SILVER MAPLE TREE IMPROVEMENT FOR BIOMASS PRODUCTION1

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Abstract --Silver maples representative of its natural range have been established at Carbondale. Micropropagated plantlets and rooted cuttings from the most vigorous trees of each provenance will provide clonal material to determine genotypic and environmental interactions at outplanting test sites in OK, MN, NY, and NH, with two IL sites, upland and lowland. One-half the trees of each outplanting will be harvested for biomass after 3 years. The 2-year coppice re-growth of these trees and the 5-year growth of the original trees will be harvested for biomass at the end of the study.

Terminal and nodal stem cuttings taken directly from stock plants can be readily rooted under mist. Shoot tip and nodal explants have been successfully cultured in vitro on Driver and Kuniyuki Walnut (DKW) medium with thidiazuron (TDZ) at 1OnM. Shoot proliferation has been rapid with single-node explants, and numerous axillary shoots have been produced. In vitro-derived microshoots have been rooted and established.

Studies have been carried out on nutrient (12 elements) accumulation by roots, stems, and leaves. Additional analyses of seasonal changes and resource partitioning will be part of a further planting for intensive growth studies at Carbondale.

Additional keywords: Acer saccharinum, micropropagation, in vitro, rooting cuttings, provenance test, nutrient content

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Rationale for biomass production using trees

Our project is part of the Short Rotation Woody Crops Programs (SRWCP) at the Oak Ridge National Laboratory. It represents a specialized kind of forestry called Short Rotation Intensive Culture (SRIC) in which selected trees are quickly grown as an energy crop. Our anticipated end product is woody fuel feedstock, with animal feed supplements and chemicals feedstock as other possibilities.

Our broad objective is maximum annual woody biomass yield per hectare. To meet this objective we have implemented a seed source selection, individual tree selection and clonal propagation program to establish a series of provenance plantations. They will be located in five geographic areas, will be harvested in short rotations, and will include assessment of coppice production.

These plantations will be established at close spacing on old fields or similar "marginal" agricultural lands. Bringing these lands into SRIC energy crop production would benefit local and national economies. With few exceptions the SRWCP funds research on different trees than those funded as timber species (Risbrudt and McDonald 1986).

Rationale for choice of silver maple in selected areas

The concept of an ideal tree (ideotype) for SRIC in a given region has been developed within the SRWCP (Meridian Corp. 1986). Promising SRIC species for energy production in various regions of the country have also been designated. Silver maple (*Acer saccharinum* L.) was selected because it has performed well in tests extending from the eastern Great Plains to the Northeast. Silver maple grows well on diverse sites (Fowells 1965), coppices readily, and is relatively pest free. The significance of the latter attribute can be seen by comparison with *Populus* species and hybrids which are subject to insect damage, and to a number of serious stem canker and foliage diseases (Meridian Corp. 1986). Silver maple has also been given a higher rating as a hydrocarbon crop than *Populus*, *Salix*, *Platanus*, and other *Acer* species (Roth et al. 1982).

Approximately one million silver maple have been planted on strip-mines in Illinois alone, and have been among the most successful species, especially in survival (Ashby et al. 1978). In a greenhouse study silver maple (53 cm) outgrew black walnut (44 cm) and sweetgum (39 cm) in height after approximately 50 days. Silver maple also had the highest shoot/root (4.1 g/g) and leaf area/plant weight (123 cm2/g) ratios and the highest (31%) percentage stem dry weight (Ashby et al. 1979). These findings encourage research on its biomass potential.

H. C. Larsson (1968), Regional Research forester, Ontario Department of Lands and Forests, Maple, Ontario, reported that silver maple appeared to be one of the most suitable species for general planting in local swamps. A program was initiated in 1958 for locating, selecting, and propagating high-quality silver maple phenotypes in southern Ontario for reforestation purposes. Geyer (1978) and Naughton (1980) in Kansas, showed silver maple to be a prime candidate for SRIC with proper weed control, spacings, and a 3-year cutting cycle.

Potential for silver maple tree improvement

Both seed source and individual tree selection have been used for tree improvement. Rink (1983) reported increases in yellow-poplar height growth up to 10% by seed source selection, and from 3 to 7% by individual-tree selection depending on whether applied with or without provenance selection. Black walnut (Bey 1973) and several other species including both sugar maple (Kriebel 1957) and red maple (Towsend 1977) have been shown to have marked provenance responses.

Kilkenny (1971) reported studies on silver maple accessions collected from Minnesota to Mississippi and Louisiana. Her results suggested that temperature was a more significant factor than photoperiod in the spring opening of leaf buds. Dormancy in fall appeared to be photoperiodically induced, and northern accessions lost their leaves more rapidly than the southern trees. Southern trees grew taller, and longer, but appeared to be winter killed.

Information will likely be available at the present conference on further provenance studies of silver maple in the midwest. The central location of southern Illinois within the natural range of silver maple is advantageous for the testing of provenance responses.

Techniques for clonal propagation of selected trees

Standard propagation techniques for woody species include layering, grafting, cuttings, and tissue culture. Larsson (1968) reported survival and growth of superior silver maple propagated by cuttings, budding, and layering. In recent years aseptic methods of micropropagation have found increasing use in horticulture (Hartmann and Kester 1983). Applications to forest trees hold much promise. Micropropagation has several advantages over cuttings including production of a great number of new plants in a short time while using little space.

The use of clonal materials leads to a reduction in genetic variability compared to seedlings from open-pollinated seed orchards. Experimental error can be reduced, thus allowing greater precision in studies, such as ours, on tree phenotype, physiology, nutrition, silviculture, and general performance (Libby 1974). Microprogated plantlets have the same advantages as other containerized stock, now widely used in Canada, the Pacific Northwest, and Southeastern U.S.

Literature on maple micropropagation has been limited. Welsh et al. (1979) reported successful axillary shoot proliferation of red maple on Abbott and Whiteley's basal medium with kinetin, benzyladenine (BA) or isopentenyladenine (2iP), or with BA in Cheng's medium. Kerns and Meyer (1986, 1987) found that the phenylurea cytokinin thidiazuron was more effective than BA, kinetin, or 2iP in promoting axillary shoot proliferation from shoot explants of *Acer* x *freemanii* (a result of a cross between *A. rubrum* and *A. saccharinum*) on Lindsmeier-Skoog (1965) medium. We are aware of no published reports specifically on micropropagation of *A. saccharinum*.

Our ultimate goal is to compare micropropagated clonal silver maples from various provenances at sites throughout midwestern and northeastern U.S. Concepts on which the project is based include,

a. Silver maple is a fast-growing, readily coppicing species which can easily be grown in tree farming.
b. Silver maple can be propagated clonally in large numbers at reasonable cost.
c. Silver maple varies genetically, in part related to provenance, and selected clones will greatly exceed average growth in several regions suitable for its use in biomass production.
d. Potential productivity of a clone can be assessed to an appreciable extent by detailed growth analyses.

MATERIALS AND METHODS

Seed/seedling/cutting populations from localities dispersed Oyer silver maple's natural range were planted in a nursery at Carbondale, Illinois at a 15 x 15-cm spacing with a 4-mil plastic mulch, and irrigation. One hundred seeds were planted per block in a randomized complete block design, with 5 plots per accession. Bags of human hair and bars of soap were hung from stakes to repel deer.

The 6 most vigorous seedlings of each population will be selected, and clonally propagated. This planting stock will be grown in containers in the greenhouse and/or lath house.

We propose to plant 50 clonal plantlets of each accession on 6 planting sites in 1988. Each site would have at least 3600 test plantlets for a total of more than 21,600 in the provenance study, plus border rows. A lowland and an upland site will be in southern Illinois near the center of silver maple's range. Other localities are planned to be in New England (New Hampshire), the Lake States (Minnesota), the Northeast (New York), and the Great Plains (Oklahoma) regions. Each planting site will be evaluated for soil conditions, amended as needed, and planted in a 1987 cover crop in preparation for tree planting the following spring. Weeds will be vigorously controlled on each site.

After establishment, each accession in the provenance study will be monitored for time of bud break, senescence, disease, drought, and other stresses. Height and diameter will be measured. One half of the plantings will be harvested for biomass productivity measurements after 3 years, and the resulting coppice growth will be similarly measured. Mycorrhizal infection will be monitored. Data will be analyzed for dry weight production, site adaptability, and coppice productivity. The 2-year coppice re-growth and the 5-year growth of the original trees will be harvested for biomass at the end of the study.

An additional 1,800 plantlets will be planted in an intensive growth study in southern Illinois. The monitoring and measurements of the provenance study will be extended for analysis of stem and limb biomass accumulation, and for evaluation of photosynthate allocation, nutrient accumulation, and coppice productivity. This study will be more highly instrumented for soil and climatic data than the provenance studies.

Propagation studies

All stock plants were seedling trees grown in a greenhouse at 25/20 ± 5° C (day/night) with night interruption from 2200 to 0200 hours with cool white fluorescent lamps. The seeds for the stock plants were collected in 1986 at Carbondale, IL; Peoria, IL; and Minneapolis, MN. They were grown in one-gallon plastic pots in a sphagnum moss peat/vermiculite/perlite (2:1:1) medium (Promix BX) and received 400 ppm N each week from a water-soluble 20-10-10 ($N-P_2O_5-K_2O$) fertilizer (Peters' peat-lite special).

Stem tip and single-node macro-cuttings (10 cm long) were placed into a 1:1 (v/v) perlite/vermiculite medium under intermittent mist (one sec. per min.) in a greenhouse under natural photoperiod. Cuttings were scored for rooting and transplant success. Cuttings were also taken from terminal shoots of young trees in areas from which we had not obtained seed.

A major study, begun in November, 1986, was conducted to learn how to micropropagate silver maple seedlings. The work was done this year in preparation for the clonal micropropagation of the selected seedlings from the provenance nursery. All cultures were initiated in 25 x 150 mm pyrex culture tubes capped with clear autoclavable lids and containing 15 ml Difco bacto agar (7 g l^{-1}) solidified medium. Magenta GA7 plastic autoclavable vessels were used for stationary liquid media. Explants were 2.5 cm softwood shoot tips or 2.5 cm nodal segments that were surface disinfested for 20 minutes in 0.5% NaC10 with 0.1% (v/v) Tween 20, followed by three 5-minute rinses in sterile distilled water.

The nutrient medium was DKW (Driver and Kuniyuki (1984) walnut) medium salts plus organics, unless otherwise noted. All media contained 30 g l^{-1} sucrose and the pH was adjusted to 5.8 + 0.1 with IN KOH or HCl prior to the addition of agar (if used).

Cultures were incubated at 22 ± 2° C with a 16-hour photoperiod and a photosynthetic photon flux (PPF) of 33-45 µmoles m -2 s -1 provided by cool white fluorescent lamps. All cultures were transferred to fresh media every 14 days. All experiments were arranged in completely randomized designs with 10 replications, and were conducted twice.

A preliminary mineral nutrient accumulation study was carried out to establish methodology and evaluate accumulation patterns. Plants were dug at --week intervals, divided into root, stem, and foliage if present, dried, weighed, ground to powder in a Wiley mill, and analyzed for 1- elements. A method (Wargo 1975) for starch accumulation was also evaluated.

RESULTS AND DISCUSSION

An unexpected result was that silver maple may not readily be found, if at all, within its mapped range (Fowells 1965), and may experience total failure of the seed crop (Table 1). Another unfortunate result was that some seed lots had very poor germination, or germinated well in the lab and not in the nursery. A striking example is the Princeton southern West Virginia (S WV) seed which had excellent germination in lab tests but emerged very

Table 1. Potential and Actual Silver Maple Sources

Seed received		Trees Found No Seed	No Native Trees Found
E Cen. AL[1]	S Ont.[1,2]	E KY	GA
E Cen. MS	Cen. Ont.[1,2]	Cen. NC[1]	Cen. NC
S IL[1]	Cen. CT[1]	SE KS	E TN
S IL[1]	Cen. RI[1]	NE KS	
Cen. IN	Cen. NH	N Cen. WI	
S WV	NW VT	Cen. OH	
Cen IA	NE PA[1]		
S Cen. WI	S Cen. NY[1]	Cuttings source	
SW VA[1]		SW VA	
		W KY	
NW PA			
SW PA		E KS	
S Cen. PA		S MN	
		Seedlings source	
		S IN	
		SE MN	
		N Cen. IL	

[1] Large yard, park, or field-edge tree
[2] Ontario

poorly in the nursery. Other seed lots also had apparently good seed which did not grow in the nursery.

Several accessions had green seed, which resulted in delayed germination. Green seed was held at room temperature to ripen and tended to rot (cen. IN was a total loss) if not watched carefully and promptly put in the cold room before planting.

Seed accessions were received over a six-week period. With the exception of S WV, all seed planted in May had 47 to 97% establishment (Table -). None of the seed planted in June had greater than -4% establishment after four or more weeks. Only limited success was obtained with planting pre-germinated seed.

Seed quality was generally better for the May accessions. Four June accessions had beetle larvae and the lower percent germinations (Table -). Only occasional seeds showed physical damage, possibly beetle-related. Some June accessions smelled fermented when the collection bag was opened.

High soil temperatures may be another reason why the June plantings had poor establishment. Also, May was relatively dry and June had heavy rain leading to saturated soils which adversely affect silver maple seedlings (Fowells 1965).

Accessions with the highest percent and to some extent most rapid germination were received early from Mississippi River Valley locations. Those accessions received later from the northeastern part of the silver maple range had relatively poor establishment. The Great Plains and Southeast--where trees were found--had seed crop failures from late freezes (Table 1). Seedlings and cuttings were used to provide geographic coverage of missing areas.

Comparative growth data are not presently available as emergence is not complete for the latest accessions, and the older plantings have grown for over two months. Substantial differences in height are evident among the seedlings within an accession and plot and presumably will provide a basis for later selection. Seedlings in the easternmost plots were stunted with small, reddish-colored leaves, evidently related to saturated soils (Fowells 1965). Foliar feeding brought better color and growth. As mentioned earlier, June had heavy rains, and occasional excess irrigation water also drained to those plots. No animal damage has been noted except for a half-dozen edge plants with tops nipped by locally abundant deer.

An unanticipated result was leaf damage in the nursery from heavy rains and strong winds. A few plantlets were uprooted. Internode elongation was likely decreased by shaking from wind (Ashby et al. 1979). Native silver maple habitats along floodplains would have lower wind velocities than our nursery site on a relatively broad east-west ridge.

Mineral nutrient content

Mineral nutrient content of forest trees is notoriously variable (Hacskaylo et al. 1969). Values for our silver maple generally fell within the range of their values for black walnut, black locust, cottonwood, and

Table 2. Percent Establishment Ten to Four Weeks After Planting 500 Seed Each

Accession	Establishment	Accession	Establishment[6]
May	%	June	%
E Cen. AL[1]	48	S. Ont.[3]	14
E Cen. MS	75	Cen. Ont.	16
S IL	97	Cen. CT[3,4,5]	0
S IL	85	Cen. RI	22
S WV[2]	2	Cen. NH[3]	12
Cen. IA	96	NW VT	20
S Cen. WI	95	S Cen. NY[3]	13
SW VA[1]	54	NE PA	24
NW PA	47		

[1] 200 seed
[2] Replanted (250 germinated seed)
[3] Beatle larvae in collection bag
[4] 100 seed
[5] Germinated when received
[6] Accessions from Cen. IN and SW and S Cen. PA with very poor establishment were eliminated

sweetgum on complete and nutrient-deficient solutions (Table 3). Exceptions are low root iron and high stem iron in our samples, and our high root and stem manganese. Nutrient contents from September to December 1986 for a different silver maple planting showed roughly comparable values after October. The boron and zinc values reported here are lower, and the managanese and root magnesium values higher.

Compared to sugar maple and four other species in a mixed-hardwood stand at Chalk River, Ontario (Carlisle and Methven 1979) our values for N, P. and K on agricultural soils are high, and those for Ca and Mg comparable. Young sugar and red maple in sand culture with complete and nutrient-deficient solutions (Erdmann et al. 1979) had comparable macro-nutrient values except for our high root magnesium in the spring 1987, and low potassium values for their red maple roots and stems.

Clonal propagation studies

In these studies single node cuttings rooted as well as stem tip cuttings. Single nodes provide more propagules per plant and were therefore used in subsequent experiments. Leafless single node cuttings took approximately 3 weeks to root. Cuttings treated with indole butyric acid (IBA) in powder rooted better than those treated with IBA or napthalene acetic acid (NAA) in 50% (v/v) ethanol/water, 53% vs 44 and -3% respectively. Equal rooting percentage was observed with all auxin concentrations (0, 1000, 3000, and 8000 ppm); however, significantly more roots formed on cuttings treated with 8000 ppm auxin. In other studies, under mist, we have had > 90% rooting from selected provenances, e.g. Virginia, when we have left the leaves on the cuttings and have treated with 1000 ppm IBA in powder.

In our preliminary micropropagation experiments silver maple shoot explants stained the medium with a small amount of dark exudate. Exudates from many woody species are autoinhibitory to explants (Compton and Preece 1986) and red maple shoots require special treatments to alleviate this problem (Welsh et al. 1979). Exudate from silver maple, however, is apparently not autotoxic and explants grow well with no special treatments.

Initial experiments conducted on Woody Plant Medium (Lloyd and McCown 1981) and Lindsmeier-Skoog (1965) medium were terminated after only one month because growth and leaf development were poor, only single shoots elongated, and the tips tended to die back. However, shoot explants on DKW (Driver and Kuniyuki 1984) medium grew considerably better, in that the leaves grew well and expanded, the apical shoots were vigorous, and axillary shoots grew. Therefore, DKW was used in subsequent experiments.

No observable growth differences have been noted among explants from the 1986 Carbondale, IL, Peoria, IL, or Minneapolis, MN seedlings. The success of our system with silver maple seedlings from a variety of provenances will be critical to cloning seedlings from our provenance nursery. We are currently conducting experiments comparing the responses of specific clones.

An experiment was conducted that consisted of a 3 x 3 factorial of benzyladenine (BA) at 1, 5 and 10 µM and IBA at 0, 0.1 and 1 µM. Previous work in our laboratory (Compton and Preece, unpublished) indicated that a small amount of auxin in the medium lessens necrosis on shoot explants of some

Table 3. Average mineral nutrient content prior to 1987 shoot growth of silver Maple seedlings on three dates

Nutrient	Roots			Stems		
	April		May	April		May
	1	15	1	1	15	1
Percent						
N	1.14	1.17	1.21	0.76	0.71	0.57
P	0.15	0.14	0.13	0.11	0.08	0.07
K	0.81	1.01	0.75	0.59	0.53	0.45
Ca	0.45	0.49	0.47	0.63	0.51	0.48
Mg	1.01	1.03	0.92	0.05	0.04	0.04
Na	0.04	0.03	0.04	0.02	0.03	0.02
ppm						
Fe	301	258	475	101	88	104
Al	435	489	884	103	99	121
Mn	120	110	129	192	151	155
B	9	14	19	8	14	12
Cu	11	14	15	10	9	11
Zn	18	17	15	21	16	17

[1]Seedlings planted on 1 April 1987 were furnished by Ware's Nursery, McMinnville TN. Ten seedlings were harvested per date. Tissue analyses by A & L Agricultural Laboratories of Memphis, TN.

woody species. BA at concentrations higher than 1 µM and IBA at 0.1 and 1 µM enhanced callus growth and inhibited shoot growth. With 1 µM BA a mean of only one axillary shoot grew from each shoot tip after two months in vitro, which is considerably fewer than the two to five axillary shoots reported from red maple shoot explants on a medium with one mg l^{-1} BA (Welsh et al. 1979).

Because of the positive effects from thidiazuron observed on maple shoot explants by Kerns and Meyer (1986, 1987), we conducted an experiment that consisted of a 2 x 6 factorial from mixed seedling sources. Two explant types (shoot tips and single nodes) and 6 concentrations (see Table 4) of thidiazuron were compared. More shoots and callus formed on single node explants than on shoot tips after one and two months in vitro (Tables 4 and 5 and Fig 1); therefore this discussion is primarily focused on results obtained with single node explants. With single nodes, after one and two months in vitro, there was a significant quadratic response to concentrations of thidiazuron for shoot number and length; the greatest stimulation was with 0.01 µM thidiazuron. After two months in vitro, single nodes on medium with 0.01 µM thidiazuron produced a mean of 6.4 shoots, 4.7 of which were longer than 5 mm (these were large enough to be excised for rooting or subculturing for clonal proliferation). The 6.4 shoots had a mean length of 10.2 mm. After one month in vitro there was a significant cubic response of nodal explants regarding callus growth.

Callus growth was relatively low at 0.01 µM thidiazuron; however, after two months nodal explants on the different concentrations of thidiazuron did not differ significantly for callus growth (Table 4). After two months in vitro the main effect of thidiazuron (averaged across explant type) on total shoot number was significant (Table 5). Again, the greatest number of shoots formed on explants cultured on medium with 0.01 µM thidiazuron. Callus at this concentration was still relatively small, but was significantly greater at the next higher concentration (0.1 µM).

Microshoots (> 2 cm long) have been excised and rooted (80 - 100% rooting) (Fig. 2) in vitro in vermiculite or in the greenhouse in peat-lite medium under shade and high relative humidity or under intermittent mist. Both in vitro and in the high humidity rooting was observed within one week, and under photometrically controlled intermittent mist in the second week. However, by three weeks after placing the microshoots in a rooting medium, rooting was equal (92%) under high humidity and the mist. Generally, rooting is equal between microshoots treated with IBA and not treated, but those in the greenhouse that were treated with 1 mM IBA had three more roots per microshoot than those that were not treated with auxin (5.4 vs. 2.4). All microshoots that rooted survived transplanting regardless of root number.

Rooted microshoots were easily acclimatized to the greenhouse conditions. Those rooted in vitro benefited from first being placed under 75% shade or intermittent mist for one week before they were moved to an unprotected greenhouse bench. Those that rooted in the greenhouse could be moved directly to an unprotected greenhouse bench 21 days after the microshoots were first placed into the rooting medium. We have transplanted 32 plants that were propagated and rooted in vitro into the nursery. They were first grown for one to two months on the greenhouse bench. After three weeks in the nursery there was 100% survival.

Table 4. The influence of thidiazuron concentration and explant type on in vitro response of silver maple after 1 and 2 months

Thidiazuron concn (μm)	Explant type	Month 1						
		Total number of shoots		Number of shoots > 5 mm		Mean shoot length (mm)		Callus volume (cm^3)
		Transformed[z]	actual number	Transformed	actual number	Transformed	actual number	
0.0001	Apical	1.3	1.6	0.8	0.1	1.2	1.3	0.19
0.001		1.4	1.5	0.7	0.0	1.1	0.4	0.07
0.01		1.7	2.6	0.9	0.4	1.7	0.9	0.27
0.1		1.2	1.3	0.7	0.0	1.1	2.5	0.34
1		1.0	0.7	0.7	0.0	0.9	0.9	0.12
10		0.9	0.4	0.7	0.0	0.7	1.3	0.00
Apical Contrast								
Linear		**		NS		**		NS
Quadratic		**		NS		**		*
Cubic		*		NS		NS		NS
0.0001	nodal	1.7	2.5	1.0	0.7	2.0	3.6	2.61
0.001		1.4	1.6	0.9	0.4	1.7	3.1	1.83
0.01		2.3	4.9	1.5	2.1	2.4	5.6	0.99
0.1		1.7	2.8	1.0	0.6	1.9	3.5	3.49
1		1.3	1.7	0.8	0.2	1.3	1.6	1.55
10		0.9	0.6	0.7	0.0	0.9	0.4	0.35
Significance[y]		**		**		*		**
5% LSD		0.25		0.16		0.29		1.124
1% LSD		0.33		0.22		0.38		1.467
Nodal Contrast								
Linear		**		**		**		*
Quadratic		**		**		**		NS
Cubic		NS		NS		NS		**

Each number represents the mean of 20-24 cultures

[z]Transformation used was $\sqrt{n + 1/2}$

[y]Significant interaction at the 1% (**) or 5% (*) level or nonsignificant (NS) according to F-test with 5 and 262 df (month 1) or 5 and 241 df (month 2)

Table 4 (continued) The influence of thidiazuron concentration and explant type on in vitro response of silver maple after 1 and 2 months

Thidiazuron concn (μm)	Explant type	Month 2						
		Total number of shoots		Number of shoots > 5 mm		Mean shoot length (mm)		Callus volume (cm^3)
		Transformed[z]	actual number	Transformed	actual number	Transformed	actual number	
0.0001	Apical	1.4	1.7	0.9	0.3	1.7	3.2	0.90
0.001		1.3	1.4	0.7	0.0	1.3	1.3	0.46
0.01		1.7	2.9	1.0	0.9	2.2	5.2	0.87
0.1		1.4	2.0	0.9	0.6	1.3	1.9	1.25
1		0.9	0.5	0.7	0.0	0.3	0.2	1.17
10		0.7	0.0	0.7	0.0	0.7	0.0	0.41
Apical Contrast								
Linear				NS		**		
Quadratic				**		**		
Cubic				NS		NS		
0.0001	nodal	1.9	3.5	1.4	1.8	3.1	10.7	3.98
0.001		2.1	4.2	1.5	1.9	2.4	5.7	4.83
0.01		2.6	6.4	2.2	4.7	3.2	10.2	3.92
0.1		2.3	5.2	1.7	2.8	2.7	7.8	6.80
1		1.5	2.2	1.0	0.8	1.4	2.1	5.76
10		1.1	1.6	0.7	0.0	0.8	0.3	2.93
Significance[y]		NS		**		**		NS
5% LSD				0.26		0.46		
1% LSD				0.35		0.60		
Nodal Contrast								
Linear				**		**		
Quadratic				**		**		
Cubic				NS		NS		

Each number represents the mean of 20-24 cultures

[z]Transformation used was $\sqrt{n + 1/2}$

[y]Significant interaction at the 1% (**) or 5% (*) level or nonsignificant (NS) according to F-test with 5 and 262 df (month 1) or 5 and 241 df (month 2)

Table 5. The main effects of thidiazuron and explant type on total shoot number and callus volume after 2 months in vitro

Treatment		Total number of shoots		Callus Volume (cm^3)
Thidiazuron concn (μm)	Explant type	Transformed[x]	actual number	
0.0001		1.6[z]	2.6	2.4
0.001		1.7	2.7	2.5
0.01		2.1	4.6	2.4
0.1		1.8	3.6	4.0
1		1.2	1.4	3.5
10		0.9	0.8	1.6
Significance[w]		**		*
5% LSD		0.25		1.36
1% LSD		0.33		1.78
Contrast				
Linear		**		NS
Quadratic		**		*
Cubic		NS		*
	Apical	1.2[y]	1.4	0.8
	Nodal	1.9	3.8	4.7
Significance[v]		**		**

[z]Each concentration number is the mean of 40-48 cultures

[y]Each explant type number is the mean of 125-138 cultures

[x]Transformation used was $\sqrt{n + 1/2}$

[w]Significant main effect at the 1% (**) or 5% (*) level according to F-test with 5 and 241 df

[v]Significant main effect at the 1% (**) level according to F-test with 1 and 241 df

CONCLUSIONS

Our experience strongly supports the conclusion that silver maple is a prime candidate for SRIC. It is readily propagated clonally, establishes well as a rule, and grows rapidly. If establishment is to be from seed, greater attention must be paid to availability of viable native seed than is evident from the literature. The marked inter-provenance and inter-tree variability holds promise for selection gains in the provenance plantations planned for next year. Use of micropropagation in supplying plantlets for those plantations appears to be very efficient. Silver maple mineral nutrient content was comparable to that of other hardwoods including maples.

Figure 1. Axillary shoot proliferation from apical (left) and single-node (right) explants on 0.1 µM thidiazuron.

Figure 2. This microshoot was dipped in 1000 ppm IBA in talc (Hormodin #1) and placed under mist for 2 weeks, Pro-mix BX medium.

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