Effect of Medium Physical Support, Shoot Length and Genotype on In Vitro Rooting and Plantlet Morphology of Sweetgum[1]

Xiaohong Lin[2], Ben A. Bergmann[3] and Anne-Marie Stomp[4]
Department of Forestry
North Carolina State University
Raleigh, NC 27695-8002

Abstract

Adventitious shoots (1 cm and 2 cm long) from 10 clones of sweetgum (*Liquidambar styraciflua* L.) were rooted on rockwool-supported Woody Plant Medium (WPM) and agar-solidified WPM *in vitro*. Shoots on rockwool-supported WPM had higher rooting frequency, shoot elongation, and leaf chlorophyll content than those on agar-solidified WPM. The data are consistent with the hypothesis that rockwool supplies more oxygen to the rooting zone. Taller shoots had significantly greater rooting frequency, more shoot elongation, and larger average area per leaf than shorter shoots. Genotype significantly influenced rooting frequency, shoot elongation, average area per leaf, chlorophyll content, and shoot fresh weight. An *in vitro* auxin pretreatment significantly increased rooting frequency over shoots grown on either agar-solidified or rockwool-supported WPM without auxin.

Index words: tissue culture, rockwool, auxin.

Significance to the Nursery Industry

Sweetgum has the potential to become a valuable hardwood tree species in the paper making industry. Its successful clonal propagation through tissue culture has been limited at the rooting stage. A rooting frequency of 65% was achieved for adventitious shoots of two clones of average rooting potential by providing an auxin pulse pretreatment and placing shoots onto rockwool in liquid woody plant medium (WPM). The significant effect of physical support on rooting frequency was independent of genotype and initial shoot length. The rooting of tissue culture shoots of other woody plants may be enhanced by applying this protocol and permit their practical clonal propagation for the nursery industry.

[1]Received for publication January 17, 1995; in revised form April 27, 1995.
[2]Graduate Student.
[3]Research Associate.
[4]Associate Professor.

Introduction

Liquidambar styraciflua L. (sweetgum) is a major deciduous tree species in the southern United States. Its economic value lies in its use for pulp blended with that of pine to make paper, core or chips for structural plywood and composite board, and basketry veneer. It is valued as an urban shade tree. Sweetgum is broadly site adapted and is highly resistant to pathogens and insects. In recent years, vegetative propagation methods, including tissue culture, have been developed in anticipation of the introduction of clonal forestry to operational plantings (1, 21, 25). Efficient tissue

The *Journal of Environmental Horticulture* (USPS Publication No. 698-330) is published quarterly in March, June, September, and December by the Horticultural Research Institute. Subscription rate is $65.00 per year for educators and scientists; $85.00 per year for others; add $25.00 for international orders. Second-class postage paid at Washington, D.C. and at additional mailing office. Send address changes to HRI, 1250 I Street, N.W., Suite 500, Washington, D.C. 20005.

culture methods are required for the development of gene transfer methods (2, 24).

Inefficient rooting can limit success of *in vitro* plant production. It is affected by environmental conditions such as culture medium pH, chemical constituents, growth regulator concentrations, and oxygen availability. Williams *et al.* (27) showed that the *in vitro* rooting frequency for 10 Australian woody species decreased when medium pH increased from 4.0 to 5.5. Soffer and Burger (20) found that increased oxygen concentration in the rooting medium reduced time to root formation and increased rooting percentages and root length in *Ficus benjamina*.

Rockwool has been used in plant propagation soil mixes to increase soil porosity (20). Lee *et al.* (9) showed that rooting of sweetgum shoots is improved using filter paper-supported liquid medium. This study was based on the hypothesis that rockwool would have the same, root-enhancing effect as filter paper but would be easier to use because it is rigid. Shoots rooted in rockwool could be transplanted directly to soil without releasing them from the solid support, thus facilitating *in vitro* to greenhouse transfer. Reduced root damage in a rockwool rooting system might also result in more rapid regrowth following transplanting. Thus, rockwool-supported medium was compared to agar-solidified medium for their influence on *in vitro* rooting and shoot morphology of adventitious sweetgum shoots. In addition to type of physical support, the influences of initial shoot length and clonal genotype were examined.

Materials and Methods

Adventitious shoots were produced from leaf pieces cultured on Woody Plant Medium (WPM) (10) with 7.5 μM benzyladenine and 0.5 μM naphthaleneacetic acid (NAA) solidified with 0.49% (w/v) washed Gibco agar. Growth chamber conditions were: 23C (73F), 16-hour photoperiod, and illumination from Gro-Lux, wide-spectrum fluorescent lights providing 40 μmol/m²/sec. The genotypes used were a subset of superior selections made in an ongoing industry-supported tree improvement program.

Two physical support treatments were tested: 1) Grodan rockwool (Single Block System) with 70 ml liquid WPM and 2) 50 ml WPM solidified with 0.49% (w/v) washed agar. Rockwool was autoclaved dry for 40 minutes at 121C before it was placed into four-inch Magenta boxes. Boxes were autoclaved for another 20 minutes before sterile liquid WPM medium was added. The medium used in this experiment, regardless of its subsequent use with agar or rockwool, was adjusted to pH 6.0 prior to autoclaving. The pH of each treatment was measured after autoclaving.

Two initial size classes of shoots were rooted, 1 cm and 2 cm. Shoots from 10 genotypes were used; 1-cm shoots were supplied by all 10 genotypes while 2-cm shoots were available for 9 of the 10 genotypes.

The bottom 0.4 cm of each shoot was submerged in agar-solidified medium or was placed in the pre-formed hole in the rockwool surface. Shoots were maintained separately by size class and genotype within physical support treatments. Each size class/genotype/physical support treatment combination was represented by one culture vessel containing nine shoots. Shoots were maintained for 9 weeks under the same environmental conditions used for shoot production.

Several variables were assessed for each shoot after 9 weeks of culture: 1) yes/no for rooting, 2) root fresh and dry weights, 3) shoot height, 4) average area per leaf (total leaf area ÷ number of leaves) measured using a Licor area meter, 5) leaf chlorophyll content, and 6) shoot fresh and dry weights. Rooting frequencies and shoot elongation were determined from these data. Rooting frequency was calculated as the percentage of rooted shoots in each culture vessel. Root fresh and dry weights were analyzed using rooted shoots only. Shoot elongation was calculated as the absolute increase in shoot height. Four shoots per treatment combination were used for chlorophyll analysis. A Minolta chlorophyll meter was used to obtain a mean reading (relative numbers without units) from three randomly-selected leaves from each sample shoot. Shoot fresh weights were determined prior to drying tissue at 65C (150F) for 36 hours to obtain dry weights.

A completely randomized experiment design with two replicates was used. The first replicate (306 shoots) included 10 genotypes, 7 that included 1- and 2-cm shoots and 3 that included 1-cm shoots only. The second replicate (342 shoots) included 10 genotypes, 9 that included 1- and 2-cm shoots and 1 that included 1-cm shoots only. Replicates were separated by 14 days. Analysis of variance tables and Duncan's critical ranges were generated using SAS (PROC GLM). The model used to analyze each variable included the main effects of replicate, physical support, shoot length, and genotype and all one-way interactions.

A separate experiment was done to test the effect of auxin combined with physical support on rooting of 2-cm sweetgum shoots *in vitro*. Two genotypes of average rooting performance were used from the 10 tested in the first experiment. Four treatment combinations were tested: rockwool with 70 ml of liquid WPM with or without an auxin pretreatment and 50 ml of WPM solidified with 0.49% (w/v) washed agar with or without an auxin pretreatment. Auxin pretreatment consisted of placing shoots into four-inch Magenta boxes containing agar-solidified WPM with 2 μM NAA for four days before transferring shoots to fresh auxin-free WPM supported by rockwool or agar.

A completely randomized experiment design was used with three replicates with 72 shoots each (i.e. 9 individuals per genotype in each treatment combination). Growth chamber conditions were as described above. After 30 days of culture, four responses were measured for each shoot: 1) yes/no for rooting, 2) root length, 3) number of roots, and 4) height. Rooting frequencies and shoot elongation were calculated from these data. Root length and number of roots were analyzed using rooted shoots only. Analysis of variance tables and Duncan's critical ranges were generated using SAS (PROC GLM). The model used to analyze each variable included the main effects of replicate, auxin pretreatment, physical support, and genotype and all one-way interactions.

Results and Discussion

Adventitious sweetgum shoots were used in an *in vitro* experiment to test the effect of medium physical support, initial shoot length, and genotype on rooting as measured by rooting frequency and root fresh and dry weight. Rooting frequency was influenced by all three factors under investigation (Table 1). The rooting frequency for shoots supported by rockwool in liquid WPM was greater than for those grown on agar-solidified WPM, that for shoots with an initial length of 2 cm was higher than for 1-cm shoots, and genotypes

Table 1. Mean value for six in vitro rooting and shoot characteristics of adventitious sweetgum shoots (10 clones) after nine weeks rooting according to type of physical support used during rooting, initial shoot length, and clones ranked lowest and highest for a given variable.

	Physical support			Shoot length			Clone		
	Rockwool[z]		Agar[z]	1 cm[y]		2 cm[x]	Lowest[w]		Highest[w]
Rooting frequency (%)	39	**	26	26	**	41	11	**	65
Root fresh weight (mg)	3.8	*	6.4	4.5		5.3	1.7	**	12.3
Shoot elongation (cm)	0.36	**	0.26	0.28	**	0.34	0.13	**	0.50
Area/leaf (cm^2)	0.38		0.37	0.33	**	0.42	0.22	**	0.47
Chlorophyll content[v]	39.5	**	37.3	38.5		38.4	35.0	**	43.8
Shoot fresh weight (mg)	69.4		63.6	47.8	**	90.1	55.5	*	81.5

[z]n = 324.

[y]n = 360.

[x]n = 288.

[w]number of observations is variable with clone.

[v]n = 4/9 the number of shoots used in other parameters.

* and ** denote a significant difference between means at the 0.05 and 0.01 levels, respectively.

differed greatly in rooting frequency. The highest rooting frequency across genotypes (51%) was obtained using 2-cm shoots supported by rockwool in liquid WPM (Fig. 1).

No significant one-way treatment interactions for rooting frequency were found. Thus, the influence of physical support and shoot length were relatively unaffected by each other and the influence of both variables were consistent across genotypes.

Root fresh weight was influenced by physical support and genotype (Table 1). Lower root fresh weight was obtained on rockwool-supported WPM as compared to agar-solidified medium. The range in root fresh weights by genotype was large. The influence of physical support, shoot length, and genotype on root dry weight followed the same pattern as root fresh weight. A significant physical support by genotype interaction was observed for root fresh weight indicating that genotype response to physical support was not uniform.

Shoot elongation was influenced by physical support, initial shoot length, and genotype (Table 1). Although the difference in elongation of shoots supported by rockwool in liquid WPM and those on agar-solidified medium was statistically significant, the difference was not important in a practical sense. Likewise, shoots with an initial length of 2 cm elongated significantly more than 1-cm shoots, but the difference is probably not important practically. The influence of genotype on shoot elongation was evidenced by the wide range of values obtained. All 10 genotypes elongated more on rockwool than on agar.

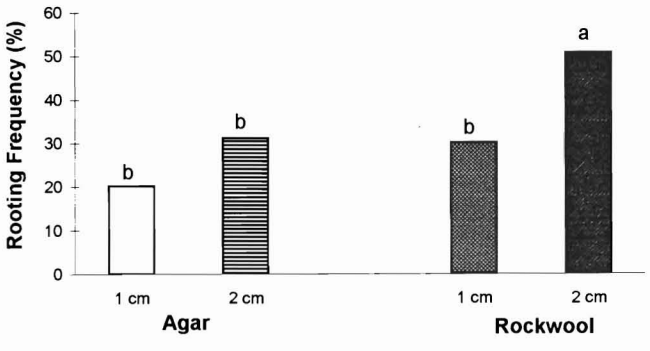

Fig 1. Effect of medium physical support (agar or rockwool) and initial shoot length (1 cm or 2 cm) on rooting frequency of adventitious sweetgum shoots in vitro. Means are composed of 18 shoots from each of nine genotypes. Bars with the same letter are not significantly different at 0.05 level.

Differences in average area per leaf were observed in the initial shoot length treatment (Table 1). Taller shoots had greater average areas per leaf in all 9 genotypes that were represented by both shoot lengths. Genotypes differed greatly in average area per leaf (Table 1).

Leaf chlorophyll content was influenced significantly by physical support and genotype (Table 1). Shoots supported by rockwool in liquid WPM had significantly higher chlorophyll content than shoots grown on agar-solidified medium. A significant physical support by genotype interaction indicated that genotype responses as measured by chlorophyll content were not uniform between physical support treatments.

Shoot fresh weight differed significantly among genotypes (Table 1). As expected, 2-cm shoots weighed significantly more than 1-cm shoots.

The second experiment showed that auxin pretreatment enhanced rooting frequency whether shoots were rooted on rockwool or agar (Fig. 2a). Number of roots per shoot did not differ among treatments. The combination of auxin pretreatment followed by rooting on rockwool-supported WPM gave the greatest shoot elongation of the four treatment combinations (Fig. 2b). The two genotypes in the auxin pretreatment experiment were chosen because they were of average rooting ability in the first experiment; in the second experiment they exhibited rooting frequencies of 74% and 56% when pretreated with auxin and supported by rockwool during rooting.

Previously published studies help to provide possible explanations for the results obtained in this work. Studies have shown that rooting efficiency can be improved by manipulating the type of matrix used for root initiation. Lee *et al.* (9) showed that rooting of adventitious sweetgum shoots in filter paper-supported liquid media was better than rooting in agar in terms of rooting percentage, rooting rate, and number of roots per plantlet. Rockwool is an inert, rock-

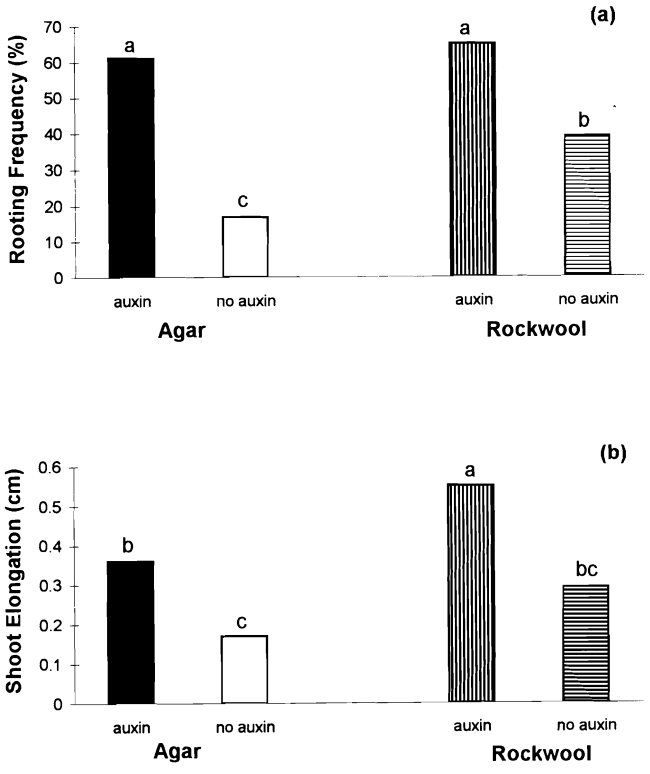

Fig 2. Effect of auxin combined with physical support (agar or rockwool) on rooting frequency (a) and shoot elongation (b) of adventitious sweetgum shoots in vitro. Means are composed of 27 shoots from each of two genotypes. Bars with the same letter are not significantly different at the 0.05 level.

based fibrous material used as a growing medium in greenhouse production that has been found useful in promoting rooting (13). We observed that rooting frequency was significantly higher for shoots supported by rockwool in liquid medium than for shoots on agar-solidified medium. The superior performance of the rockwool-supported medium for rooting and growth of adventitious shoots could be related to: 1) higher oxygen tension in rockwool than in agar, 2) a pH shift in liquid medium supported by rockwool, or 3) inhibitory substances in the agar.

Rooting media studies have shown that oxygen concentration influences root formation and growth. Lowering the dissolved oxygen concentration was shown to increase the time required to form adventitious roots and to reduce rooting frequency, number of roots formed per cutting, and average root length (20). Rockwool is characterized by a large amount of pore space that can hold both nutrient solution and air. In this work, 70 ml of liquid medium was placed in Magenta boxes such that only ⅔ of the rockwool was submerged in liquid allowing nutrient solution availability and good aeration at the top of the rockwool. The higher air content in the upper portion of the rockwool where shoot bases were inserted could provide a more favorable environment for root establishment and account for the higher rooting frequencies of shoots placed on rockwool.

Root formation could be related to oxygen diffusion rate as well as air content. Gislerod (5) found that poinsettia cuttings developed fewer roots in Grodan rockwool compared to cuttings planted in another medium with lower air content. Gislerod reported that two of the media provided higher oxygen diffusion rates than rockwool at the same air content. Since air content and oxygen diffusion rates were not measured in this study, it is uncertain whether one, the other, or both determined the oxygen supply within the rooting zone.

The observed enhanced rooting with rockwool relative to agar could have resulted from differential nutrient uptake due to differences in medium pH. Rockwool initially has a slightly basic pH (15) and has been observed to increase the pH of soil mixes (4). In this work the liquid medium with rockwool had a pH of 6.0 while the agar solidified medium had a pH of 5.3 after autoclaving. Rooting and shoot growth are known to be affected by pH differences in soil mixes and tissue culture media (7, 19, 23). Inhibition of nutrient uptake and root growth have been associated with low pH (11, 12), and it is known that availability of most plant nutrients decreases with increasing pH from 4.3 to 7.8 (14). Since pH changes can either inhibit or promote nutrient uptake, and nutrient uptake was not measured during this experiment, a conclusion can not be made concerning whether the higher pH or potentially higher oxygen tension of rockwool-supported medium relative to agar-solidified medium was responsible for the differences in rooting variables and shoot morphology.

Alternatively, the lower rooting frequency on agar-solidified WPM could have been due to inhibitory compounds in the agar. Agar is a commonly used gelling agent in tissue culture. According to Pochet *et al.* (16) it is not a pure polysaccharide; it is a complex mixture of polysaccharides ranging from those that are heavily sulfated or pyruvated to those carrying little or no charge. The type of agar used to solidify culture media can markedly affect plant tissue cultures (6, 16). It has also been shown with *in vitro* shoots of woody plant species that decreasing agar concentration can increase shoot dry weight (18) and shoot growth (26). Less highly purified agars may have detrimental effects (3, 18). The explanation for these effects is still lacking, but one explanation proposed by Kohlenback and Wernicke (8) is that some inhibitors are present in agar. The agar we used for these experiments was a commercial plant tissue culture agar that was rinsed repeatedly with distilled water before use to reduce soluble impurities.

Rockwool has traditionally been described as a totally inert medium with no available nutrients. Calcium is added during rockwool production as a fluxing agent to aid in melting and producing fiber from rock or slag materials. Since calcium increases the water solubility of rockwool, iron oxides are also added to provide strength and decrease solubility (17). These iron oxides may subsequently serve as a source of iron to plants growing in rockwool. Sonneveld and Voogt (22) have shown that plants grown in rockwool required less iron than those grown in nutrient film. According to Rupp and Dudley (17), when chlorotic rose cuttings were placed in loose rockwool, the chlorotic conditions disappeared in actively growing leaves with or without the addition of iron to the nutrient solution. This observation suggests that increased iron availability may be related to the observation presented here that higher chlorophyll levels occur in sweetgum shoots grown in rockwool as compared to those grown on agar-solidified media.

The significant interactions found between genotype and physical support or shoot length indicated that the positive

effects of rockwool and longer initial shoots differ in magnitude among clones and that these variables may be more critical for some clones than for others. Thus, it may be possible to devise superior rooting protocols that are specifically designed for given genotypes.

These two experiments suggest that rooting of sweetgum adventitious shoots could be enhanced by using 2-cm-long shoots pretreated on agar-solidified WPM with 2 μM NAA for 4 days prior to transfer to auxin-free liquid WPM supported by rockwool for 30 days. Rooting success will vary by genotype, but this work shows that a rooting rate of at least 65% can be achieved with genotypes of average rooting.

Literature Cited

1. Brand, M.H. and R.D. Lineberger. 1988. *In vitro* adventitious shoot formation on mature- phase leaves and petioles of *Liquidambar styraciflua L.* Plant Sci. 57:173–179.

2. Chen, Z.Z. 1991. Nodular culture and *Agrobacterium*-mediated transformation for transgenic plant production in *Liquidambar styraciflua L.*(sweetgum), PhD Dissertation. North Carolina State University, Raleigh.

3. Debergh, P.C. 1983. Effect of agar brand and concentration on the tissue culture medium. Physiol. Plant. 59:270–276.

4. Fonteno, W.C. and P.V. Nelson. 1990. Physical properties of and plant responses to rockwool-amended media. J. Amer. Soc. Hort. Sci. 115:375–381.

5. Gislerod, H.R. 1983. Physical conditions of propagation media and their influence on the rooting of cuttings. Plant and Soil 75:1–14.

6. Goldfarb, B., G.T. Howe, L.M. Bailey, S.H. Strauss and J.B. Zaerr. 1991. A liquid cytokinin pulse induces adventitious shoot formation from douglas-fir cotyledons. Plant Cell Rep. 10:156–160.

7. Harbaugh, B.K. and S.S. Woltz. 1991. *Eustoma* quality is adversely affected by low pH of root medium. HortScience 26:1279–1280.

8. Kohlenback, H.W. and W. Wernicke. 1978. Investigations on the inhibitory effect of agar and the function of active carbon in anther culture. Z. Pflanzenphysiol. 86:463–472.

9. Lee, N., H.Y. Wetzstein, and H.E. Sommer. 1986. The effect of agar vs. liquid medium on rooting in tissue-cultured sweetgum. HortScience 21:317–318.

10. Lloyd, G. and B. McCown. 1980. Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot tip culture. Combined Proc. Inter. Plant Prop. Soc. 30:421–427.

11. Mahler, R.L. and R.E. McDole. 1987. Effect of soil pH on crop yield in northern Idaho. Agron. J. 79:751–755.

12. Moore, D.P. 1974. Physiological effects of pH on roots. p 135–151. *In*: E.W. Carson (Editor). The Plant Root and Its Environment. Univ. Press of Virginia, Charlotteville.

13. Olympios, C.M. 1992. Soilless media under protected cultivation rockwool, peat, perlite and other substrates. Acta Hort. 323:215–230.

14. Peterson, J.C. 1982. Effects of pH upon nutrient availability in a commercial soilless root medium utilized for floral crop production. Ohio Agric. Res. Dev. Center Res. Circular. 268:16–19.

15. Pierik, R.L.M. 1991. Micropropagation of ornamental plants. Acta Hort. 289:45–51.

16. Pochet, B., V. Scoman, M.M. Mestdagh, B. Moreau, and P. Andre. 1991. Influence of agar gel properties on the *in vitro* micropropagation of different clones of *Thuja plicata.* Plant Cell Rep. 10:406–409.

17. Rupp, L.A. and L.M. Dudley. 1989. Iron availability in rockwool may affect rose nutrition. HortScience 24:258–260.

18. Romberger, J.A. and C.A. Tabor. 1971. The *Picea abies* shoot apical meristem in culture. I. Agar and autoclaving effects. Amer. J. Bot. 58:131–140.

19. Schubert, S., E. Schubert, and K. Mengel. 1990. Effect of low pH of the root medium on proton release, growth, and nutrient uptake of field beans (*Vicia faba*). Plant and Soil 124:239–244.

20. Soffer, H. and D.W. Burger. 1988. Effects of dissolved oxygen concentrations in aero-hydroponics on the formation and growth of adventitious roots. J. Amer. Soc. Hort. Sci. 113:218–221.

21. Sommer, H.E. and C.L. Brown. 1980. Embryogenesis in tissue cultures of sweetgum. For. Sci. 26:257–260.

22. Sonneveld, C. and W.Voogt. 1985. Studies on the application of iron to some glasshouse vegetables grown in soilless culture. Plant and Soil 85:55–64.

23. Stoffella, P.J., M.L. Dipaola, A. Parodossi, and F. Tognoni. 1991. Rhizosphere pH influences early root morphology and development of bell peppers. HortScience 26:112–114.

24. Sullivan, J. and L.M. Lagrimini. 1993. Transformation of *Liquidambar styraciflua* using *Agrobacterium tumefacients.* Plant Cell Rep. 12:303–306.

25. Sutter, E.G. and P.B. Barker. 1985. *In vitro* propagation of mature *Liquidambar styraciflua.* Plant Cell Tiss. Org. Cult. 5:13–21.

26. Von Arnold, S. and T. Erickson. 1984. Effect of agar concentration on growth and anatomy of adventitious shoots of *Picea abies* (L.) Karst. Plant Cell Tiss. Org. Cult. 3:257–264.

27. Williams, R.R., A.M. Taji, and J.A. Bolton. 1985. Specificity and interaction among auxins, light, and pH in rooting of Australian woody species *in vitro*. HortiScience 20:1052–1053.