# Micro- and Cutting Propagation of Silver Maple. II. Genotype and Provenance Affect Performance

John E. Preece<sup>1</sup> and Carl A. Huetteman<sup>2</sup>

Department of Plant and Soil Science, Southern Illinois University, Carbondale, IL 62901

## W. Clark Ashby<sup>1</sup>

Department of Botany, Southern Illinois University, Carbondale, IL 62901

## Paul L. Roth<sup>1</sup>

Department of Forestry, Southern Illinois University, Carbondale, IL 62901

Additional index words. Acer saccharinum, in vitro, tissue culture, biomass, energy, short-rotation woody crops

Abstract. During 1987, we selected the six fastest-growing seedlings or clones from each of 15 provenances that represented the natural distribution range of silver maple (*Acer saccharinum* L.). Shoots from all 90 trees were cut into nodal segments, rooted as cuttings, and maintained as clonal stock plants in the greenhouse. Rooting was generally excellent and more than half of the clones rooted  $\geq 90\%$ . At the same time, explants were obtained from these field-grown trees and many were established in vitro as aseptic cultures by first pretreating with benomyl and rifampicin. Single-node explants from the greenhouse-grown clonal stock plants were also established and multiplied in vitro. There was a significant effect of clone within provenance on all in vitro growth characteristics. All clones proliferated axillary shoots, but not all at the same rates. Although statistically significant, low correlation coefficients indicated that micropropagation results were not good predictors of nursery performance of the populations from which the clones were selected, nor of the climatic conditions at the site of origin of the trees. The micropropagation system reported herein, therefore, should be applicable to a wide variety of silver maple genotypes. Chemical name used: methyl [1-[butylamino)carbonyl]-1H-benzimidazol-2-yl]carbamate(benomyl).

Silver maple increasingly has been recognized as having an important role in the energy future of the United States (Ashby et al., 1987; Ellis and McCown, 1988; Kopp et al., 1988; Meridian Corp., no date; Ranirey et al., 1987, 1988). It grows well on a variety of sites (Fowells, 1965), can be propagated easily (Ashby et al., 1987; Preece et al., 1991), coppices readily, and is relatively free from serious pest problems. This last criterion is important in that members of the presently used *Populus* genus are susceptible to serious stem and leaf diseases and have a variety of insect pests. In addition, Roth et al. (1982) analyzed 508 species as renewable energy resources and rated silver maple higher than *Populus, Salix, Platanus,* and other *Acer* species.

Silver maple can be clonally propagated by rooting stem cuttings (Ashby et al., 1987; Larsson, 1968; Kling and Meyer 1983; Preece et al., 1991), budding and layering (Larsson, 1968), and by micropropagation (Ashby et al., 1987; Preece et al., 1991). The advantages of the use of clonal stock, rather than seedlings from open-pollinated seed orchards were discussed in a companion paper (Preece et al., 1991).

The use of seedlings from different provenances allows for initial selections to be made under uniform nursery conditions at one location. Juvenile plants are generally easier to propagate clonally than adult forms (Hartmann and Kester, 1983; Libby, 1983), and clonal testing can be very effective when starting with young seedlings (Libby, 1983). Selected clones can then be used in replicated studies at different locations to separate the components of genotype and environment.

<sup>1</sup>Professor. <sup>2</sup>Researcher.

J. Amer. Soc. Hort. Sci. 116(1):149-155. 1991.

This study was conducted to assess the propagation responses of silver maples as they were being multiplied clonally for planting in several states of the United States for growth assessment relating to biomass production. We were interested in the relationships between propagation response and 1) seedling growth in the nursery and 2) climatic conditions at the site of origin of the trees. If strong relationships existed, propagation attributes might then be useful as a predictor of origin or field performance and many genotypes might be screened rapidly in limited space. If relationships were not strong, it would verify that this propagation system is applicable to a wide variety of silver maple genotypes and that the system would not need modification if one wished to clone a new silver maple tree.

#### **Materials and Methods**

Silver maple seeds/plants/cuttings representative of the tree's reported distribution range were collected and established in a nursery in Carbondale, Ill., during Spring and Summer 1987. The nursery was arranged in a randomized complete-block design with 200 seeds from each location (provenance) sown (two per planting hole) 15 cm apart. Upon emergence, seedlings were thinned to one per planting hole. Provenances within a block were separated by 90 cm and blocks were 1.5 m apart. There were five blocks, each containing up to 100 trees from each site of origin. Exceptions to this were the Virginia, Kansas, and Minnesota trees because of the lack of a seed crop in these areas during 1987. The Virginia and Kansas trees were propagated by cuttings collected from native trees in these states, and the Minnesota seedlings were dug from the floor of a native stand. Trees from these three provenances were established in the greenhouse. This plan resulted in 16 provenances established from seed, one from seedlings, and two from cuttings. The focus of this research was narrowed to 15 provenances to give a wide representation of the native distribution range of silver maple and avoid redundancy of provenances in close proximity to each other.

During Aug. 1987, when most growth had slowed in the

Received for publication 18 Dec. 1989. Research performed under Subcontract no. 86x-95908C with Oak Ridge National Laboratory under Martin Marietta Energy Systems, Inc. Contract no. DE-AC05-840R21400 with the U.S. Dept. of Energy. We thank Nor-Am Chemical, Wilmington, Del., for providing the thidiazuron. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement solely* to indicate this fact.

| Provenance<br>(south to north)    | Clone<br>no.               | Ht<br>(cm)                             | Field<br>Caliper <sup>y</sup><br>(cm)  | Cuttings<br>(no.)                | Rooting<br>(%)                          | Explants<br>(no.)  | Aseptic<br>explants<br>surviving<br>(%) |
|-----------------------------------|----------------------------|--|--|----------------------------------|---|--|---|
| East-central<br>Mississippi 01    | 1<br>2<br>3<br>4<br>5<br>6 | 150<br>152<br>144<br>129<br>122<br>144 | 1.0<br>1.4<br>1.5<br>1.2<br>1.3<br>1.5                                       | 21<br>23<br>19<br>18<br>27<br>15 | 90<br>96<br>84<br>100<br>89<br>87       | 2<br>12<br>3<br>18<br>10<br>7  | 0<br>0<br>67<br>100<br>80<br>100        |
| Virginia 18                       | 1<br>2<br>3<br>4<br>5<br>6 | 42<br>46<br>56<br>48<br>56<br>46       | $\begin{array}{c} 0.6 \\ 1.0 \\ 0.6 \\ 0.6 \\ 0.6 \\ 0.6 \\ 0.6 \end{array}$ | 14<br>23<br>29<br>12<br>11<br>23 | 86<br>96<br>100<br>82<br>96             | 7<br>10<br>10<br>4<br>2<br>8   | 100<br>10<br>80<br>25<br>100<br>62      |
| South-central<br>West Virginia 07 | 1<br>2<br>3<br>4<br>5<br>6 | 43<br>46<br>77<br>55<br>46<br>44       | 0.6<br>0.9<br>0.7<br>0.6<br>0.7  | 6<br>5<br>25<br>15<br>12<br>11   | 100<br>100<br>92<br>93<br>100<br>100    | 3<br>6<br>5<br>2<br>6  | 100<br>100<br>100<br>60<br>50<br>0      |
| Southern<br>Illinois 04           | 1<br>2<br>3<br>4<br>5<br>6 | 150<br>132<br>124<br>175<br>141<br>146 | 1.4<br>1.3<br>1.2<br>1.5<br>1.7<br>1.4                                       | 13<br>10<br>18<br>25<br>33<br>11 | 100<br>100<br>100<br>100<br>100<br>100  | 4<br>2<br>3<br>5<br>8<br>4   | 25<br>100<br>100<br>100<br>88<br>50     |
| South-central<br>Indiana 05       | 1<br>2<br>3<br>4<br>5<br>6 | 150<br>140<br>120<br>148<br>148<br>145 | 1.7<br>1.6<br>1.2<br>1.2<br>1.9<br>1.5                                       | 29<br>35<br>51<br>83<br>62<br>44 | 96<br>100<br>98<br>89<br>98<br>98<br>91 | 8<br>6<br>10<br>10<br>7<br>6   | 38<br>100<br>50<br>100<br>43<br>67      |
| Northeastern<br>Pennsylvania 11   | 1<br>2<br>3<br>4<br>5<br>6 | 49<br>46<br>28<br>33<br>17<br>49       | 0.4<br>0.6<br>0.3<br>0.4<br>0.2<br>0.5                                       | 7<br>6<br>10<br>6<br>1<br>7      | 100<br>83<br>100<br>100<br>100<br>100   | 3<br>3<br>2<br>3<br>3  | 0<br>100<br>33<br>100<br>100<br>0       |
| Central<br>Iowa 06                | 1<br>2<br>3<br>4<br>5<br>6 | 110<br>103<br>97<br>96<br>83<br>107    | 1.2<br>0.9<br>1.1<br>1.1<br>1.1<br>1.2                                       | 10<br>9<br>17<br>17<br>15<br>9   | 100<br>100<br>88<br>100<br>100<br>100   | $     \begin{array}{r}       10 \\       3 \\       5 \\       \\       3 \\       3     \end{array} $ | 30<br>67<br>80<br><br>67<br>33          |
| South-central<br>New York 17      | 1<br>2<br>3<br>4<br>5<br>6 | 50<br>47<br>53<br>48<br>46<br>37       | 0.6<br>0.6<br>0.5<br>0.6<br>0.5<br>0.5                                       | 9<br>6<br>10<br>9<br>7<br>3      | 100<br>100<br>100<br>78<br>71<br>100    | 5<br>4<br>2<br>3<br>3<br>1   | 0<br>100<br>100<br>100<br>100<br>100    |
| South-central<br>Wisconsin 08     | 1<br>2<br>3<br>4<br>5<br>6 | 117<br>136<br>98<br>135<br>82<br>129   | 1.3<br>1.3<br>0.6<br>1.5<br>0.9<br>1.2                                       | 10<br>14<br>18<br>21<br>15<br>19 | 80<br>100<br>100<br>67<br>93<br>26      | 2<br>8<br>3<br>5<br>3  | 100<br>75<br>100<br>100<br>100          |

Table 1. Rooting of nodal stem cuttings of silver maple clones selected from a provenance nursery.<sup>z</sup>

(continued)

| Table 2. Clone within provenance eff | ects on in vitro growth o | f silver maple from single-node | explants after 1 and 2 months. |
|--------------------------------------|---------------------------|---------------------------------|--------------------------------|
|--------------------------------------|---------------------------|---------------------------------|--------------------------------|

|                                |               |                   | Month 1    |                |                |                              | Month 2 |              |       |                |                |                              |         |
|--------------------------------|---------------|-------------------|------------|----------------|----------------|------------------------------|---------|--------------|-------|----------------|----------------|------------------------------|---------|
|                                |               |                   | Shoots     | s >5 mm        | Shoot          | Callus                       |         |              | Shoot | s >5 mm        | Shoot          | Callus                       |         |
| Provenance<br>(south to north) | Clone<br>no.  | Shoot<br>no.      | No.        | Length<br>(mm) | length<br>(mm) | volume<br>(mm <sup>3</sup> ) | n       | Shoot<br>no. | No.   | Length<br>(mm) | length<br>(mm) | volume<br>(mm <sup>3</sup> ) | n       |
| East-central                   |               |                   |            |                | •              |                              |         |              |       |                |                |                              |         |
| Mississippi 01                 | 1             | 1.7               | 0.7        | 10.2           | 5.4            | 227                          | 10      | 3.1          | 1.6   | 53.5           | 25.5           | 880                          | 10      |
|                                | 2             | 2.5               | 1.0        | 8.5            | 4:9            | 141                          | 10      | 2.6          | 2.0   | 19.4           | 16.5           | 202                          | 10      |
|                                | 3             | 2.1               | 0.7        | 5.5            | 3.4            | 529                          | 10      | 2.3          | 1.8   | 7.5            | 6.4<br>8.6     | 485                          | 10      |
|                                | 4             | 4./               | 1.5        | 0.1            | 3.7<br>5 7     | 192                          | 10      | 4.1<br>2 9   | 2.0   | 21.9           | 20.0           | 134                          | 10      |
|                                | 6             | 0.9               | 0.0        | 0.0            | 1.0            | 307                          | 10      | 1.9          | 0.6   | 9.4            | 4.9            | 326                          | 10      |
| Southwestern                   | Ť             |                   |            |                |                | •                            |         |              |       |                |                |                              |         |
| Virginia 18                    | 1             | 1.7               | 0.3        | 10.0           | 3.4            | 163                          | 10      | 3.7          | 2.4   | 19.2           | 10.9           | 989                          | 10      |
|                                | 2             | 1.9               | 0.9        | 12.1           | 7.0            | 98                           | 10      | 5.6          | 4.4   | 27.7           | 19.9           | 863                          | 10      |
| ۰.                             | 3             | 1.8               | 0.4        | 11.8           | 4.9            | 188                          | 10      | 2.3          | 1.3   | 20.5<br>26.4   | 14.1<br>18 7   | 631                          | 10      |
|                                | 4             | 1.0               | 0.1        | 12.0           | 2.8<br>6.9     | 401                          | 10      | 4.4          | 4.0   | 20.4           | 18.6           | 1226                         | 10      |
| · •                            | 6             | 1.5               | 0.8        | 10.4           | 7.0            | 271                          | 10      | 3.3          | 2.1   | 18.4           | 12:0           | 843                          | 10      |
| South-central                  |               |                   |            |                |                |                              |         |              |       |                |                |                              |         |
| West Virginia 07               | 1             | 2.4               | 1.8        | 10.3           | 7.0            | 280                          | 10      | 5.1          | 4.2   | 28.1           | 22.8           | 728                          | 10      |
|                                | $\frac{2}{2}$ | 2.1               | 1.0        | 12.0           | 5.8            | 300                          | 10      | 3.0          | 2.0   | 10.2           | 12.3           | 499                          | 10      |
|                                | 3<br>4        | 1.5               | 0.5        | 8.0            | 1.9            | 200<br>502                   | 10      | 2.8          | 2.0   | 18.8           | 6.2            | 1024                         | 10      |
|                                | 5             | 1.0               | 1.1        | 11.9           | 9.0            | 136                          | 10      | 5.1          | 4.1   | 25.2           | 22.3           | 301                          | 10      |
|                                | 6             | 2.0               | 0.5        | 2.4            | 3.2            | 11                           | 10      | 2.3          | 0.7   | 4.5            | 5.1            | 19                           | 10      |
| Southern                       |               |                   |            |                | ~ 1            | 1.00                         | 10      |              |       |                | 10.4           | 107                          | 10      |
| Illinois 04                    | 1             | 1.8               | 0.1        | 5.0            | 2.1            | 160                          | 10      | 2.1          | 1.4   | 14.1           | 12.4           | 187                          | 10      |
|                                | 23            | $\frac{1.7}{2.0}$ | 0.0        | 5.0            | 2.0            | 35                           | 10      | 2.3          | 1.5   | 15.4           | 9.5            | 99                           | 10      |
|                                | 4             | 2.1               | 0.1        | 6.0            | 2.0            | 52                           | 10      | 3.3          | 1.9   | 17.6           | 11.5           | 162                          | 10      |
|                                | 5、            | 1.2               | 0.1        | 0.7            | 2.3            | 9                            | 10      | 1.4          | 0.7   | 9.7            | 10.4           | 131                          | 10      |
|                                | . 6           | 1.8               | 0.4        | 8.3            | 2.9            | 298                          | 10      | 2.4          | 1.3   | 16.6           | 9.9            | 381                          | 10      |
| South-central                  | 1             | 1.0               | 12         | 11 /           | 0 2            | 65                           | 0       | 1 1          | 3.6   | 22.9           | 18.6           | 843                          | 9       |
| Indiana 05                     | 2             | 2.6               | 1.5        | 63             | 5.2            | 178                          | 10      | 3.8          | 3.6   | 15.5           | 14.9           | 1002                         | 10      |
|                                | 3             | 2.8               | 1.5        | 15.8           | 5.7            | 220                          | 10      | 3.8          | 2.8   | 17.4           | 10.5           | 599                          | 10      |
|                                | 4             | 2.6               | 1.3        | 8.1            | 5.6            | 223                          | 10      | 3.3          | 2.8   | 15.0           | 12.4           | 672                          | 7       |
|                                | 5             | 2.0               | 0.8        | 11.5           | 6.2            | 141                          | 10      | 3.3          | 2.2   | 22.9           | 15.8           | 258                          | 4       |
| Cantral                        | 0             | 1.9               | 0.1        | 8.0            | 3.0            | 297                          | 10      | 2.0          | 1.4   | 15.4           | 0.0            | 750                          | 10      |
| Kansas 20                      | 1             | 1.3               | 0.2        | 9.5            | 2.5            | 201                          | 10      | 2.0          | 1.1   | 15.5           | 8.5            | 1120                         | 10      |
|                                | 2 .           | 2.3               | 1.0        | 7.6            | 4.4            | 136                          | 10      | 5.0          | 3.6   | 18.8           | 13.3           | 754                          | 10      |
|                                | 3             | 1.3               | 0.3        | 21.3           | 4.9            | 326                          | 10      | 3.0          | 2.7   | 13.7           | 11.4           | 1172                         | 10      |
|                                | 4             | 2.0               | 1.1        | 8.7            | 4.5            | 284                          | 10      | 5.3          | 3.8   | 17.3           | 12.0           | 1138                         | 10      |
|                                | 5             | 1.4               | 0.5        | 5.8<br>8.4     | 5.0<br>4 4     | 220<br>67                    | 10      | 2.1          | 21    | 20.4           | 20.3           | 644                          | 10      |
| Northeastern                   | 0             | 1.0               | 0.5        | 0.4            |                | 07                           | 10      | 5.1          | 2.1   | 2011           |                |                              |         |
| Pennsylvania 11                | 1             | 4.6               | 2.8        | 7.4            | 5.3            | 199                          | 10      | 6.7          | 6.0   | 12.7           | 11.8           | 617                          | 10      |
|                                | 2             | 1.5               | 0.2        | 5.0            | 2.6            | 234                          | 6       | 3.2          | 1.3   | 9.7            | 6.1            | 552                          | 6<br>10 |
|                                | 3             | 2.1               | 0.9        | 14.3           | 6.4<br>5 3     | 279<br>QQ                    | 10      | 4.4          | 3.2   | 10.4           | 14.0           | 286                          | 10      |
|                                | 5             | 0.6               | 0.0        | 8.5            | 3.0            | 239                          | 10      | 1.3          | 0.6   | 19.3           | 11.0           | 252                          | 10      |
|                                | 6             | 3.4               | 1.6        | 6.8            | 3.8            | 44                           | 9       | 5.1          | 3.6   | 12.8           | 9.5            | 153                          | 10      |
| Central                        |               |                   |            |                |                |                              |         |              |       | 54.0           | 14.1           | 1161                         | 10      |
| Iowa 06                        | 1             | 1.9               | 1.0        | 7.8            | 4.8            | 428                          | 10      | 2.6          | 2.4   | 51.8           | 40.4           | 1404                         | 10      |
|                                | 2             | 1.0               | 0.3        | 8.U<br>0.0     | 5.0<br>5.5     | 303                          | 10      | 3.2<br>2 7   | 1.7   | 25.4           | 14.6           | 991                          | 10      |
|                                | 4             | 2.0               | 0.7        | 9.9            | 5.1            | 176                          | 10      | 4.1          | 2.6   | 17.8           | 11.0           | 969                          | 9       |
|                                | 5             | 2.2               | 1.0        | 24.8           | 11.0           | 459                          | 10      | 2.6          | 2.3   | 33.9           | 26.0           | 1513                         | 10      |
|                                | 6             | 2.6               | 1.5        | 8.7            | 6.9            | 348                          | 10      | 3.2          | 2.6   | 21.2           | 17.5           | 1094                         | 10      |
| South-central                  | 1             | 2.2               | 1 1        | 16 0           | 0.0            | 247                          | 10      | 12           | 3.0   | 11 0           | 28 7           | 917                          | 10      |
| New FORK 1/                    | 1<br>2        | 2.2<br>1.8        | 1.1<br>0 0 | 6.1            | 9.0<br>4.6     | 152                          | 10      | 3.7          | 3.0   | 18.1           | 15.8           | 514                          | 10      |
|                                | 3             | 2.1               | 1.0        | 15.6           | 9.0            | 206                          | 10      | 3.0          | 2.1   | 44.0           | 28.9           | 742                          | 10      |
|                                | 4             | $2.\bar{1}$       | 0.8        | 6.2            | 4.0            | 127                          | 10      | 2.9          | 1.8   | 22.3           | 17.0           | 674                          | 10      |
|                                | 5             | 2.0               | 1.0        | 21.0           | 11.1           | 204                          | 10      | 2.4          | 1.5   | 62.0           | 39.2           | 622                          | 10      |
|                                | 6             | 1.9               | 0.6        | 23.5           | 10.5           | 325                          | 10      | 2.9          | 1.5   | 32.1           | 14.7           | 780                          | 10      |

(continued)

| Table 2. | (continued) |
|----------|-------------|
|----------|-------------|

|                  |       |                   | Month 1 |         |            |            | Month 2 |            |       |         |        |             |      |
|------------------|-------|-------------------|---------|---------|------------|------------|---------|------------|-------|---------|--------|-------------|------|
|                  |       |                   | Shoots  | s >5 mm | Shoot      | Callus     |         |            | Shoot | s >5 mm | Shoot  | Callus      |      |
| Provenance       | Clone | Shoot             |         | Length  | length     | volume     |         | Shoot      |       | Length  | length | volume      |      |
| (south to north) | no.   | no.               | No.     | (mm)    | · (mm)     | (mm³)      | n       | no.        | No.   | (mm)    | (mm)   | (mm³)       | n    |
| South-central    |       |                   |         |         |            |            |         |            |       |         |        |             |      |
| Wisconsin 08     | 1     | 1.9               | 1.3     | 10.2    | 8.1        | 298        | 10      | 3.6        | 2.3   | 23.5    | 17.3   | 904         | 10   |
|                  | 2     | 2.3               | 1.3     | 8.5     | 5.7        | 236        | 10      | 4.3        | 3.6   | 19.0    | 14.9   | 707         | 7    |
|                  | 3     | 1.0               | 0.2     | 1.5     | 1.6        | 20         | 10      | 1.6        | 0.8   | 10.7    | 7.6    | 15          | 10   |
|                  | 4     | 0.9               | 0.3     | 11.7    | 3.2        | 163        | 10      | 1.9        | 0.8   | 32.3    | 12.6   | 386         | 10   |
| •                | 5     | 1.7               | 0.6     | 21.5    | 7.4        | 674        | 10      | 2.5        | 1.8   | 41.0    | 28.8   | 2295        | 10   |
| ~ .              | 6     | 1.5               | 0.3     | 10.0    | 3.8        | 88.2       | 10      | 1.6        | 1.2   | 23.1    | 15.0   | 435         | 10   |
| Central          |       |                   |         |         |            |            |         |            |       |         |        |             |      |
| New Hampshire 15 | 1     | 1.9               | 0.8     | 10.0    | 5.6        | 128        | 10      | 2.3        | 1.5   | 34.3    | 22.6   | 163         | 10   |
| ۰.               | 2     | 3.4<br>5 1        | 2.3     | 22.0    | 0.9        | 307        | 10      | 4.9        | 3.6   | 32.5    | 21.4   | 1894        | 10   |
|                  | 3     | 5.1               | 3.7     | 13.6    | 9.6        | 481        | 10      | 5.4        | 5.2   | 26.8    | 26.2   | 1145        | 10   |
| `                | 4     | 2.3               | 1.2     | 14.1    | 8.0<br>7.2 | 352        | 10      | 2.9        | 1.9   | 25.4    | 19.2   | 630<br>1060 | 10   |
| •                | 5     | 4.0               | 2.2     | 13.4    | 1.5        | 234<br>107 | 10      | 1.4        | 0.2   | 24.0    | 21.8   | 700         | 10   |
| South-central    | 0     | 4.5               | 2.0     | /.4     | 5.2        | 197        | 10      | 4./        | 5.5   | 19.2    | 12.0   | /00         | 10   |
| Ontario 12       | 1     | 5 8               | 43      | 9.0     | 74         | 370        | 10      | 64         | 57    | 22.0    | 20 7   | 765         | 10   |
|                  | 2     | 1.8               | 0.8     | 9.1     | 5.8        | 309        | 10      | 33         | 2.0   | 12.9    | 84     | 023         | 10   |
|                  | 3     | 1.0               | 13      | 30.8    | 3.8        | 287        | 10      | 34         | 1.0   | 54 2    | 34 7   | 423         | Q 10 |
|                  | 4     | 2.6               | 1.8     | 16.2    | 13.1       | 313        | 10      |            |       |         |        |             |      |
|                  | 5     | 2.5               | 1.5     | 10.6    | 7.3        | 288        | 10      | 3.6        | 3.3   | 28.9    | 20.2   | 980         | 10   |
|                  | 6     | 1.1               | 0.5     | 18.4    | 6.5        | 317        | 10      | 1.8        | 1.4   | 32.6    | 16.4   | 738         | 10   |
| Northwestern     |       |                   |         |         |            |            |         |            |       |         |        |             |      |
| Vermont 16       | 1     | 1.4               | 1.0     | 23.8    | 15.3       | 584        | 10      | 3.6        | 2.2   | 44.1    | 22.3   | 1618        | 10   |
|                  | 2     | 1.1               | 0.9     | 10.4    | 4.7        | 258        | 10      | 3.0        | 2.1   | 33.3    | 18.4   | 809         | 10   |
|                  | 3     | 2.0               | 1.2     | 11.6    | 8.4        | 262        | 10      | 3.2        | 1.7   | 16.8    | 8.9    | 616         | 10   |
|                  | 4.    | 0.2               | 0.1     | 8.0     | 0.6        | 308        | 10      | 0.7        | 0.4   | 20.0    | 2.6    | 1450        | 10   |
|                  | • 5   | 1.9               | 0.9     | 9.8     | 6.1        | 557        | 10      | 3.9        | 2.9   | 32.3    | 20.8   | 1256        | 10   |
| <b>T</b>         | 6     | 1.4               | 0.7     | 9.8     | 6.5        | 601        | 10      | 2.0        | 1.1   | 36.4    | 24.4   | 1292        | 10   |
| East-central     | 1     | 1.0               | 0.0     | 0.4     | 7.0        | E E A      | 10      | 4.1        |       | 10.4    | 0.2    | 0/74        | 10   |
| Minnesota 19     | 1     | 1.9               | 0.9     | 9.4     | 7.2        | 254        | 10      | 4.1        | 2.3   | 12.4    | 9.3    | 20/4        | 10   |
|                  | 23    | 1.0               | 1.0     | 21.0    | 173        | 200<br>532 | 10      | 2.0<br>3.5 | 3.2   | 19.3    | 15.4   | 405         | 10   |
|                  | 4     | $\frac{2.0}{2.0}$ | 1.7     | 11 3    | 55         | 285        | 10      | 40         | 3.2   | 10 0    | 15 1   | 800         | 10   |
|                  | 5     | 1.8               | 1.0     | 12.6    | 87         | 891        | 10      | 2.0        | 12    | 49.9    | 28.6   | 1846        | 10   |
| 98 - F           | 6.    | 2.7               | 1.5     | 11.2    | 7.0        | 352        | 10      | 4.9        | 3.9   | 22.8    | 15.7   | 1365        | 10   |
| Central          | Ū     |                   | 1.0     |         |            | 202        | 10      |            | 0.0   |         | 10.7   | 1000        | 10   |
| Ontario 13       | 1 .   | 2.0               | 0.8     | 13.7    | 5.7        | 272        | 10      | 3.8        | 3.2   | 25.0    | 18.6   | 916         | 10   |
|                  | 2     | 1.2               | 0.6     | 9.8     | 3.8        | 444        | 10      | 2.5        | 1.9   | 9.6     | 7.1    | 1068        | 10   |
|                  | 3     | 3.0               | 2.0     | 11.0    | 7.7        | 342        | 10      | 3.6        | 2.9   | 28.3    | 18.0   | 916         | 10   |
|                  | 4     | 2.1               | 0.7     | 9.8     | 4.5        | 296        | 10      | 3.7        | 2.5   | 20.6    | 12.1   | 766         | 10   |
|                  | 5     | 1.5               | 0.4     | 12.8    | 5.3        | 347        | 10      | 2.0        | 1.3   | 25.5    | 18.7   | 593         | 10   |
|                  | 6     | 2.4               | 1.0     | 9.2     | 5.1        | 312        | 10      | 3.4        | 2.6   | 16.1    | 14.7   | 941         | 10   |
| Significance     |       | **                | **      | * *     | **         | **         |         | * *        | **    | * *     | * *    | **          |      |
| lsd 5%           |       | 1.0               | 0.9     | 7.7     | 5.2        | 165.6      |         | 1.6        | 1.5   | 13.3    | 9.7    | 474.4       |      |
| LSD 1%-          |       | 1.3               | 1.2     | 10.1    | 6.8        | 217.8      |         | 2.1        | 1.9   | 17.5    | 12.8   | 624.0       |      |

Explants were cultured on DKW medium with 10 nM TDZ.

\*\*Significant effect at P = 0.01 according to F test.

each provenance; however, we did not obtain 100% aseptic cultures for all six clones from any provenance. Five of the six clones from south-central New York resulted in 100% aseptic establishment; however, none of the explants from the other New York clone survived.

All single nodes from greenhouse-grown stock plants of the selected clones performed well in vitro (Table 2). Callus volume ranged from 12 to 674 mm<sup>3</sup> and 99 to 2674 mm<sup>3</sup> after 1 and 2 months, respectively, in vitro. We reported that there was no apparent relationship between callus formation and shoot proliferation from silver maple explants exposed to various doses of TDZ (Preece et al., 1991). Likewise, there was no significant correlation between callus and total shoot number of these selected clones after 1 month in vitro, and although this correlation was significant after 2 months, the low correlation coefficient indicates a weak relationship (Table 3). There were significant positive but low correlations between callus formation and shoot length and number of shoots >5 mm long. The additional callus may have aided in absorption of nutrients and other materials from the medium.

After 1 month in vitro, each single-node explant had at least one axillary shoot (Table 2). Although some were as short as 1 mm, explants from six of the 90 clones had a mean shoot length >1 cm. After 1 month, the explants of 40 of the 90 clones had one or more axillary shoots >5 mm long. When only these shoots were considered, many cultures had mean shoot lengths

Table 3. Correlations among in vitro growth characteristics from single-node explants after 1 and 2 months, across 84 silver maple clones.<sup>2</sup>

|   |              | Month               | 1                    |  | Month 2      |                     |                                  |                                  |  |  |
|---|--------------|---------------------|----------------------|--|--------------|---------------------|----------------------------------|----------------------------------|--|--|
| Characteristic                                | Shoot<br>no. | No. shoots<br>>5 mm | Shoot<br>length      | Callus<br>volume                             | Shoot<br>no. | No. shoots<br>>5 mm | Shoot<br>length                  | Callus<br>volume                 |  |  |
| Shoot no.<br>No. shoots >5 mm<br>Shoot length |              | 0.7538**            | 0.1093**<br>0.3366** | 0.0391 <sup>NS</sup><br>0.1484**<br>0.2739** |              | 0.8749**            | 0.0511 <sup>NS</sup><br>0.1944** | 0.1047**<br>0.1368**<br>0.1919** |  |  |
| shoots >5 mm                                  | -0.1700**    | -0.1572**           | 0.8712**             | 0.2179**                                     | -0.1304**    | -0.1917**           | 0.8137**                         | 0.1542**                         |  |  |

Pearson correlation coefficients (R values). \*\*, <sup>SS</sup>Significant Correlation with probability > [R] under HO: $R_{HO}$ = 0/N = 66 at P = 0.01 or nonsignificant, respectively.

Table 4. Correlations between phonological variables of silver maple seedlings grown in a nursery at 15  $\times$  15-cm spacings and growth from single-node explants after 2 months in vitro.<sup>\*</sup>

| Year | Condition<br>in nursery      | No.<br>shoots      | No.<br>shoots       | Mean<br>length     | Shoot<br>length    | Callus<br>volume    |
|------|------------------------------|--------------------|---------------------|--------------------|--------------------|---------------------|
| 1987 | Height                       | 0.01 <sup>NS</sup> | -0.01 <sup>NS</sup> | 0.37**             | 0.29*              | 0.43**              |
|      | Caliper                      | $-0.02^{NS}$       | $-0.03^{NS}$        | 0.29*              | 0.24 <sup>NS</sup> | 0.36**              |
| 1988 | Height                       | 0.03 <sup>NS</sup> | -0.03 <sup>NS</sup> | 0.11 <sup>NS</sup> | 0.07 <sup>NS</sup> | -0.04 <sup>NS</sup> |
|      | Caliper                      | 0.03 <sup>NS</sup> | $-0.14^{NS}$        | $-0.05^{NS}$       | $-0.14^{NS}$       | $-0.21^{NS}$        |
| 1987 | Terminal bud sety            | 0.16**             | 0.14**              | 0.08 <sup>NS</sup> | 0.02 <sup>NS</sup> | -0.01 <sup>NS</sup> |
|      | Leaf abscission <sup>x</sup> | 0.13**             | 0.10**              | 0.10 <sup>NS</sup> | $-0.01^{NS}$       | -0.17**             |
| 1988 | Terminal bud sety            | -0.13**            | -0.16**             | $-0.01^{NS}$       | -0.09*             | -0.28**             |
|      | Leaf abscission <sup>x</sup> | 0.02 <sup>NS</sup> | 0.26**              | $-0.01^{NS}$       | $-0.02^{NS}$       | -0.16**             |
|      | Bud flush <sup>w</sup>       | $-0.07^{NS}$       | 0.10 <sup>NS</sup>  | $-0.07^{NS}$       | $-0.03^{NS}$       | $-0.01^{NS}$        |

<sup>z</sup>Pearson correlation coefficients (R values).

Terminal bud set was calculated as the number of days from 1 Oct. until 75% of the terminal buds per plot were set.

\*Leaf abscission was calculated as the number of days from 1 Oct. until 75% of the leaves were off the trees in each plot.

"Bud flush was calculated as the number of days from 1 Apr. 1988 until 75% of the provenance's buds were at least 3 cm long.

\*,\*\*,<sup>NS</sup> significant correlation with probability > [R] under HO:R<sub>HO</sub> = 0/N = 66 at P = 0.05or 0.01, or nonsignificant, respectively.

between 1 and 2 cm. This "rapid, successful explant establishment lessens the time needed to obtain proliferating shoot cultures and to meet production deadlines.

Explants of most clones had two or more shoots after 2 months in vitro, and 46 clones had at least three axillary shoots. The range was as high as a mean of 7.4 shoots for a New Hampshire clone, six of which were > 5 mm long. There were good correlations between total shoot number and number of shoots > 5mm long after 1 and 2 months in vitro (Table 3). Therefore, if axillary shoot outgrowth began, it tended to progress rapidly.

Microshoots have been continuously harvested from these cultures for rooting. Initially, based on excellent rooting results under intermittent mist (Preece et al., 1991), microshoots were placed into a 1 perlite : 1 vermiculite (v/v) medium. Because of uneven coverage by the mist system, a 10-m bench full of microcuttings contained areas where rooting was extremely poor and many shoots died. Conversely, microcuttings in other locations rooted well. Because of this spotty rooting, which was not related to clone, subsequent root initiation in vitro has been on DKW medium with 1 µM NAA. Rooting of all clones was =80% with this system (data not presented).

Once microcuttings rooted, they were transplanted into RootMaster six-chamber multiple containers with a 2 sphagnum peat : 1 perlite : 1 vermiculite (by volume) (Premix BX; Premier Brands, New

Rochelle, N.Y.) medium. They were placed under intermittent mist in a shaded greenhouse for the first week to begin acclimatization. The plantlets were moved to a sunlit bench while remaining under mist for another week. They were then moved to a shaded bench without mist for 1 week, and then finally moved to fully sunlit benches until a height of 30 cm was reached (=2 months after the plantlets were removed from the laboratory). The entire acclimization and growing-on stages were conducted under natural photoperiods supplemented with a night interruption with cool-white fluorescent lamps from 2200 to 0200 HR.

We were interested in learning if there was a relationship between nursery performance of the populations from which the clones were selected and in vitro growth of the single-node explants. If such relationships existed, micropropagation of silver maple might be a useful method for rapidly screening genotypes before making selections for field testing. Although there were statistically significant positive correlations between shoot number, shoot length, callus volume, and several conditions measured in the nursery (Table 4), the low correlation coefficients indicate that micropropagation results with silver maple would not be an appropriate selection system for speed of growth of phenotypes. The best correlations were between callus volume and 1987 height and caliper; however, this relationship was not significant for 1988.

Table 5. Correlations between the climatic conditions of the provenance and growth from single-node explants after 2 months in vitro.<sup>\*</sup>

|                                       |                     | Shoots           | >5 mm          |                 |                  |
|---------------------------------------|---------------------|------------------|----------------|-----------------|------------------|
| Climatic condition <sup>y</sup>       | No.<br>shoots       | No.<br>shoots    | Mean<br>length | Shoot<br>length | Callus<br>volume |
| Mean annual<br>temperature            | -0.11**             | <i>-</i> →0.13** | -0.11**        | -0.11**         | -0.23**          |
| Mean January<br>temperature           | -0.07 <sup>NS</sup> | -0.09*           | -0.10*         | -0.11**         | -0.29**          |
| Mean July<br>temperature              | -0.17**             | -0.19**          | -0.09*         | -0.10**         | -0.15**          |
| Number<br>frost-free days             | -0.15**             | -0.17**          | -0.18**        | -0.16**         | -0.27**          |
| Mean annual precipitation             | -0.05 <sup>NS</sup> | -0.08*           | -0.18**        | -0.16**         | -0.32**          |
| Nursery planting<br>date <sup>x</sup> | 0.24**              | 0.25**           | 0.128**        | 0.10*           | 0.17**           |

Pearson correlation coefficients (R values).

<sup>9</sup>Mean temperatures, frost-free days, and annual precipitation are for the sites of provenance origin.

\*Seeds were sown in the nursery during May and June 1987.

\*,\*\*,<sup>NS</sup>Significant correlation with probability > [R] under HO: $R_{H0} = 0/N = 66$  at

P = 0.05 or 0.01, or nonsignificant, respectively.

We were also interested in correlations between micropropagation results and climatic conditions of the provenance. If such relationships existed, micropropagation might be useful in determining origin of unknown seedlings. With silver maple, this might be useful since the tree has Been transported thousands of kilometers by humans for use in landscapes. Knowledge of origin might facilitate a better understanding of general tree performance, including cold hardiness. Except for date of planting, there were significant negative correlations between micropropagation results and climatic conditions of the area where the seeds originated (Table 5). The low correlation coefficients indicate, however, that these relationships were not strong, and micropropagation results were not very good predictors of the location of seed origin. Date of planting also was included as a climatic condition. The seeds were collected from the various locations as they reached maturity, which generally followed a south-to-north latitude gradient. They were planted immediately in the nursery because of the short period of silver maple seed viability. The best correlation was between callus volume and mean annual precipitation. The negative correlation indicates that there was a tendency for explants from provenances with less rainfall to produce more callus.

The excellent micro- and macropropagation responses of the wide assortment of silver maple genotypes in this propagation system is encouraging. The lack of strong correlations between growth characteristics and origin of the stock plants and in vitro results indicates that micropropagation has wide-ranging implications for clonal propagation of a variety of silver maple genotypes. This rate of success means that new propagation protocols do not need to be developed for various silver maple clones, thus simplifying propagation and facilitating the production of many clonal trees for biomass studies.

#### Literature Cited

Ashby, W. C., J.E. Preece, C.A. Huetteman, D.F. Bresnan, and P.L. Roth. 1987. Silver maple tree improvement for biomass production. Proc. 5th North Central Tree Imp. Conf. p. 6-23.

- Driver, J.A. and A.H. Kuniyuki. 1984. In vitro propagation of paradox walnut rootstock. HortScience 19:507-509.
- Ellis, D.D. and B.H. McCown. 1988. The potential of biotechnology in the biomass production program. Oak Ridge National Laboratory, Oak Ridge, Term. ORNL/M-606, Environ. Sci. Div. Pub]. 3172.
- Fowells, H.A. 1965. Silvics of forest trees of the United States. U.S. Dept. Agr. For. Serv. Agr. Hdbk. 271.
- Haldeman, J. H., R.L. Thomas, and D.L. McKamy. 1987. Use of benomyl and rifampicin for in vitro shoot tip culture of *Camellis* sinensis and C. japonica. HortScience 22(2):306-307.
- Hartmann, H.T. and D.E. Kester. 1983. Plant propagation: Principles and practices. 4th ed. Prentice-Hall, Englewood Cliffs, N.J.
- Kling, G.J. and M.M. Meyer, Jr. 1983. Effects of phenolic compounds and indoleacetic acid on adventitious root initiation in cuttings of *Phaseolus aureus, Acer saccharinum,* and *Acer griseum*. Hort-Science 18(3):352-354.
- Kopp, R. K., W.A. Geyer, and W.R. Lovett. 1988. Silver maple seed sources for increased biomass production. Northern J. Applied For. 5:180-184.
- Larsson, H.C. 1968. Some methods of selecting and propagating asexually high quality phenotypes of silver maple (*Acer saccharinum* L.), p. 75-84. In: E.J. Schreiner (cd.). Proc. 15th Northeastern For. Tree Imp. Conf.
- Libby, W.J. 1983. The clonal option. Norwegian For. Res. Inst., Ås, Norway.
- Meridian Corporation. (@ 1986). Short-rotation intensive culture of woody crops for energy: Principles and practices for the Great Lakes Region. Falls Church, Va.
- Preece, J. E., C.A. Huetteman, W.C. Ashby, and P.L. Roth. 1991. Micro- and cutting propagation of silver maple. I. Results with adult and juvenile propagules. J. Amer. Soc. Hort. Sci. 116:142-148.
- Ranney, J. W., A.R. Ehrenshaft, P.A. Layton, W.A. McNabb, and L.L. Wright. 1988. Short rotation woody crops program annual progress report for 1987. Oak Ridge National Laboratory, Oak Ridge, Term. 0RNL-6440, Environ. Sci. Div. Publ. 3030.
- Ranney, J. W., L.L. Wright, and P.A. Layton. 1987. Hardwood energy crops: The technology of intensive culture. J. For. 85:17-28.
- Roth, W. B., I.M. Cull, R.A. Buchanan, and M.O. Bagby. 1982. Whole plants as renewable energy resources: Checklist of 508 species analyzed for hydrocarbon, oil, polyphenol, and protein. Trans. Illinois State Acad. Sci. 75:217–231.