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- AUSTRALIAN REGION
- EASTERN REGION, NORTH AMERICA
- REGION OF GREAT BRITAIN AND IRELAND
- IPPS JAPAN REGION
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- SCANDINAVIAN REGION
- SOUTHERN AFRICAN POTENTIAL REGION
- SOUTHERN REGION OF NORTH AMERICA
- WESTERN REGION

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# Development of a Subirrigation System with Potential for Hardwood Tree Propagation®

**Mark V. Coggeshall**

Center for Agroforestry, University of Missouri, Columbia, Missouri 65211-7270 U.S.A.

**J. W. Van Sambeek**

USDA Forest Service, North Central Research Station, Columbia, Missouri 65211-7260 U.S.A.

## INTRODUCTION

The successful propagation of many desirable hardwood tree species by means of traditional stem cutting propagation remains an elusive goal. A number of species within genera such as *Juglans* and *Quercus* are highly prized for their timber value, and in the case of black walnut (*J. nigra*) and pecan (*Carya illinoensis*) also produce marketable edible nuts. Black walnut, pecan, and several species of oak are also important tree species in agroforestry-based systems in Missouri (Garrett and Reitveld, 1995). Clonal propagation of superior trees on their own root systems is highly desired; however, most species from these three genera are usually classified as difficult-to-root species (Coggeshall and Beineke, 1997; Zaczek et al, 1997).

Mezitt (1978) first proposed the use of the subirrigation method as a propagation technique for difficult-to-root plants. Subsequent papers by Holt and Maynard (1997) and Regan and Henderson (1999) indicated that a broad range of genera could successfully be propagated, at least to a limited degree, by using subirrigation. Systems for subirrigation allow for the upward movement of water from a reservoir through the rooting media by means of capillary action, rather than providing water from above by means of a standard mist or fog system. The advantages of subirrigation systems include lower cost, reduced water use and leaching of nutrients, easier maintenance, and avoidance of water-logging of the rooting media (Regan and Henderson, 1999). For subirrigation, the physical properties of the rooting media, such as particle size, while important, are not as critical as the ease with which water can move through it (capillarity). Perlite has been the basic media of choice for most subirrigation systems because of the high surface-to-volume ratio needed for aeration and water movement and its thermal insulation characteristics (Cook and Dunsby, 1978; Holt and Maynard, 1997).

An array of environmental and physiological factors may influence propagation success including: rooting media composition, bottom heat, shading levels, auxin formulation, and the stage of cutting growth. Difficult-to-root species can be especially sensitive to these factors, which makes their successful propagation more challenging. The purpose of our study was to examine the feasibility of developing a simple, inexpensive subirrigation system as a propagation method for rooting a number of difficult-to-root tree species over an array of environmental and physiological treatment combinations.

## MATERIALS AND METHODS

Our subirrigation experiments were done in the greenhouse at the University of Missouri Horticulture and Agroforestry Research Center in New Franklin, Missouri. Bench light was approximately 50% of full sunlight and supplemented with high-pressure sodium lamps to provide 16-h photoperiod. Our subirrigation system was constructed from two 15¼ inch × 15¼ inch × 5-inch-deep propagation flats, as manufactured by Anderson Die and Manufacturing Company (Portland, Oregon). The top tray contained thirty-six 2½ inch × 2½ inch × 5-inch plant bands and was lined with shade cloth to reduce media loss from plant bands. The top tray was nested into the bottom (reservoir) tray allowing the base of the plant bands to be immersed in water to a depth of 1½ inches. The reservoir tray was lined with 6-mil clear polyethylene sheeting. Reservoir trays had water added daily through an empty plant band to overflow and were flushed once per week as a means of providing aeration. The top tray with cuttings was placed on four stacks of 2½ inch × 2½ inch × 3-inch plastic pots, which served as 3½-inch-tall pedestals to maintain the proper height of the cutting tray within the reservoir tray.

Plant bands within each subirrigation tray were filled with one of the following media: perlite, perlite and peat (9 : 1, v/v), or perlite and crushed limestone (9 : 1, v/v). One tray containing each media was randomly placed in one of six treatment combinations consisting of three shade treatments with or without bottom heat. Shade structures to cover three subirrigation trays were constructed from 1-inch PVC pipe and covered with white 4-mil polyethylene sheeting, 50% shade cloth, or 80% shade cloth. For treatments with bottom heat, the three subirrigation trays were set on an electric heating pad (Phytotronics, Inc., Earth City, Missouri). Thermostats were set for a minimum of 78°F during the study. The minimum and maximum temperatures were recorded daily inside one plant band filled with perlite within each shade × bottom heat treatment combination. Gas heaters maintained a minimum temperature of 75°F and evaporative coolers started when the maximum temperature exceeded 85°F within the greenhouse.

Five different plant sources were utilized to produce 6- to 9-inch soft to semihardwood cuttings throughout the growing season. We began by sticking black walnut cuttings in early June obtained from several stock types including seedling stump sprouts or forced grafted shoots taken from 1-year-old potted grafts. In mid-summer, shoots were again harvested from the same potted seedlings. We also harvested branch tips from 6-year-old trees planted in New Franklin, Missouri, that were established as a field trial to evaluate growth and early flowering traits of RPM seedlings as described by Lovelace (1998). Cuttings were collected from black walnut (*J. nigra*), bur oak (*Q. macrocarpa*), swamp white oak (*Q. bicolor*), and northern red oak (*Q. rubra*).

We tried five different auxin treatments in 50% ethanol including 3000 ppm IBA (15 mM IBA), 2000 ppm IBA and 1000 ppm NAA by diluting Dip 'n Grow (DNG) (Astoria-Pacific, Clackamas, Oregon), 2000 ppm IBA and 1000 ppm NAA by diluting Wood's Rooting Hormone (WOODS) (Earth Science Product Corporation, Wilsonville, Oregon), 9000 ppm IBA, and an ethanol control. All cuttings were treated for 5 sec to a depth of 0.5 inches and randomly assigned to one of the 18 treatment combinations for shade, bottom heat, and media. Cuttings were stuck to a depth of 1.5 inches.

Dead or diseased cuttings were removed as found and presence/absence of basal rot was recorded. Approximately 1 month after sticking, remaining live cuttings were evaluated for retention of green foliage. Approximately 2 months after

**Table 1.** Percent of cuttings with basal rot and surviving for 1 month, and percent of live cuttings retaining green foliage or producing callus in a subirrigation system by source of cuttings, shade levels with and without bottom heat, rooting medium, and auxin treatment.

	Cuttings treated (no.)	Basal rot (%)	1 month survival (%)	Green foliage (%)	Callus (%)
Source of cutting:					
Spring stump sprouts	60	43	35	70	30
Summer stump sprouts	156	50	43	53	48
Shoots from grafts	30	38	58	72	47
6-year old RPM walnuts	65	22	61	23	6
6-year old RPM oaks	298	81	16	22	18
Significance:	---	**	**	**	*
5% least significant difference:		19	18	30	28
Shade and bottom heat:					
Polyethylene None	106	34	60	54	35
50% Shade None	90	69	22	47	30
80% Shade None	115	59	26	37	26
Polyethylene Yes	108	61	26	52	25
50% Shade Yes	93	71	26	37	36
80% Shade Yes	102	85	8	7	20
Significance: Shade	---	**	**	*	ns
Significance: Heat	---	**	**	ns	ns
Significance: Shade × Heat		**	*	ns	ns
5% least significant difference:		11	14	30	26
Rooting medium:					
Perlite and peat (9:1 v/v)	203	60	34	39	17
Perlite only	205	36	52	60	55
Perlite and lime (9:1 v/v)	206	58	34	44	20
Significance:	---	**	*	ns	**
5% least significant difference:		11	12	22	21



	Cuttings treated (no.)	Basal rot (%)	1 month survival (%)	Green foliage (%)	Callus (%)
Auxin treatment:					
50% ethanol	166	41	54	52	36
3000 ppm IBA	135	35	55	42	25
3000 ppm auxin (DNG)	135	67	22	38	33
3000 ppm auxin (Woods)	138	56	30	55	32
9000 ppm IBA	40	73	21	57	30
Significance:	----	*	**	ns	ns
5% least significant difference:		19	18	30	28

ns, \*, \*\* - Nonsignificant or significant by  $P < 0.05$  or  $0.01$ , respectively.

sticking, remaining cuttings were evaluated for presence/absence of basal rot, visible callus, and adventitious roots greater than 0.2 inches long. Presence/absence data for individual cuttings were converted to percentages by calculating averages for treatment combinations by source of cutting, auxin treatment, and media or by amount of shade, use of bottom heat, and media. Percentages were subjected to 3-way ANOVA with all interactions having  $p > 0.01$  for a significant  $F$ -value pooled into the error for calculation of least significant difference (LSD) value at the 0.05 probability level.

## RESULTS AND DISCUSSION

The source of cuttings had a significant impact on the percentage of cuttings with basal rot, survival percentage, and percentage of live cuttings retaining some green foliage or producing visible callus (Table 1). While the RPM walnut cuttings had the least incidence of basal rotting, they also had the lowest percentage of surviving cuttings with callus. In contrast, the RPM oak cuttings had the highest incidence of basal rot as well as the lowest percentage of cuttings with callus. Successful propagation of oaks has been found to be highly associated with the timing of cutting collection (Teclaw and Isebrands, 1987). Our results suggest more knowledge is needed on the best time for making cuttings, especially for some of the oak species. The relatively high incidence of basal rot and low cutting survival may reflect a problem associated with our subirrigation system, which essentially had a noncirculating water reservoir. Rein, et al (1991) discussed the need to maintain high oxygen levels in the media to stimulate rooting and reported that rooting success is highly dependent on the cutting growth stage of *Ilex*, when comparing spring versus late summer cutting dates. We also found a higher percentage of surviving cuttings with callus from the summer stump sprouts compared to the spring stump sprouts.

Shading and bottom heat, and their interaction, significantly influenced the incidence of basal rot and cutting survival (Table 1). Cuttings covered with polyethylene sheeting and without bottom heat have the lowest incidence of basal

rot and highest survival. Also cuttings covered with polyethylene sheeting had less wilting after sticking resulting in less leaf drop 1 month later. An interaction for percentage of cuttings with basal rot and cutting survival existed between use of bottom heat and with 50% and 80% shade. Differences in the minimum temperature within the perlite medium averaging 0.8°F in the 50% shade treatment with and without bottom heat and 2.8°F for the 80% shade treatment with and without bottom heat. However, we found substantial differences in maximum temperature of the medium between treatments that may partially explain the interaction. The difference in average maximum temperature in the 80% shade treatment with and without bottom heat was 8.9°F and a 26% greater incidence of basal rot. In comparison, the difference in average maximum temperature in the 50% shade treatment with and without bottom heat was only 1.2°F and a nonsignificant difference in incidence of basal rot. In general, bottom heat tended to increase incidence of basal rot and cutting mortality without increasing the percentage of cuttings with callus that maybe necessary for successful rooting.

Of the three rooting media, perlite had the lowest incidence of basal rot and highest percentage of cuttings that survived for 1 month, and produced callus (Table 1). Our results confirm earlier observations that perlite is an excellent rooting medium (Holt and Maynard, 1997; Regan and Henderson, 1999; Al-Salem and Karam, 2001). The addition of either peat to decrease pH or limestone to increase the pH of medium failed to improve our results over perlite alone. Apparently, the addition of peat or limestone resulted in increased water-holding capacity and decreased oxygen level in the media. Both of these factors have been shown to lead to increased incidence of fungal infection and basal rot (Loach, 1988; Rein, et al, 1991).

The auxin treatments resulted in a variable response as would be expected with different auxins and carriers (Table 1). We were unable to confirm earlier reports that black walnut stump sprouts could be rooted with 8000 ppm IBA in 50% ethanol (Shreve, 1974). The treatment with the highest incidence of basal rot and fewest surviving cuttings after 1 month was 9000 ppm IBA suggesting the auxin concentration was too high for our softwood and semihardwood cuttings. Dirr and Heuser (1987) previously reported that high auxin concentrations could predispose cuttings to basal rotting. Our data also suggest that mixtures of IBA and NAA at 3000 ppm were also too high resulting in unacceptable disease levels. The least incidence of basal rot and highest percentage of surviving cuttings with callus on our softwood and semihardwood cuttings of walnut and oak was found with IBA at 3000 ppm or 50% ethanol.

Although more than 600 cuttings either died within 1 month of sticking or failed to initiate roots, we did have one swamp white oak and three black walnut cuttings that each initiated one elongating adventitious root. All four cuttings retained 2 or 3 green leaves 1 month after sticking and rooted in the perlite medium with no consistency for response to shade or bottom heat. Three of the four cuttings had been treated with Wood's Rooting Hormone and one with 9000 ppm IBA.

Our results confirm previous observations that the most promising subirrigation system for rooting hardwood cuttings will likely use coarse perlite as the rooting medium. We also found that our subirrigation system constructed by nesting heavy-duty plastic trays designed to hold open bottom plant bands was relatively inexpensive, could be easily constructed and maintained, and was easily managed if one plant band was left empty. We are currently pursuing approaches to increase aeration of the rooting medium. Future developments will evaluate the effective-

ness of shade treatments to reduce cutting stress during rooting. In preliminary experiments, we have evaluated several approaches to precondition, etiolate, or blanch stock plant shoots to increase cutting survival or rootability (Bassuk, et al., 1987). Other trials have included the greenhouse forcing of epicormic sprouts with juvenile traits from dormant buds on branch segments cut from basal limbs as a source of softwood or semihardwood cuttings (Van Sambeek, et al., 1998).

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