceptions, loblolly pine (*Pinus taeda* L.) is currently propagated exclusively by seed which results in undesirable genotypic and phenotypic variation. Vegetative propagation would eliminate much of this variation and permit cloning of superior trees. In recent years considerable work has been conducted to propagate the species by micropropagation and somatic embryogenesis. Although some progress has been reported, much additional research needs to be conducted before such techniques aimed at vegetative propagation of elite trees can be utilized commercially. As a result, interest in clonal propagation of loblolly pine has shifted in part to propagation by stem cuttings.

Nitrogen availability affects stock plant physiological status, which in turn influences subsequent rooting of stem cuttings taken from that plant. Nitrogen deficiency (not stress) within the stock plant generally promotes root formation of cuttings, presumably due to restricted metabolism of stock plant carbohydrates. These stored carbohydrates may then be available to support root formation on stem cuttings. A low nitrogen status relative to available carbohydrates (high C/N ratio) results in a tendency for stored carbohydrates and current photosynthate to be directed into adventitious root formation. Hyun and Hong (3) reported that clones of pitch pine (*Pinus rigida* Mill.) which rooted easily had higher C/N ratios than clones which were difficult to root. Differences in the C/N ratios were attributed to the nitrogen content, with the easy-to-root clones containing less nitrogen than the difficult-to-root clones.

Although precise endogenous and exogenous relations between nitrogen and adventitious rooting have not been established, carbohydrate to nitrogen ratios can be manipulated by varying nitrogen fertilization rates provided to the stock plants. Henry et al. (2) reported optimal growth of eastern redcedar (*Juniperus virginiana* L.) when the stock plants were provided weekly with 180 ppm N. However, optimal rooting occurred when the stock plants received only 20 ppm N. Therefore, our objective was to determine if nitrogen availability to hedged stock plants of loblolly pine may show a similar response with respect to adventitious rooting.

In May 1993, six-month-old seedlings were moved to a container pad located at the Horticulture Field Laboratory, Raleigh, N.C. The experimental design was a randomized complete block with 4 blocks each containing 4 families (B, G, R, and W) and 6 nitrogen treatments (including a peat:vermiculite:perlite control) arranged in a complete factorial. Trees for the five N treatments were grown in 3 gal pots with a medium of 6 perlite : 4 sand (v/v) and the control trees were grown in a medium of 2 peat : 2 coarse vermiculite : 1 perlite (v/v) amended with 2.1, 0.24, and 1.0 $kg \cdot m^{-3}$ (3.6, 0.4, and 1.7 $lb \cdot yd^{-3}$) of Osmocote 18-6-12, Micromax, and dolomitic lime. The four families were all full-sib families (controlled pollinations where both parents were known). The six N

treatments consisted of the peat culture control and five levels of N (0, 5, 10, 20, and 40 ppm N) supplied as NH_4NO_3 through an automated irrigation system. All other mineral nutrients were supplied at optimal levels.

Initial hedging in January 1994 involved decapitating trees 20 cm (8 in) above the rim of the pot and removing all remaining terminal buds from branches such that half the length of any individual branch remained intact. Trees were hedged to keep them in the juvenile growth phase as cuttings taken from physiologically adult trees are often difficult or even impossible to root (1). During May 94 (spring softwood), July 94 (summer softwood), and January 95 (hardwood), stem cuttings were taken from the hedged stock plants for rooting. These dates also coincided with rehedging of stock plants back to a height of 20 cm as described previously. Osmocote was reapplied [24 g (0.8 oz) per pot] in March 1994 and following hedging in July 1994. The basal 2 cm (0.75 in) of 9 cm (3.5 in) long terminal cuttings were inserted into flats containing a medium of 1 perlite : 1 coarse vermiculite (v/v) and placed in a greenhouse under intermittent mist. Cuttings were not treated with auxin. After 12 weeks, cuttings were harvested and percent rooting, number of roots per cutting, total root length, and root area were recorded. Means were subjected to analysis of variance procedures and regression analysis.

Results and Discussion: There were significant differences among seasons, nitrogen treatments, and families, as well as a family x nitrogen interaction in regards to rooting percentages. The season in which the cuttings were taken had a significant impact on rooting. When averaged over families, significantly greater rooting percentages ocurred for spring softwood cuttings than summer softwood or winter hardwood cuttings (Fig. 1). Maximum rooting within each season was 69% at 20 ppm N for spring softwood, 59% at 40 ppm N for summer softwood, and 51% at 20 ppm N for winter hardwood cuttings.

For winter hardwood cuttings, rooting of families B and G increased linearly with increasing levels of N (Fig. 2). Families R and W showed a quadratic response with maximum rooting of 42% and 81%, respectively, at 20 ppm N . For all families, rooting was significantly lower for the peat:vermiculite:perlite control. Actual N concentrations present in the soil solutions analyzed by the Virginia Tech Extraction Method (4), indicated the peat:vermiculite:perlite control treatment contained the highest level of N in the soil solution (33%) compared to the other N treatments (1%, 2%, 4%, 8%, and 18% for 0, 5, 10, 20, and 40 ppm N, respectively, in the irrigation water). This suggests that rooting of families B and G may have also exhibited a quadratic response if supplied with higher rates of N. These results emphasize that differences in rooting response may be genetic, even within the same species. The number of roots per rooted cutting showed a quadratic response with a maximum of 2.2 roots produced at 20 ppm N (data not presented). Root area increased linearly with increasing N concentration for all families (data not presented). The sharpest increase occurred in family B which had a root area of 8.3 cm^2 (1.3 in^2) at the highest N level (40 ppm). Root length showed a similar response to root area (data not presented).

Significance to Industry: The time of year in which cuttings are taken from stock plants (actually the growth stage), nitrogen fertility of stock plants, and genetic variation all have a major influence on rooting success of loblolly pine. Spring softwood cuttings rooted in the highest percentages, followed by winter hardwood, and summer softwood cuttings. The best rooting occurred at N levels of 20 to 40 ppm, but this varied depending on the particular family.

Literature Cited

1. Hackett, W.P. 1988. Donor plant maturation and adventitious root formation, p.11-60. In: T.D. Davis, B.E. Haissig, and N. Sanhla (eds.). Adventitious root formation in cuttings. Dioscorides Press, Portland, Ore.

2. Henry, P.H., F.A. Blazich, and L.E. Hinesley. 1992. Nitrogen nutrition of containerized eastern redcedar. II. Influence of stock plant fertility on adventitious rooting of stem cuttings. J. Amer. Soc. Hort. Sci. 117:568-570.

3. Hyun, S.K. and S.O. Hong. 1968. Fundamental mechanism of root formation in the cuttings of forest trees. Inst. For. Genet. Suwon, Korea, Res. Feg. 6:1-52.

4. Wright, R.D. 1987. The Virginia Tech liquid fertilizer system for container grown plants. Va. Tech College Agr. Life Sci. Info. Ser. 86-5.

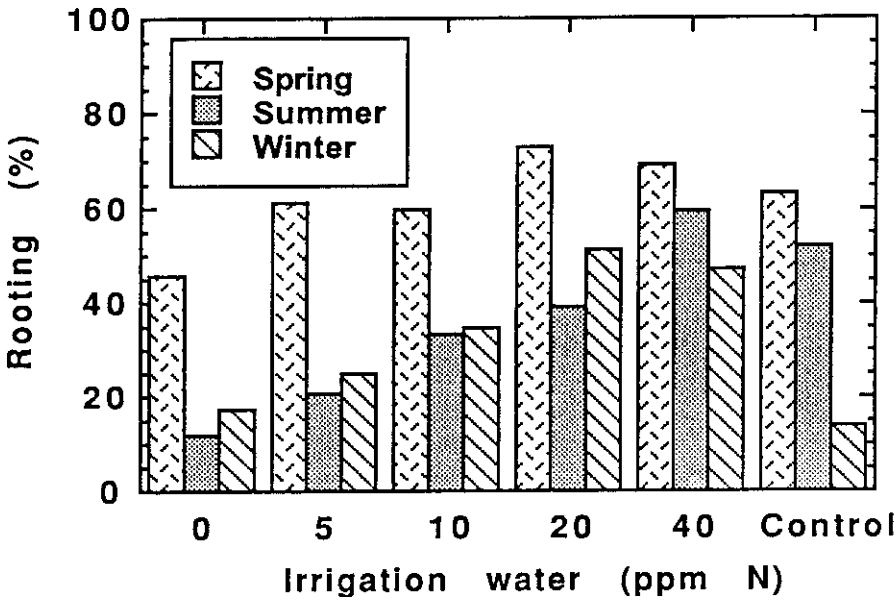


Fig. 1. Effect of stock plant nitrogen fertilization on rooting stem cuttings of loblolly pine taken at three growth stages (spring softwood, summer softwood, and winter hardwood). Data are averaged over families. Each bar represents a mean of 144 observations.

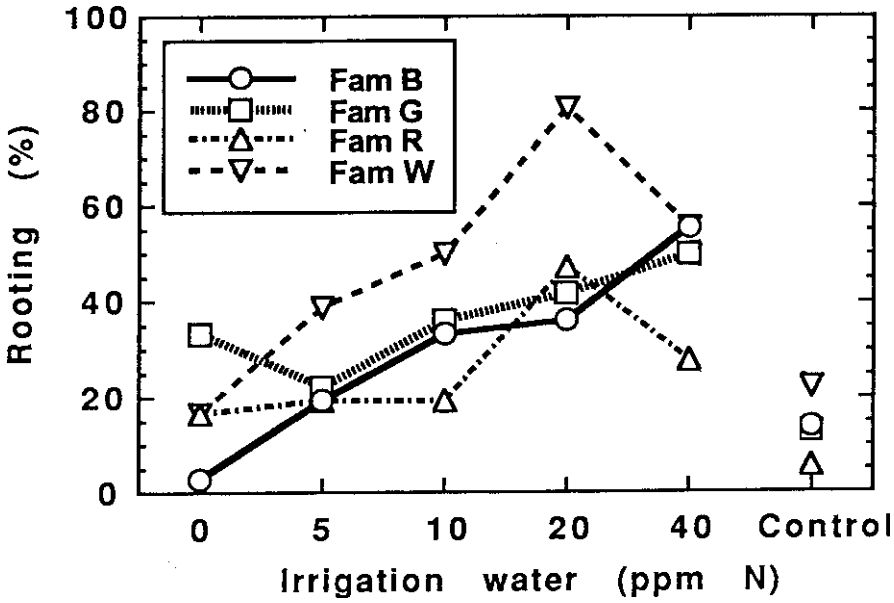


Fig. 2. Effect of stock plant nitrogen fertilization on rooting winter hardwood stem cuttings of loblolly pine taken from four families (Fam B, G, R, or W) of hedged stock plants. Each symbol is based on 36 observations.

In Vitro Culture of Oak

N.D. Camper and Kimberly S. Grumbine
South Carolina

Nature of Work: Oaks are predominant trees in our residential environment as an urban or landscape tree and are important in lumber production. Oak wilt disease, caused by *Ceratocystis fagacearum,* has been a potentially serious threat to oaks for a long time. Studies designed to better understand the nature of the disease, screen for resistant plant material and possibly establish the mechanism of fungal action require a responsive bioassay system. In vitro culture systems can be used to study host-pathogen relationships; e.g., involvement of fungal toxins in the disease symtomology and the identification of resistant tissues or cell lines. Initial in vitro culture of oak was reported by several investigators (Chalupa, 1990; Maataoui et al., 1990; Romano, 1992; Gingas, 1991; Gingas and Lineberger, 1989). The objective of this study was to develop an in vitro culture system for several different oak species.

White oak *(Quercus alba)* acorns were collected the first week of August. The pericarp was removed from the cotyledonary tissue and the immature embryo. Cotyledonary tissue and the embryo were surface disinfested by successive treatments with antibacterial soap, 70% ethanol, 15% Clorox and several sterile water rinses. Immature embryos were excised from the cotyledonary tissue and placed in vials containing 10 ml of culture medium. Three media were tested: Murashige and Skoog (MS), woody plant (WP) and Gresshoff and Doy (GD). Each medium was supplemented with 20 g/l sucrose and 8.0 g/l agar. Hormone combinations included 1.0 and 3.0 mg/l of either naphthaleneacetic acid (NAA) or 2,4dichlorophenoxyacetic acid (2,4-D) with 1.0 mg/l benzyladenine (BA). Cultures were incubated under a 16-hr photoperiod at 26°C (79 °F). Scarlet *(Quercus coccinea)* and water (Ouercus nigra L.) oak acorns were collected in August, washed with antibacterial soap and a 50% Clorox solution, and stored at 4°C (39°F) until used.

The pericarp was removed from the acorn along with most of the cotyledonary tissue. The embryos were removed from remaining cotyledonary tissue, disinfested and placed on WP medium with combinations of 0, 1.0 and 3.0 mg/l 2,4-D and 1.0 mg/l BA. Embryo cultures were placed under fluorescent lights at 26°C. Growth and morphological development were rated weekly.

Results and Discussion: Immature embryos exhibited radicle elongation but failed to generate seedlings. Radicle elongation was stimulated on all media tested (Figure 1). Gradual growth increases followed imbibition; greening of remaining cotyledon tissue was observed in some cultures. Shoots formed on WP medium in some cultures. No significant differences were observed on media containing different hormone combinations (Figure 2). Embryos isolated from immature acorns of white oak produced embryogenic callus on all three media with either NAA or 2,4-D in combination with BA. Somatic embryos were best initiated and maintained on GD media containing 2,4-D and BA.

Mature scarlet and water oak embryos produced seedlings with complete root and shoot systems when cultured on hormone-free medium. Additions of hormones was not necessary for in vitro germination and growth of these two species of oak. The culture of immature and mature embryos, production of embryogenic callus lines and nonembryogenic callus tissue will facilitate studies of the host-pathogen relationships involved in the oak wilt disease phenomenon.

Significance to Industry: A protocol for the in vitro culture of white, scarlet and water oaks will aid in the study of host-pathogen relationships for the oak wilt disease syndrome. These studies will provide information about the disease mechanism, facilitate disease management strategies and perhaps enable identification of resistant plant materials.

Literature Cited

1. Chalupa, V. 1990. Plant regeneration by somatic embryogenesis from cultured immature embryos of oak (*Quercus robur* L.) and linden (*Tilia cordata* Mill.). Plant Cell Reports. 9:398-410.

2. Gingas, V.M. 1991. Asexual embryogenesis and regeneration from male catkins of Ouercus. HortSci. 26:1217-1218.

3. Gingas, V. and R. Lineberger. 1989. Asexual embryogenesis and plant regeneration in Ouercus. Plant Cell, Tissue and Organ Cult. 17:191-203.

4. Maataoui, J.A., H. Espagnac and N. Michaus-Ferriere. 1990. Histology of callogenesis and somatic embryogenesis induced in stem fragments of cork oak (*Quercus suber*) cultured in vitro. Annal. Bot. 66:183-190.

5. Romano, A., C. Noronha and M. Marinis-Loucao. 1992. Influence of growth regulators on shoot proliferation in Ouelcus L. Annal. Bot. 70:531-536.

6. SC Agricultural Experiment Station Publication No. 4101.

Figure1. Growth response of immature white oak embryos after 7 weeks time on three different media: MS, WPM and GD. Data represented as average rating; error bars represent one standard deviation.

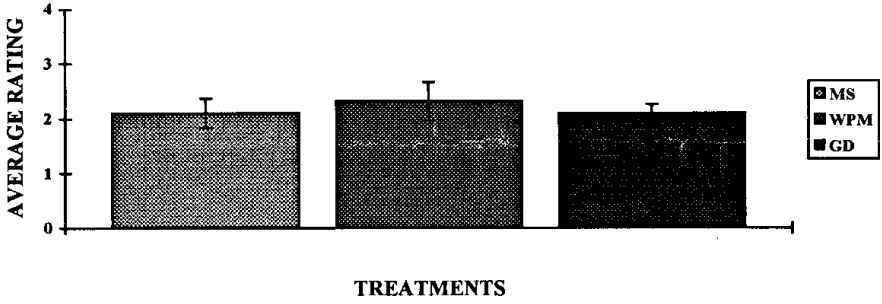
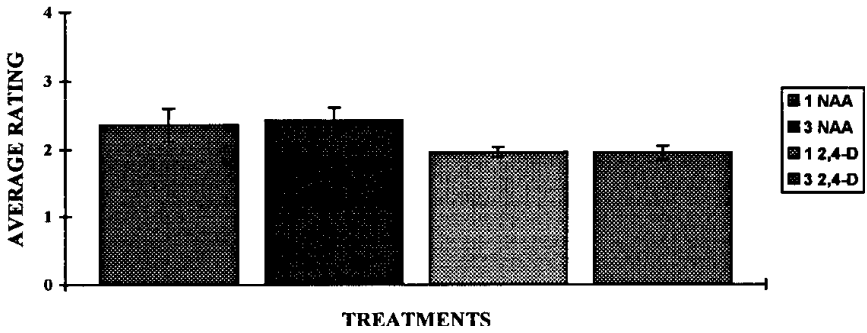


Figure 2. Growth response (average rating) of white oak immature embryos after 7 weeks time on four different hormone treatments. The treatments were 1.0 and 3.0 mg/ 1 NAA or 2, 4-D in combination with 1.0 mg/1 BA. Error bars represent one standard deviation.



Three Alternatives to Peat in the Propagation and Growth after Transplanting of Buddleia

James H. Aldrich, Joshua L. Roddenberry and Jeffrey G. Norcini
Florida

Nature of Work: The increasing cost of sphagnum peat and local availability of alternatives to peat, especially recycled materials, have resulted in numerous studies on peat substitutes. Peat has been substituted with municipal solid waste compost (MSWC) (5), composted yard waste (3), spent mushroom compost (MC) (1), and wood waste (2). Although most studies have dealt with use of peat alternatives in potting media for 1-gal or larger containers, there has been interest in use of alternatives in propagation media (4). The objective of this study was to determine if replacing sphagnum peat with MC, MSWC, or knots and chives (KC; a paper mill by-product consisting of incompletely digested pine tree knots) would affect rooting of *Buddleia davidii* or growth of rooted cuttings after transplanting to 1-gal containers.

Canadian sphagnum peat, MC, MSWC, or KC were mixed 1:1 (by vol.) with coarse perlite. On 9 Apr. 1994, each of the four media were placed in 12 2x2x3" cups. Softwood cuttings (4-5") from *Buddleia davidii* at the North Florida Res. & Ed. Ctr. in Monticello were scored on opposite sides at the base and dipped (3 sec) into 3000 ppm K-IBA and placed into the pots. The pots were placed in a randomized complete block design on a heating pad (70°F) and under intermittent mist (9 secs/15 mins; 12 hrs/day) in a greenhouse. On 27 Apr. 1994, 6 of the 12 replications from each medium were evaluated for rooting quality (0 = no rooting to 3 = heavy rooting), number of primary roots, and root fresh and dry weights (dried at 150°F for 4 days). The remaining rooted cuttings were transplanted on 28 Apr. into 1-gal containers with a 3:1:1 (by vol.) pine bark:sand:Canadian sphagnum peat medium amended (per yd^3) with 3.8 lb triple superphosphate, 10 lb Osmocote 18-6-12, and 2.4 lb Micromax. An additional 0.4 oz Osmocote 18-6-12 was top-dressed on 28 Apr. The plants were arranged in a randomized complete block design on black plastic in full sun with daily overhead irrigation (0.3"). Plant growth indices [(height+ ((widest point + width perpendicular to widest point)/2))/2] were recorded on 21 May and 8 June 1994. Additionally on 8 June, the plants were harvested for shoot and root fresh and dry weight determination.

Percent air space, water holding capacity, and weight were determined for 4 samples per medium. Three samples of each of the 4 media were analyzed by the Univ. of Fla., Soil Analytical Res. Lab. for pH, electrical conductivity (EC), and macro and micro elemental determination.

Results and Discussion: All alternative media components evaluated were probable replacements for Canadian sphagnum peat when used 1:1 with perlite to root *Buddleia* cuttings, since root fresh and dry weights, quality, and number of primary roots were not significantly different compare to the peat-based medium (Table 1). In a review article by Shiralipour et al. (5), use of MSWC at 50% or greater in container media resulted in suppressed plant growth, while lowering the percentage improved plant growth.

Shiralipour et al. recommended matching the various composts with the requirements of various crops. Additionally, some production practices might require modification in order to use MSWC. The MSWC-based medium was the closest to sphagnum peat-based medium in physical characteristics, except medium with MSWC had a lower water holding capacity (Table 2). The chemical characteristics of KC-based medium was identical to peat-based except the pH for KC was significantly higher (Table 3).

On 21 May, there were no differences in growth indices for any treatment (data not shown). However by 8 June (41 days after transplanting), the cuttings originally propagated in the KC-based medium were significantly smaller (growth index and shoot and root fresh and dry weights) than those propagated in any other media (Table 4). Since water was not a limiting factor and the chemical properties of the KC-based medium were similar to the peat-based medium, it is possible that KC may contain some growth-inhibiting substance(s). The growth-inhibiting substance(s) may be in the raw product or may have resulted from processing the raw product.

Significance to Industry: Municipal solid waste compost, mushroom compost, and knots and chives could be good replacements for peat in perlite-based medium (1:1, by vol.) used to propagate *Buddleia davidii*. Statistically, cuttings rooted equally well in all three media; however, growth after transplanting into 1-gal containers was similar only for MSWC and MC. Moreover, the local cost of one cubic yard of MSWC and MC is 1/15th and 1/20th the cost of Canadian sphagnum peat, respectively.

Literature Cited

1. Chong, C., R.A. Cline, and D.L. Rinker. 1994. Bark- and peat-amended spent mushroom compost for containerized culture of shrubs. HortScience 29:781-784.

2. Lord, W.G., T.E. Bilderback, and L. Hight. 1993. Wood waste compost as a potting medium amendment. Proc SNA Res. Conf. 38:75-77.

3. McConnell, D.B. and R. Harrell. 1992. Commercial production of landscape plants using yard waste compost. Proc. SNA Res. Conf. 37:72-74.

4. Poole, R.T. 1969. Rooting response of four ornamental species propagated in various media. Proc. Fla. State Hort. Soc. 82:393- 397.

5. Shiralipour, A., D.B. McConnell, and W.H. Smith. 1992. Uses and benefits of MSW compost: a review and an assessment. Biomass and bioenergy 3:267-279.

Florida Agricultural Experiment Station Journal Series No. N-01095.

Table 1. Effect of 3 alternative media components and peat (combined 1:1 with coarse perlite, by vol.) on rooting of *Buddleia*.

Media Component	Root fresh wt (oz)	Root dry wt (oz)	Root quality[z] (oz)	No. primary roots
Peat	0.025	0.0035	1.67	47.50
MSWC	0.001	0.0004	0.67	8.83
Knots & chives	0.041	0.0049	2.33	61.83
Mushroom compost	0.050	0.0035	1.67	54.00
LSD (P = 0.05)	0.043	0.0039	1.05	46.04

[z] Root quality = 0 - no rooting, 1 - light, 2 - moderate, and 3 - heavy rooting.

Table 2. Physical characteristics of rooting media composed of coarse perlite combined 1:1 (by vol.) with peat or a peat alternative.

Media Component	% Air space	Water holding capacity (fl oz)	Media weight (oz)
Peat	5.8	2.19	3.08
MSWC	5.8	1.85	3.24
Knots & chives	25.8	1.68	2.44
Mushroom compost	33.8	1.48	2.44
LSD (P = 0.05)	7.0	0.23	0.25

Table 3. Chemical properties of rooting media with peat or a peat alternative combined with coarse perlite 1:1 (by vol.).

Media Component	pH	E.C. dS/m	NO_3-N ppm	P ppm	K ppm	Ca ppm	Mg ppm
Peat	4.2c	0.2c	0.5c	7.5a	16.4c	3.3c	2.0b
MSWC	7.5a	3.0a	34.0a	0.1a	162.7a	144.7a	24.7a
Knots & chives	6.1b	0.3c	0.2c	0.9a	8.0c	12.0c	3.3b
Mushroom compost	7.4a	1.2b	8.7b	6.0a	124.7b	97.7b	26.3a
LSD (P = 0.05)	0.9	0.2	4.4	7.9	25.7	10.3	4.0

Table 4. Effect of four media components (combined with coarse perlite 1:1, by vol.) on the growth of *Buddleia*, 41 days after transplanting into 1-gal containers.

Media Component	Growth index[z] (in)	Shoot fresh wt (oz)	Shoot dry wt (oz)	Root fresh wt (oz)	Root dry wt (oz)
Peat	21.7	3.24	0.76	0.72	0.08
MSWC	19.7	3.10	0.74	0.66	0.09
Knots & chives	18.5	1.93	0.42	0.37	0.04
Mushroom compost	20.8	2.84	0.68	0.64	0.08
LSD (P = 0.05)	2.7	0.57	0.18	0.16	0.02

[z] Growth index = (height + ((widest point + width perpendicular to widest point)/2))/2.