IBA. The bases are quick-dipped in 1.2% K-IBA. Callus appears in 4 to 5 weeks with rooting taking place in 7 to 9 weeks.

Cuttings from *Quercus shumardii* are taken in late July from the current year's growth and are 3 to 4 in. long. The wood is not as brittle nor as hard as the wood on the other oak species. We quick-dip the cuttings in 1% K-IBA. Callus appears in 4 to 5 weeks with rooting taking place in 7 to 9 weeks.

CONCLUSION

At Simpson Nurseries we have found softwood cutting propagation to be the preferable method for propagation of certain species of oak, magnolia, crabapple and dogwood. Because of the tremendous success we have experienced, we intend to expand our experimentation and utilization of the process. In our opinion, the potential and merit of softwood cutting propagation have been well documented and demonstrated.

TISSUE CULTURE OF OAKS AND REDBUDS

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Abstract. A micropropagation method for *Quercus shumardii* is described. Stem sections were utilized as explants and shoot multiplication was promoted with WPM amended with BA. BA and 2iP were tested in a range of 0.0 to 5.0 mg/liter with 2.0 mg/liter BA supporting optimal shoot growth. After 6 weeks shoots could be divided and subcultured on a combination of BA, IBA, and GA_3. Shoots were simultaneously rooted and acclimatized after a 15-minute dip in 500 ppm IBA. The methods presented required only minor refinements for the micropropagation of three other *Quercus* species and of *Cercis canadensis*.

REVIEW OF LITERATURE

The list of tissue-cultured woody perennials available to nurseries is increasing. Among these availabilities are: *Amelanchier*, apple, azalea, birch, blueberry, blackberry, dogwood, kiwifruit, *Magnolia*, *Nandina*, poplar, raspberry, *Raphiolepis*, *Rhododendron*, rose, and *Syringa*.

One problem with this list is that for many of the cultivars plants are only available one to two months of the year, and orders must be booked a year ahead. A second problem is that there are no oaks on the list. Oaks are a very highly valued tree, both as timber and as landscape plants.

Oaks are usually sexually propagated since clonal propagation has been limited (2,3,8). Seed propagation of oaks is plagued

with problems. Weevils often attack acorns and the seed must be heat-treated to destroy them. Seeds lose viability if allowed to dry out, and seed crops vary widely from year to year (2). The biggest problem with seed propagation of oaks is that they are so highly heterozygous that uniformity is next to impossible.

For this reason Dr. Fred Davies, Professor at Texas A&M University, and I chose oaks as a genus that could benefit from research and development of micropropagation techniques. If an efficient protocol for clonal reproduction could be developed, the problems associated with seed propagation could be avoided.

We were not the first to attempt oak tissue culture. Oak micropropagation has been reported by Seckinger, *et al.* (7) in 1979, Lineberger (4) in 1980, Pardos (6) in 1981, and Vietez, *et al.* (9) in 1985. None of these researchers established plants out of culture conditions.

Vietez (9) achieved the most success of these researchers. Stem sections and embryonic axes of *Quercus robur* were established on Gresshoff-Doy medium with 0.1 to 1.0 mg/liter BA. Less than 75% of the cultures formed shoots, and senescence was a problem in subculturing. Although 20 to 83% rooting was obtained, no mention of acclimatization success was made.

MATERIALS AND METHODS

Sterilization. Single-node stem sections of *Quercus shumardii* served as explants. Sterilization consisted of a four-step procedure of 1% Liquinox for 20 min., 70% ethanol for 2 min., 10% Clorox for 15 to 20 min., and 70% ethanol for 1 to 2 min. This was followed by three rinses in sterile distilled water.

Multiplication. Explants were placed in test tubes with 3 ml of WPM (5), 2% sucrose, and either benzylamino purine (BA) or isopentyl-adenine (2iP) at 0.0, 0.5, 1.0, 2.0, 3.0, or 5.0 mg/liter. Previous studies found WPM superior to MS medium and liquid superior to agar-based medium (1). The pH was adjusted to 5.3 prior to autoclaving and cultures were maintained under Sylvania Gro-Lux fluorescent lights with a 16-hour photoperiod at 26°C.

After 6 weeks, shoots were divided into single-node pieces and subcultured on agar-based WPM amended with BA (2.0 mg/liter), indole-3-butyric acid (IBA) (0.5 mg/liter), and gibberellic acid (GA_3) (1.0 mg/liter).

Rooting. Shoots 10 mm or greater in length were used for rooting. A variety of rooting experiments, both *in vitro* and *in vivo*, were attempted to maximize rooting. Some of the experiments included:

A) 1 to 14 days on 1 to 5 mg/liter IBA followed by transfer to WPM without growth regulators on either agar or filter paper bridges;

B) exposure to IBA, naphthaleneacetic acid (NAA), or combinations of both (0.1 to 1.0 mg/liter) for 6 weeks;

C) quick dips in 500 ppm IBA followed by transfer to agar, sterile vermiculite with WPM, or non-sterile Jiffy-7 pellets; and

D) quick dips in 500 ppm IBA for 0 to 30 min. followed by insertion into Jiffy-7 pellets. Humidity was maintained by clear plastic covers.

After rooting, plants were acclimatized by gradually removing the clear plastic and decreasing the relative humidity. Plants were then potted into a peat:perlite mix with 1.2 kg/m^3 Osmocote (18-6-12) and placed in the greenhouse under mist. After 3 weeks plants were removed from the mist and placed on the greenhouse bench with no special care other than routine watering and pest control.

RESULTS

Sterilization. Depending on the time of year, contamination ranged from 0 to 50%. The lowest contamination occurred in late winter and early spring when the relative humidity was lowest. Single-node stem sections were a better explant source than apical shoot tips because the apical tips produced a whorl of buds that tended to trap fungi.

Multiplication. BA proved to be a more effective cytokinin than 2iP. Increasing BA concentration increased the number of shoots produced, and as many as 10 shoots were produced at 5 ppm BA (Table 1). These shoots, however, showed signs of cytokinin toxicity such as swelling, stunting, and leaf abnormalities. BA at 2.0 ppm proved to be the optimal concentration for shoot multiplication. This concentration allowed for 7 shoots to be produced, of which 2 were large enough for rooting while the others could be subcultured. The rapid transfer technique originally used is not necessary with oaks. Plants need only be transferred at day 7 and again in

Table 1. Effect of growth regulator on shoot growth of shumard oak.[z]

Growth regulator	Concentration (mg/liter)	No. shoots (10 mm)	Total shoot number	Total leaf number
BA	0.1	1.10	1.58	2.19
	0.5	1.06	1.82	2.86
	1.0	1.17	3.65	5.12
	2.0	1.70	7.20	6.48
	3.0	1.32	9.90	6.72
	5.0	0.85	10.48	7.68
2iP	0.0	1.05	1.75	2.40
	0.5	0.92	1.20	1.98
	1.0	0.92	1.07	2.00
	2.0	0.92	1.18	2.15
	3.0	0.92	1.15	2.25
	5.0	0.70	1.10	2.38

[z]Means represent 80 and 40 explants for each BA and 2iP concentration respectively.

2 to 3 weeks. Small shoots and single-node sections could be subcultured when supplied with BA, IBA, and GA_3. Shoots could be subcultured only for 3 passages. After the third subculture a decline in shoot growth occurred. If the shoots were transferred to the basal medium without growth regulators and placed in a low light situation, approximately half would resume growth and could be subcultured again.

Rooting. Shoots could be rooted *in vitro*, but rooting was not consistent (Figure 1). The treatment that produced optimal rooting one time often produced little rooting when repeated.

Because of inconsistent rooting *in vitro* and the high cost of a single rooting stage, shoots are best treated as mini-cuttings (Figure 2). A 15-minute dip in 500 ppm IBA was optimal and resulted in 73% rooting (Table 2). After the relative humidity was gradually lowered, 86% of the rooted plantlets survived the transition to the greenhouse. Although not quite as vigorous as seedlings in the early stages, there was little difference at 5 months, and most are still actively growing after 2 years.

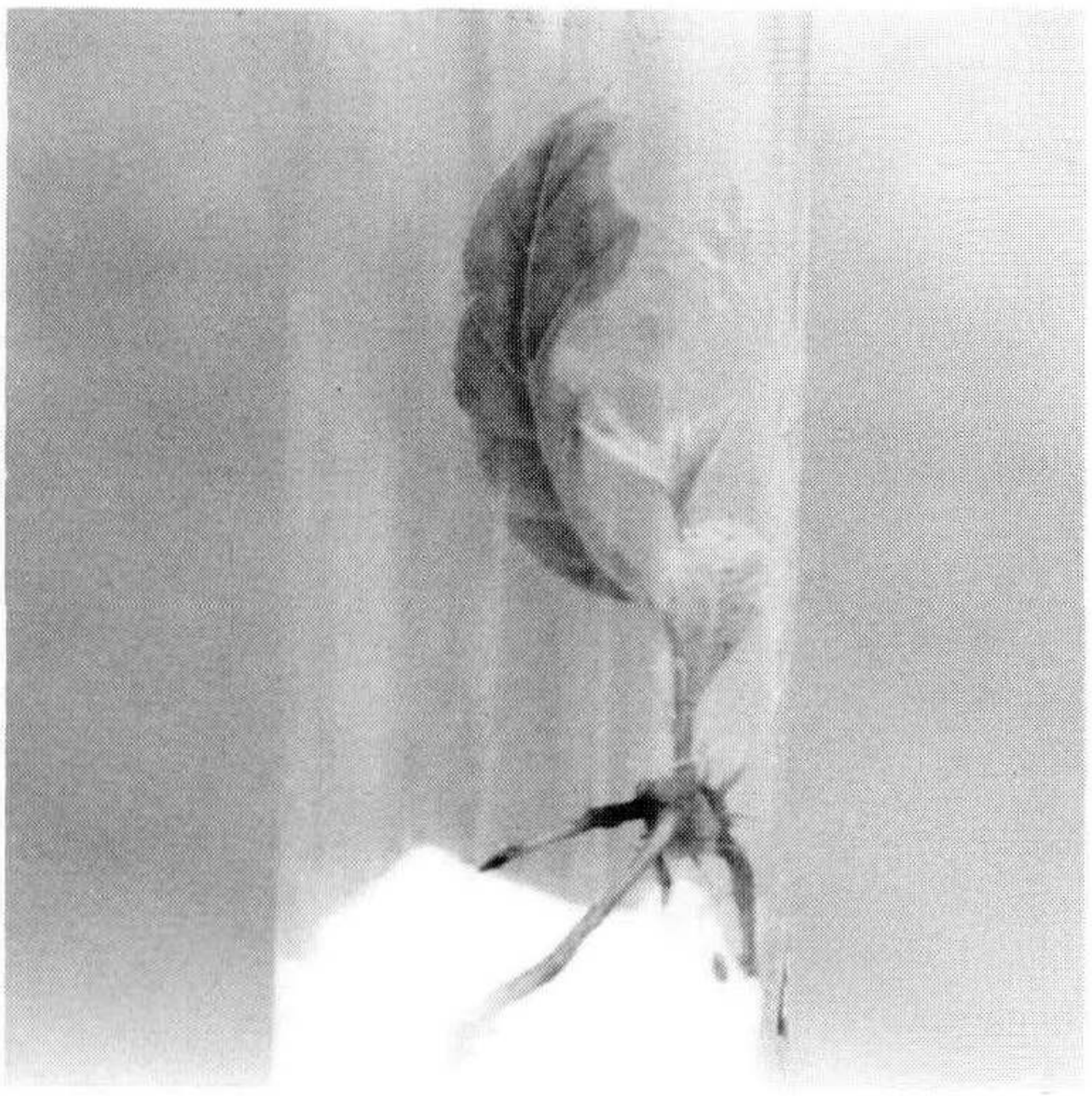

Figure 1. Shumard oak rooted *in vitro*.

Table 2. Effect of time in 500 ppm IBA on percent rooting of shumard oak.[z]

Time (min.)	Percent rooting
0	0.0
5	7.0
10	27.0
15	73.0
30	43.0

[z]Means represent 30 shoots/treatment.

Although the methods presented here are far from perfected, they work not only on shumard oak but also on water oak (*Quercus nigra*), pin oak (*Quercus palustris*), and live oak (*Quercus virginiana*). Work is being continued and procedures will soon be completed for mature oaks.

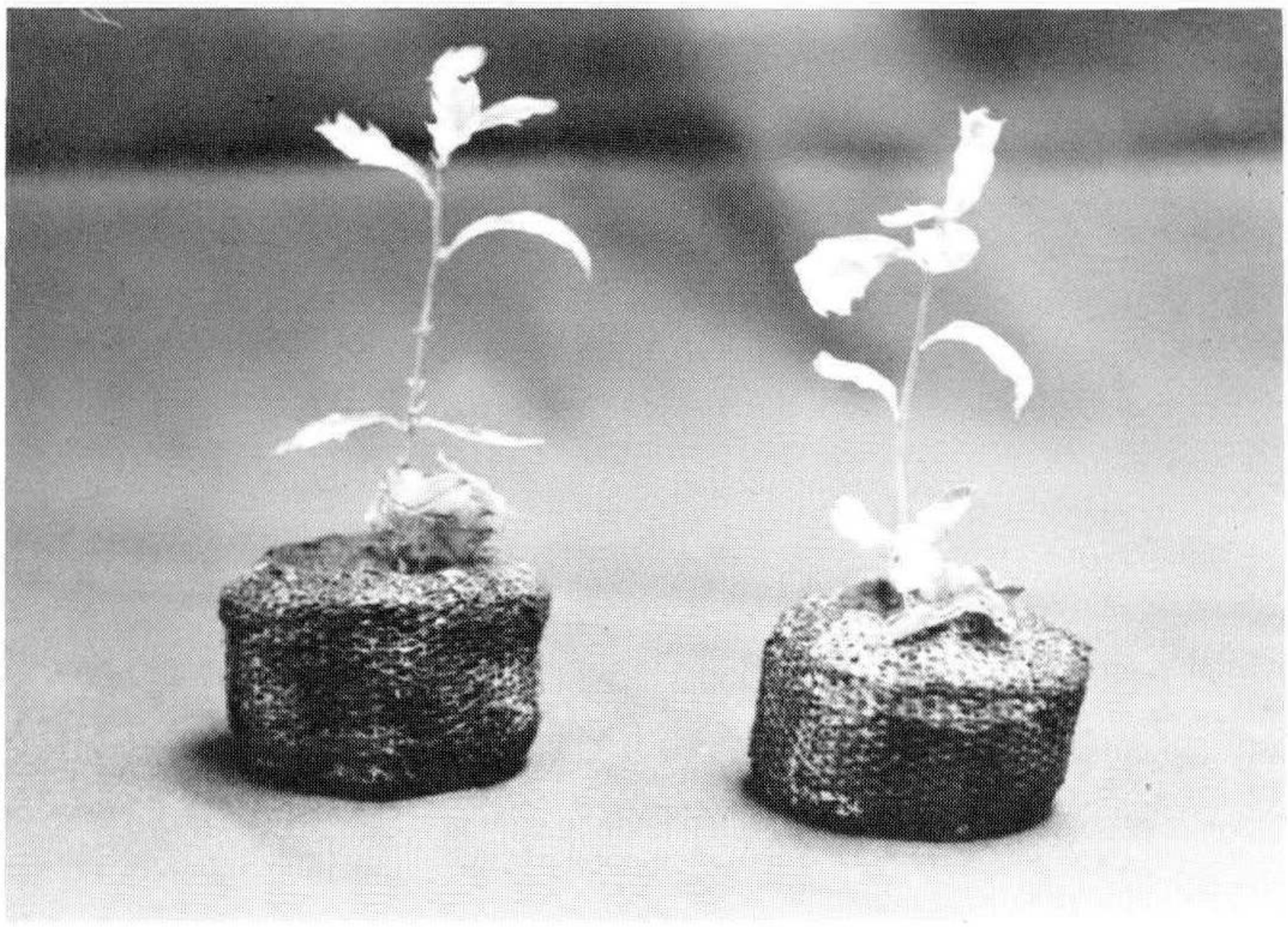

Figure 2. Shumard oak rooted in Jiffy-7 pellet after 15 min. dip in 500 ppm IBA.

REDBUD TISSUE CULTURE

Redbuds are another woody species that will soon be appearing on tissue culture price lists. A selection of *Cercis canadensis* from Mexico with thick wavy leaves and a weeping habit has been successfully cultured on WPM with 5 mg/liter BA (Figure 3). Shoot growth is extremely rapid and sectioning is often required monthly to prevent crowding. These plants have been in culture for over 2 years with no apparent ill effects.

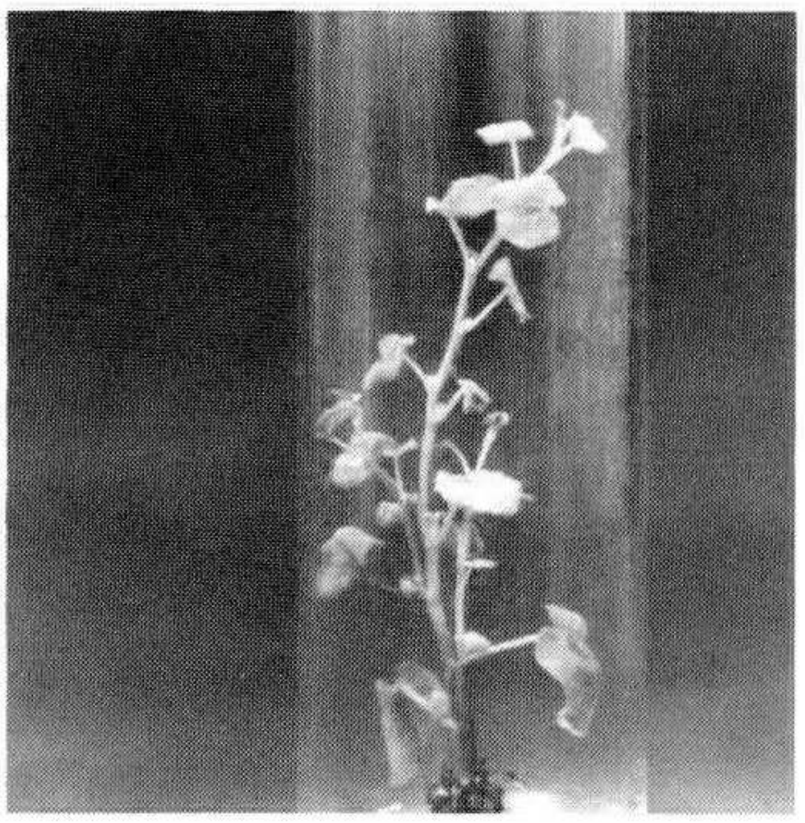

Figure 3. Shoot growth of *Cercis canadensis* var. *mexicana*.

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