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Micro- and Cutting Propagation of Silver Maple. I. Results with Adult and Juvenile Propagules

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Abstract. **Clonal micropropagation studies with silver maple *(Acer saccharinum* L.) included experiments with various shoot. explant types, cytokinins, and stock plant maturation levels. These trials led to successful explant establishment, axillary shoot proliferation, rooting of microshoots, and establishment of plantlets in the greenhouse. Overall, the best cytokinin tested was the phenylurea derivative TDZ. Shoot proliferation on juvenile explants was poor with kinetin, 2iP, and BA. Only zeatin at 10 µM was comparable to TDZ. TDZ at 10 nM was optimal for both juvenile and adult nodal explants. Juvenile explants that were held in vitro for 4 months commonly had at least 60 axillary shoots that could be subculture or excised for rooting. Microshoots rooted within 2 weeks. Following rooting, silver maple plantlets could be transplanted into a growing medium and placed directly onto a greenhouse bench. Studies were also conducted on rooting stem cuttings (macropropagation). Single nodes from juvenile plants rooted under intermittent mist, regardless of auxin application; however, shoot-tip cuttings from adult trees rooted best when auxin in ethanol solution was applied. Chemical names used: *N*- phenyl- *N′* -1,2,3 -thiadiazol-5-ylurea (thidiazuron, TDZ), *N*-(2-furanylmethyl)-1H-purin-6-amine (kinetin), isopentenyladenine (2iP), benzyladenine (BA), (E)-2-methyl-4-(1H-purin-6-ylamino)-2-buten-1-ol (zeatin).**

Silver maples are valued in the landscape because of their attractive foliage with its typical "maple" shape and silvery underside, the graceful inverted vase shape of the mature trees, rapid growth that provides summer shade in a reasonably short time, and adaptability to a wide variety of soil types. The rapid juvenile growth rate is an attribute where fast shade is required (Dirr, 1977) and is also desirable in species grown under short-rotation culture conditions for the production of energy (Ranney et al., 1986).

-Appropriate selection and documentation of performance for woody biomass species that grow well on secondary farmland are important to the energy future of the United States. As fossil fuels become depleted, renewable resources can be used to convert the sun's energy into biomass. Silver maple has been selected as a model species for research under the Short Rotation Woody Crops (SRWC) program, sponsored by the U.S. Dept. of Energy's Biofuels and Municipal Waste Technology Division, because of its rapid juvenile growth and other attributes as a potential woody biomass species (Ranney et al., 1986).

Research that uses clonal planting stock offers advantages for the study of tree genotypes, as compared to using seedlings from open-pollinated seed orchards. Individual tree seedlings can present problems because the components of genotype and environment are very difficult to separate when one is attempting to interpret the performance of a particular phenotype. Members of clones are genetically identical and, as such, can allow for the testing of each selected tree genotype in different environments in a replicated study. By using clones to reduce genetic variability (compared to seedlings from open-pollinated seed orchards), experimental error can be minimized. This allows greater precision in studies of tree phenotype, physiology, nutrition, silviculture, and general performance (Libby, 1974). Juvenile plants are generally easier to propagate clonally than adult forms (Hartmann and Kester, 1983; Libby, 1983).

Juvenile silver maple (Ashby et al., 1987) and a red-silver maple hybrid (Kerns and Meyer, 1986, 1987) have been micropropagated successfully using thidiazuron (TDZ) in the medium. Rooted plantlets were obtained in both reports.

Silver maple stem cuttings root reasonably well. Larsson (1968) found that stem cuttings, collected in early July from coppice of 5-year-old trees, rooted well (63%) after 6 weeks when they were soaked for 60 min in 500 ppm indolebutyric acid (IBA) and placed in a sand and vermiculite medium. Whalley and Loach (1982) took stem cuttings from hedged, 6-year-old silver maple stock plants in February and dipped them for 15 sec in 1500 ppm IBA. They reported > 50% rooting, but noted a poor survival percentage of good-quality plants, unless they were misted under a polyethylene tunnel. Kling and Meyer (1983) found that the phenolic compound catechol stimulated rooting in softwood stem cuttings from silver maple seedlings. Although stem cutting propagation has been successful, more cuttings per stem would result if single nodes were used as propagules.

The objectives of this study were to determine the conditions for clonal micro- and cutting propagation of juvenile and adult silver maple. These techniques could then be applied to the production of clonal trees for field plantings of various silver maple genotypes (Preece et al., 1991).

Materials and Methods

Juvenile trees were propagated from seeds collected at Carbondale, Ill.; Peoria, Ill.; and Minneapolis. The genotypes were

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mixed in the experiments reported herein because no observable growth differences were noted among explants from the Illinois and Minnesota seedlings (Ashby et al., 1987). The adult trees used for rooting studies were >15 m tall and native to southern Illinois. These rooted cuttings were transplanted and their new growth was used as adult explant material for in vitro-studies.

Except for the full-sized adult trees, stock plants were grown in a greenhouse at 25/20 ± 5C (day/night). During September through March, nights were interrupted from 2200 to 0200 HR with cool-white fluorescent light. The plants were in plastic pots in a 2 sphagnum peat : 1 vermiculite : 1 perlite medium (by volume} (Premix BX; Premier Brands, New Rochelle, N. Y.) and received 400 ppm N each week from a water-soluble 20N–4P–8K fertilizer.

Explants for micropropagation studies were 2.5-cm softwood shoot tips or 2.5-cm nodal segments that were surface-sterilized for 20 min in 0.5% NaClO with 0.1% (by volume) Tween 20, followed by three 5-min rinses in sterile distilled water. Explants were initiated in 25 × 150-mm borosilicate glass culture tubes capped with clear autoclavable lids and containing 15 ml Difco-bacto agar (7 g·liter^{-1})-solidified medium. After 2 months in vitro, cultures were transferred to 120-ml glass (baby food) jars or to Magenta (Chicago) GA7 plastic autoclavable vessels. All juvenile explants were transferred to fresh medium every 2 weeks and all adult explants were transferred monthly.

The nutrient medium was DKW (Driver and Kuniyuki, 1984) with 30 g sucrose and 7 g Difco-bacto agar/liter. The pH was adjusted to 5.8 ± 0.1 with 1 N KOH or HC1 before adding agar and autoclaving. Stock solutions of all cytokinins, including TDZ, were solubilized in a small amount of 1 N KOH and brought to final volume with water. Auxins were in 50% ethanol : water solutions (v/v).

Cultures were incubated at 22 ± 2C with a 16-h photoperiod and a photosynthetic photon flux of 33–45 μmol·s^{-1}·m^{-2} provided by cool-white fluorescent lamps. All in vitro experiments were arranged in completely randomized designs with at least 10 replications.

Rooting studies with microshoots and stem cuttings from trees were under intermittent mist in a greenhouse. In separate experiments, juvenile microshoots were rooted in 1 sphagnum peat :1 vermiculite :1 perlite (by volume) medium and stem cuttings and adult microshoots were rooted in a 1 vermiculite : 1 perlite (v/v) medium.

Rooted microshoots and stem cuttings were gradually acclimatized to ambient greenhouse conditions by first transplanting into the same peat-lite medium that was used for the stock plants. Plants were then moved to areas of the mist bench receiving less coverage by the mist for ≈2 weeks. They were then moved to benches without intermittent mist,

Data were subjected to analyses of variance for the factorial combinations of simple and main effects. The General Linear Model was used because of the unequal number of replications of uncontaminated cultures within each treatment combination. The $(y + ½)^{½}$ was used when there were many zero values (Steele and Torrie, 1980).

Results and Discussion

Micropropagation. We compared the responses of seedling shoot tip explants to four amino purine cytokinins (Table 1). After 1 and 2 months in vitro, zeatin was the most active. Explants exposed to 10 μM zeatin produced the most shoots, shoots >5 mm long (the minimum length that can be excised for rooting), and the longest shoots. There was little response to BA or kinetin. Compared to BA and kinetin, 1 μM 2iP stimulated shoot elongation; however, shoot proliferation remained low.

Table 1. Effects of cytokinins and their concentration on in vitro performance of 2.0-cm silver maple seedling shoot tips after 1 and 2 months.[z,y]

Cytokinin	Concn (μM)	No. shoots	No. shoots >5 mm	Shoot length (mm)	Callus volume (cm^3)
		After 1 month			
BA	1	1.0	0.0	2.5	0.28
	10	0.3	0.0	0.2	0.12
Kinetin	1	1.3	0.0	1.4	0.23
	10	1.0	0.1	1.2	0.28
Zeatin	1	1.7	0.3	3.8	0.47
	10	3.5	0.3	5.5	0.89
2iP	1	1.5	0.1	3.2	0.60
	10	0.4	0.0	0.4	0.10
Significance		**	NS	**	**
LSD 5%		0.61		2.26	0.32
LSD 1%		0.80		2.98	0.42
		After 2 months			
BA	1	0.9	0.0	2.7	1.24
	10	0.1	0.0	0.1	0.21
Kinetin	1	1.7	0.3	5.0	0.66
	10	1.3	0.1	3.5	1.32
Zeatin	1	2.6	0.5	7.4	0.67
	10	4.4	1.4	8.0	3.13
2iP	1	1.3	0.1	10.6	1.58
	10	0.3	0.0	0.2	0.70
Significance		**	**	**	**
LSD 5%		0.98	0.43	4.61	1.13
LSD 1%		1.29	0.56	6.10	1.49

[z]Each datum represents the mean of 20 to 23 (month 1) or 15 to 16 (month 2) cultures. There was no response when cytokinin was excluded from the medium.

[y]Data on shoot number and length were transformed for analysis using $(y + ½)^{½}$; nontransformed data are presented.

**, NS Significant interaction at $P = 0.01$ or nonsignificant, respectively, according to F test with 3 and 166 df for month 1 and 116 df for month 2.

We were aware of the success that Kerns and Meyer (1986, 1987) had when they used TDZ combined with BA on a red-silver maple hybrid. We had also reported (Ashby et al., 1987) that this substituted phenylurea compound effectively stimulated axillary shoot proliferation of silver maple. We therefore conducted a study in which we compared the four amino purine cytokinins with TDZ in a lower range of concentrations, from 1 to 500 nM (0.001–0.5 μM) (Table 2). After 2 months in vitro, the main effects of cytokinin and concentration were significant with those shoots on media containing TDZ and having the greatest number of shoots >5 mm long. The best concentration across all cytokinins for number of shoots >5 mm long was 100 nM (data not presented). The greatest mean shoot length and length of shoots longer than 5 mm was on media containing 10 to 100 nM TDZ (Table 2). The longer shoots are more desirable than short shoots because they are easier to harvest for rooting or subculture. Callus growth was greatest on media containing the highest concentrations of TDZ and zeatin. We have found that when 10 nM TDZ was in the medium, there was a significant but low correlation between callus and shoot growth of several silver maple genotypes (Preece et al., 1991).

We were interested in the long-term effects of continuous exposure to TDZ compared to shorter exposures (pulses) fol-

Table 2. Effect of cytokinin and concentration on in vitro growth of single-node explants of seedling silver maple after 2 months.[z,y]

Cytokinin	Concn (nM)	No. shoots	Mean length of shoots >5 mm: Length	n	Shoot length (mm)	Callus volume (mm^3)
BA	1	1.8	12.2	5	6.3	97
	5	1.5	10.8	6	5.3	44
	10	1.9	5.5	4	3.7	20
	50	1.9	10.0	3	4.6	214
	100	1.6	6.7	6	5.1	104
	500	1.7	12.8	5	6.5	1075
Kinetin	1	1.5	10.5	5	6.4	46
	5	1.6	12.9	4	6.6	42
	10	1.9	10.5	8	8.9	35
	50	1.8	5.8	6	4.3	14
	100	1.5	10.5	5	6.7	24
	500	1.7	16.5	6	10.3	296
Thidiazuron	1	1.7	13.4	9	9.9	419
	5	1.8	8.4	7	5.6	596
	10	1.8	24.5	10	19.7	2114
	50	1.8	40.2	8	24.8	5075
	100	2.5	46.4	10	38.4	7278
	500	2.3	23.7	6	11.3	5618
Zeatin	1	1.8	8.6	5	4.1	253
	5	1.8	14.0	5	5.5	379
	10	2.1	10.3	7	6.3	153
	50	1.9	13.8	9	9.6	1445
	100	1.8	11.6	8	8.8	1797
	500	1.6	9.1	7	5.8	2597
2iP	1	1.7	14.6	5	7.7	130
	5	1.7	9.7	6	5.2	108
	10	1.7	12.6	4	6.0	52
	50	2.0	11.6	9	8.3	673
	100	1.8	9.5	7	6.7	235
	500	1.7	10.2	7	7.4	314
Significance		NS	**		**	**
LSD 5%			14.03		7.20	925
LSD 1%			18.43		9.46	1217

[z]Except where otherwise noted, each datum represents the mean of 10 cultures.
[y]Data on mean length of shoots. > 5 mm were transformed for analysis using $(y + ½)^{½}$; nontransformed data are presented.
**, [NS]Significant two-way interaction at $P = 0.01$ or nonsignificant, respectively, according to F test.

Table 3.Effects of thidiazuron exposure periods on in vitro performance of seedling silver maple single-node explants after 2 months.[z,y]

Exposure to thidiazuron (weeks)	No. shoots	No. shoots >5 mm[x]	Shoot length (mm)	Callus volume (cm^3)
0	3.3	0.7	2.7	2.1
2	3.5	2.1	5.3	4.2
4	4.8	2.1	5.5	4.4
6	5.5	3.1	7.4	3.8
8	6.2	3.8	8.8	4.7
Alternate weeks thidiazuron/BA	4.7	2.5	7.4	4.6
Significance	NS	**	**	NS

[z]Each datum represents the mean of 15 cultures.
[y]Explants were placed onto DKW medium with 10 nM thidiazuron for the designated length of time and were then placed on medium with 10 μM BA. Alternate weekly treatments were transferred between these two cytokinin media.
[x]Data on number of shoots >5 mm were transformed for analysis using $(y+ ½)^{½}$; nontransformed data are presented.
** ,[NS]Significant at $P = 0.01$ or nonsignificant, respectively, according to F test with 5 and 84 df.

Table 4. Effects of thidiazuron exposure periods on in vitro performance of seedling silver maple single-node explants after 3 and 4 months.

Exposure to thidiazuron[z] (weeks)	No. cultures[y]	No. shoots	No. axillary shoots >5 mm	Shoot length[x] (mm)	Callus volume (cm^3)
After 3 months					
0	10	1.0		0.8	0.3
2	10	4.8		10.8	1.7
4	10	7.4		20.9	2.5
6	10	5.0		17.6	1.5
8	10	10.7		39.5	2.7
12	10	17.5		43.6	1.9
Alternate weeks	9	6.7		28.5	1.5
Alternate months	10	7.6		23.6	1.4
Significance		**		**	NS
After 4 months					
0	5	0.0	0.0	0.0	0.8
2	5	6.4	4.4	9.6	6.1
4	5	12.6	9.2	10.2	6.3
6	5	6.6	4.0	12.1	3.9
8	5	19.0	12.2	13.2	7.4
16	5	63.4	47.8	14.4	3.8
Alternate weeks	5	5.4	4.4	6.2	1.9
Alternate months	5	18.8	15.0	9.7	5.1
Significance		**	**	NS	NS

[z]Explants were placed on DKW medium with 10 nM thidiazuron for the designated length of time and then placed on medium with 10 μM BA. Alternate weekly and monthly treatments were transferred between these two cytokinin media at the stated intervals.
[y]Because of contamination, five replications were measured after 4 months.
[x]For month 3, shoot length is the mean of the three longest shoots. At month 4, cultures were destructively evaluated and all shoots lengths were recorded.
**, [NS]Significant at P = 0.01 Or nonsignificant, respectively, according to F test with 7 and 71 df (3 months) and 7 and 32 df (4 months).

lowed by transferring explants to medium containing the more commonly used BA. We believed that the potent TDZ might become toxic over time. In this study, single-node explants were exposed to TDZ (10 nM), followed by, or compared to, BA (10 μM) (Tables 3 and 4). After 2 months, explants produced significantly more and longer axillary shoots (> 5 mm long) without a significant increase in callus growth when they had been continuously exposed to TDZ, compared to those on medium with BA (Table 3, Fig. 1). Even after 3 and 4 months in vitro, there were no significant differences in callus growth among treatments (Table 4). After 3 months, explants that had been exposed to TDZ continuously had more and longer axillary shoots than those with shorter exposure times or those shifted between medium with TDZ and BA on alternate weeks or months.

After 4 months in vitro, a nearly 4-fold increase occurred in the number of axillary shoots in our cultures, compared to the previous month. After 2 months, the explants were placed in GA7 vessels. We have observed that if explants remain in the culture tubes, this increase does not occur. There was an increase from 17 shoots after 3 months to 63 shoots after 4 months (Table 4). This happened consistently with our silver maple

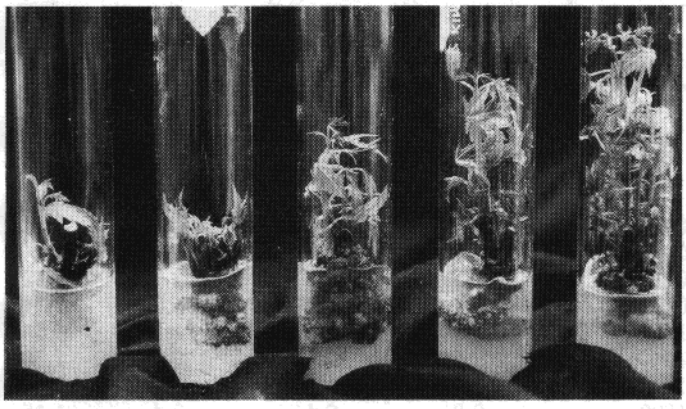

Fig. 1. Two-month-old single-node cultures from juvenile trees on medium with 10 μM BA. The cultures had been exposed to 0.01 μM thidiazuron for **(left to right)** 0, 2, 4, 6, or 8 weeks.

Fig. 2. Proliferating mass of silver maple axillary shoots after 4 months in vitro. The original single-node explant was from a juvenile tree. Scale bar = 1 cm.

cultures. We have frequently counted >100 shoots, all long enough to be rooted after 4 months in culture.

We believe that this rapid increase in axillary shoot formation results from a combination of factors: 1) the long-term exposure of silver maple explants to TDZ seemed beneficial; 2) we physically decapitated the longest shoots that reached the tops of the culture vessels, thus releasing apical dominance; and 3) we had developed a good callus base to support the cultures and to take up nutrients and organics from the medium. We refer to this massive shoot proliferation as the "turf" stage because it resembles a plug of lawn grass placed into a culture vessel (Fig. 2). Once the shoots reached the turf stage, they were easy to maintain because they then were stabilized shoot cultures. Shoots can be subculture or excised for rooting. Our data support continuous exposure to TDZ for maximum proliferation rates and indicate that BA is not a suitable cytokinin for micropropagation of this species.

To ensure that there will be a sufficient number of axillary shoots for rooting, it is standard practice in micropropagation to harvest microshoots and place them individually on shoot proliferation medium. We conducted an experiment to determine the best dose of TDZ for such reculture shoots. Unlike the primary explants, reculture shoots do not perform best with 10 nM TDZ, but produce the most axillary shoots on medium with 100 nM TDZ (Table 5). They produced more than twice as many shoots in 2 months as with any other dose of TDZ. Callus production is substantial with this concentration, but this does not hamper shoot production.

The formation of callus on shoot cultures is of concern to many workers in micropropagation. It is widely thought that shoot proliferation rates will suffer when much callus is produced. Callus tissue may also give rise to adventitious shoot organogenesis, which, because of somaclonal variation, is highly undesirable when propagating clonal material. In the many studies that we have conducted on micropropagation of silver maple, as well as on other tree species, axillary shoot proliferation rates are higher when there is some callus produced and lower when there is little or no callus. There is a limit to this, however, since at very high growth regulator rates, only callus grows and few, if any, shoots elongate. We believe that it is beneficial to have some callus present to provide physical support for the silver maple explants that grow much better in stationary liquid than agar-solidified medium. In addition, It is not clearly understood how nutrients and other substances are taken up by explant material. The beneficial effects of callus noted above suggest that this tissue may be important in nutrient and growth substance uptake. There has been no evidence to suggest that any of the shoots we obtained were adventitious. All shoots had visibly originated at the nodes.

We occasionally noticed fasciated shoots in proliferating cultures (Fig. 3). These fascia ted shoots appeared to originate in leaf axils. Apparently, this phenomenon is a physiological response to the phenolic cytokinin. Srivastava and G1ock (1987) reported that the phenolic compound p-fluorophenylalanine (FPA) induced fascination in birch shoots in vitro. They speculated that the fascination in birch may have been the result of the influence of FPA on the expression of a gene responsible for this phenotypic trait. We routinely excised and discarded the fasciated maple shoots. All other shoots in these cultures appeared normal and, when rooted, resulted in uniform normal members of clones.

Single-node and shoot-tip explants were harvested from greenhouse-grown silver maples grown from rooted cuttings of adult trees. These soft, succulent shoots were harvested from stock plants that had been pruned at least six times. The explants generally performed similarly juvenile shoots (Table 6). The shoot tips performed poorly and produced a dark exudate that discolored the medium. This exudate appeared to be a sign, not a cause, of explant stress because frequent transfers did not increase shoot tip survival or the shoot multiplication rate. Single-node explants responded in a cubic manner after 1 and 2

Table 5. Effects of thidiazuron concentrations on in vitro performance of microshoots excised from proliferating shoot cultures after 2 months in vitro.[z]

Thidiazuron concn (nM)	No. shoots	No. shoots >5 mm	Shoot length (mm)	Callus volume (cm^3)
0.001	2.4	1.5	10.8	0.1
0.01	5.1	3.2	6.9	1.2
0.1	2.9	2.0	8.3	0.4
1	5.2	3.7	9.0	1.0
10	4.7	3.5	9.1	1.1
100	10.7	7.3	8.6	5.8
1000	3.9	2.5	8.0	5.6
10000	2.5	0.9	3.9	9.8
Significance	**	**	**	**

[z]Each datum represents the mean of 18 to 20 cultures.
**Significant at $P = 0.01$ according to F test with 7 and 139 df.

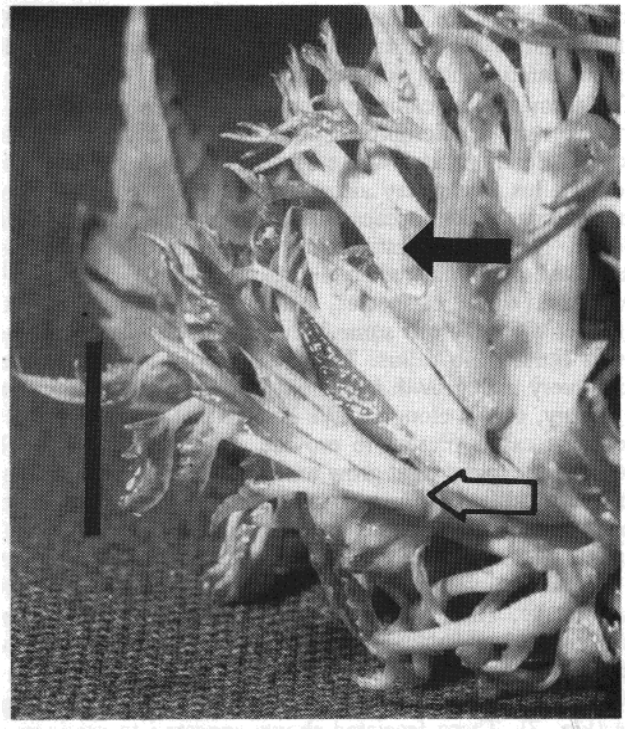

Fig. 3. Shoot mass showing normal shoot (open arrow) and fasciated shoot (closed arrow). Scale bar = 1 cm.

months in vitro for total number of shoots, number of shoots > 5 mm long, shoot length, and callus volume, with those exposed to 1 μM TDZ performing more poorly than those at higher and lower concentrations (Table 6). The best shoot growth without excessive callus was from explants on medium with 10 nM TDZ. In a preliminary experiment, single nodes collected from shoots that were somewhat more woody generally did not respond as well as the succulent shoots. TDZ concentrations most favorable for shoot outgrowth were from 0.01 to 10 nM.

When single-node and apical rnicroshoots of adult origin were rooted with O, 1, and 10 mM IBA, very few nodal segments rooted. Up to 70% of adult apical microshoots rooted when 1 mM IBA was applied as a 15-sec dip. Rooting was <2070 on the adult apical microshoots when no auxin was applied.

Microshoots were harvested from proliferating juvenile shoot cultures and rooted in vitro in the greenhouse. When applied as a 15-sec liquid dip to microshoots, IBA at 1 and 10 mM did not stimulate rooting compared to controls (data not shown). These shoots rooted well without the addition of auxin, and we have observed rooting of the proliferating shoots in vitro when cultures were not transferred frequently. Although there were no statistical differences in rooting between apical and single-node microshoots, we have since placed several thousand of both types of microshoots under mist in a greenhouse. The nodal microshoots have consistently rooted and acclimatized poorly. They are not a good choice for producing silver maple microplants.

There was a highly significant linear increase in rooting of juvenile apical microshoots with increasing length of microcuttings (Table 7). Microcuttings 21.5 cm long rooted best and those < 1.5 cm long rooted less well. Although 5-mm-long shoots can be rooted, the lower success rate and difficulty in handling makes them impractical. If few specimens of a particular clone are available, the ability to root short shoots may be critical.

Table 6. Influence of thidiazuron concentration on in vitro response of originally adult silver maple single-node explants after 1 and 2 months.[z,y]

Thidiazuron concn (nM)	No. shoots	No. shoots >5 mm	Shoot length (mm)	Callus volume (cm^3)
		After 1 month		
0.000	1.5	0.7	4.5	0.94
0.001	1.7	1.0	4.8	1.94
0.01	1.5	0.6	2.7	0.72
0.1	2.5	1.3	5.0	0.71
1	0.8	0.3	1.8	1.60
10	3.5	2.6	9.1	1.38
100	2.1	1.4	4.5	3.28
1000	0.8	0.4	2.4	1.15
10000	0.2	0.0	0.3	1.65
Significance[x]	**	**	**	**
Contrasts[w]				
Linear	**	NS	**	**
Quadratic	**	**	**	NS
Cubic	**	**	**	**
		After 2 months		
0.000	1.8	1.6	9.0	0.34
0.001	1.9	1.9	12.5	0.40
0.01	2.4	1.9	9.5	0.48
0.1	3.3	3.2	10.6	0.37
1	2.1	2.1	10.8	2.14
10	4.5	4.5	14.2	1.34
100	2.9	2.7	11.0	9.63
1000	0.8	0.7	3.7	6.24
10000	0.2	0.0	0.3	8.02
Significance[x]	**	**	**	**
Contrasts[w]				
Linear	**	**	**	**
Quadratic	**	**	**	**
Cubic	**	**	**	**

[z]Each datum represents the mean of 20 cultures.
[y]Data on shoot number and length were transformed for analysis using $(y + ½)^{½}$; nontransformed data are presented.
[x]Significant at $P = 0.01$ according to F test with 8 and 170 df.
[w]Significant contrast at $P = 0.01$ (**) or nonsignificant (NS) according to F test with 1 and 170 df.

Table 7. Effects of the size of juvenile apical microcuttings on rooting under intermittent mist in the greenhouse.[z]

Microcutting length (cm)	No. microcuttings	No. roots	Root length (mm)	Rooting percentage
0.5	20	1.4	7.7	65
1.0	20	2.6	10.0	75
1.5	20	3.5	22.9	95
2.0	20	3.4	18.9	85
2.5	20	4.6	28.8	100
3.0	19	4.8	28.5	100
Significance[y]		**	**	
Contrasts[x]				
Linear		**	**	
Quadratic		NS	NS	
Cubic		NS	NS	

[z]The cut end of each microcutting was treated with 1000 ppm IBA in talc.
[y]Significant at $P = 0.01$ (**) according to F test with 5 and 113 df.
[x]Significant contrast at $P = 0.01$ (**) or nonsignificant (NS) according to F test with 1 and 113 df.

We conducted an experiment to determine if harvested microshoots could be stored and then rooted later to facilitate scheduling both our tissue culture workers and the plantlet production. The microshoots were harvested and stored moist in a dark refrigerator (3 ± 2C) for various periods (Table 8). As time of storage increased, rooting response, as measured by root number, length, and percentage, decreased linearly. This result demonstrates that storage is feasible, but is best for only short periods (up to 3 days).

Microshoots that rooted under intermittent mist could be placed directly on an uncovered greenhouse bench with excellent survival (>90%) in the greenhouse. For acclimatization, it was best when the silver maple plantlets developed new leaves in the mist bench. Those with leaves that had grown only in' vitro were less likely to survive the move to the greenhouse bench without humidity control.

Excessive basal branching was not a problem on the micropropagated plants, and they appeared normal in every way (Fig. 4). We planted >750 micropropagated juvenile silver maple trees in the field. When planted, those trees ≤15 cm tall had poor survival, those ≥30 cm tall had a ≥75% survival rate. Rabbit and deer browse was a problem with the young, tender plants. When we interplanted micropropagated plants with bareroot seedlings from Indiana, growth was nearly equal between the two sources of plants (data not shown). The micropropagated plants grew with straight leaders and normal apical dominance.

Rooting stem cuttings. Our first experiments with rooting stem cuttings were with apical (terminal 10 cm of shoot growth) and single-node cuttings (same length) from seedling stock plants. Because single-node cuttings (leaf-bud cuttings) rooted as well as apical cuttings under intermittent mist and we could obtain many more single-node cuttings per plant, we concentrated our experiments on single nodes. Although auxin did not significantly affect rooting of juvenile cuttings, we have consistently applied 1000 ppm IBA in talc and have generally achieved ≥90% rooting.

able 8. The effects of refrigerated storage (4 ± 2C) on the subsequent rooting of silver maple microshoots.[z,y]

Days in storage	No. roots	Root length (mm)	Rooting percentage
0	4.4	13.4	80
1	3.2	7.8	100
2	3.0	13.9	80
3	5.2	17.0	100
4	2.4	2.9	80
5	0.8	7.2	40
6	1.4	7.2	40
7	0.2	0.8	20
8	2.0	4.7	60
9	4.6	9.7	80
10	0.8	1.6	20
Significance[x]	**	*	
Contrasts[w]			
Linear	**	**	
Quadratic	NS	NS	
Cubic	NS	NS	

[z]Each datum is based on five replications.
[y]Cuttings were treated with 1000 ppm IBA in talc and placed into peatlite medium (Pro-mix BX) under intermittent mist. Data were collected after 2 weeks.
[x]Significant at $P = 0.05$ (*) or 0.01 (**) according to F test with 10 and 44 df.
[w]Significant contrast at $P = 0.01$ (**) or nonsignificant (NS) according to F test with 1 and 44 df.

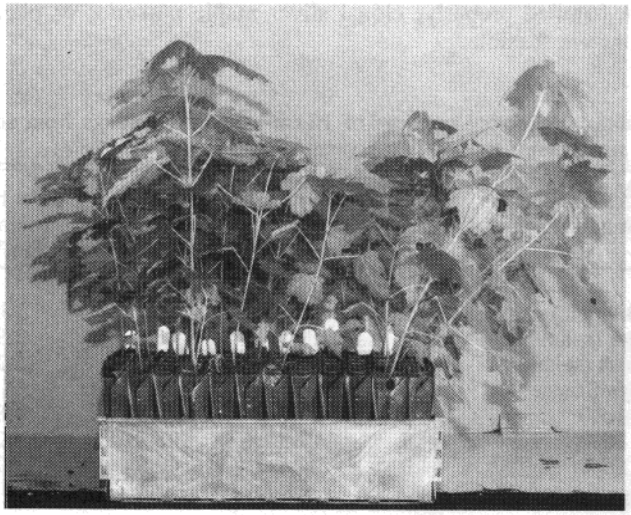

Fig. 4. Flat of micropropagated silver maple trees sufficiently large to be planted in the field. These plants had been out of culture for 3 months, 1 month of acclimatization under fog plus 2 months on a greenhouse bench. For scale, the planting flat was 15 cm tall.

Cuttings taken directly from local adult trees, however, did respond favorably to applied auxin (Table 9). During 1987, the best rooting was on cuttings treated with naphthaleneacetic acid (NAA) in a *50%* (v/v) ethanol : water solution. These cuttings had the most roots, the longest roots, and the greatest root ball volume than with IBA in talc or ethanol solution. Roots were longest and root ball volume was greatest on cuttings treated with 8000 ppm auxin.

Results were somewhat different during 1988. During that summer, there was a severe drought in southern Illinois. The new growth on the trees was only ≈5 to 10 cm long. Cuttings, therefore, included both 1987 and 1988 wood. The cuttings were generally less vigorous than those rooted during 1987. During 1988, cuttings rooted significantly better when auxin was applied in ethanol solution than in talc. Cuttings responded in a linear manner to increasing level of auxin, with the best percent rooting, root number per cutting, and longest roots on those cuttings treated with 8000 ppm auxin.

Silver maple can now be routinely propagated clonally by using in vitro methods or traditional cutting propagation. These techniques work well for both juvenile and adult forms. Juvenile forms are easier to root as cuttings, but both forms respond well in vitro. It is important to be able to clonally propagate both juvenile and adult forms. Juvenile trees tend to grow more rapidly than adult forms, and clonally propagating juvenile silver maples may be crucial for biomass plantations. Additionally, clonal populations of seedling-origin plants allow for replications of seedling genotypes for studies designed to separate the components of genotype and environmental influences on tree growth. Adult forms are important to clone because only by observing the adult tree can one know its ultimate form and habit on a given site.

The use of the cytokinin TDZ is important to in vitro success with silver maple. Continuous exposure at the proper dosage does not result in decline of the cultures or reduced microshoot size. The use of auxin, especially in ethanol solution, is important for success of rooting of adult cuttings. If propagules

are handled properly, clonal silver maple nurseries or plantations are feasible.

Table 9. Rooting response of 10-cm-long stem cuttings from adult. silver maple trees during 2 years. [z,y,x].

Main effects Auxin/concn (ppm)	Percent	No. roots	Root length (mm)	Root ball volume (cm^3)
		1987		
IBA (talc)	52.0	11.0	20.1	2.6
IBA (solution)	50.0	11.5	26.5	2.9
NAA (solution)	72.7	34.6	50.8	15.2
Significance[w]	*	**	**	**
1000	50.0	15.0	28.1	4.8
3000	60.7	17.5	28.8	4.2
8000	64.0	24.6	40.5	11.7
Significance[w]	NS	NS	*	**
		1988		
IBA (talc)	15.0	1.8	1.3	
IBA (solution)	30.5	7.9	2.5	
NAA (solution)	30.0	9.1	2.4	
Significance[v]	**	**	**	
0000	5.3	0.5	0.4	
1000	18.7	3.9	1.3	
3000	36.7	9.5	2.9	
8000	40.0	11.4	3.7	
Significance[u]	**	**	**	
Contrasts				
Linear	**	**	**	
Quadratic	NS	NS	NS	

[z]Each datum represents the mean of 30 cuttings for 1987, and for 1988, 80 cuttings for auxin and 60 cuttings for concentration.

[y]Dataon rooting percentage were transformed for analysis using arcsin (% ÷ 100); nontransformed data are presented.

[x]Data on root number and length were transformed for analysis using $(y + ½)^{½}$; nontransformed data are presented.

[w]Significant at $P = 0.05$ (*) or 0.01 (**), or nonsignificant (NS) according to F test with 2 and 72 df.

[v]Significant main effect at P = 0.01 according to F test with 2 and 99 df.

[u]Significant main effect at P = 0.01 according to F test with 3 and 99 df.

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