ARRESTING PLANT MATURATION TO MAINTAIN HIGH PROPAGATION SUCCESS WITH AMERICAN SYCAMORE CUTTINGS

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Abstract.--Loss of rooting potential with maturation in sycamore limits clonal propagation of selected clones by conventional cuttings. By the time the clones can be identified in progeny tests, they have already lost much of their juvenility (and thus the rooting ability of their cuttings has declined). Data from four studies conducted during 1991-95 are presented to show how serial propagation can arrest the maturation process, thereby maintaining high propagation success until progeny tests are completed. Three-month survival and sprout growth in an on-going field trial of cuttings from different serial stages of propagation of the same clones are given.

Keywords: *Platanus occidentalis* L., vegetative propagation, serial propagation

INTRODUCTION

Clonal plantation forestry has a genetic advantage over seedling plantations by being able to utilize all of the superiority of a selected genotype, rather than just the additive component. Conventional unrooted hardwood cuttings, stecklings (rooted cuttings), or *in* vitro-derived propagules have been used, but conventional cuttings may be more cost competitive with seedling planting stock. The problem for forest geneticists is that rooting ability of vegetative propagules from many woody plant species declines with age of the seedling-derived mother plant (ortet) (Hackett 1988). By the time seedlings have been progeny tested and selected, they may have lost much of their potential to root. As a result, they cannot be clonally propagated with commercially acceptable efficiency.

Several methods have been successfully used with some species to arrest or reverse (rejuvenate) the maturation process in order to obtain large numbers of cuttings with high rooting potential. These include (a) severe pruning or hedging, (b) serial grafting of mature scions on juvenile rootstocks, (c) *in vitro* rejuvenation by serial subculturing of mature explants, (d) use of cuttings from adventitious origin, and (e) recovery of existing juvenile material and placement in stock block plantings with serial propagation (Hackett 1988).

American sycamore (*Platanus occidentalis* L.) will root easily from conventional cuttings taken from seedlings (Land 1983), but cuttings from mature woody tissue of this species are difficult to root (Hare and Land 1982). There is some evidence that severe hedging may partially rejuvenate cuttings from sprouts on mature grafts (Land et *al.* 1995). The objectives of the studies reported in the present paper were to determine if serial propagation [similar to method (e) above] can be used to (a) rejuvenate mature clones and (b) arrest

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the maturation of juvenile material, thereby maintaining high propagation success until progeny tests are completed.

METHODS

1991 Nursery Cutting Study

Cuttings were collected from one-year-old sprouts on eight-year-old hedged trees for a 1988 Nursery Cutting Study (Land *et al.* 1995), and the resulting stecklings were planted in a field test at Mississippi State University (MSU) on December 19, 1988. Some of the two-year-old stecklings were detopped (coppiced) to a 15-cm stump height on April 10, 1990.

Three rootstock types and six of the clones from the 1988 study were used as sources of 25-cm (10-inch) cuttings for the 1991 Nursery Cutting Study. These cuttings were planted in 2.8-liter pots in an irrigated nursery at MSU on March 20, 1991. The three rootstock types were (a) one-year-old sprouts on the three-year-old stecklings that had been coppiced in 1990; (b) limbs from the upper crowns of other three-year-old stecklings that had not been coppiced; and (c) three-year-old sprouts on the 11-year-old ortets that had originally provided the stecklings. The six clones came from six seedlings of Mississippi origin that had been obtained from the state nursery and planted for grafting rootstock at MSU. Since the same clones were used in all three rootstock types, the genetic makeup of the three types was the same.

The 25-cm cuttings were prepared by making the basal-end cut at 1.2 cm (one-half inch) below a bud node, were selected to have at least two nodes within the lower 20 cm of the cutting, and were chosen to have a basal diameter between 16 and 21 mm [based on results of Land *et al.* (1995)]. These cuttings were basally dipped for three seconds in 50% ETOH followed by a dip in 5% Captan powder, and they were planted with only the top 2.5 cm remaining above ground.

The experimental design in the nursery consisted of randomized complete blocks (RCB) with four replications. The three rootstock types served as treatments. A rootstock treatment plot within a replication contained 12 cuttings (two cuttings from each of the six clones). Measurements for number, height, and diameter of sprouts per cutting were taken monthly from May through July, again in September, and finally on November 23, 1991. Analyses of variance were conducted on a plot-mean basis to test for differences among rootstock types in percent sprouted cuttings (percent survival) and sprout height.

1993 Nursery Cutting Study

Twelve clones, representing two seedling ortets from each of six progeny families, were selected from a two-year-old (from seed) progeny test at MSU. Cuttings were taken from the lower limbs of the seedlings and cut into 25-cm lengths such that the first (lowest) bud node was within two cm above the basal cut. Basal diameter of cutting, height above ground of the limb/stem junction for the limb where the cutting was collected, length from basal end of cutting to second node up the cutting, and number of nodes per 25-cm cutting were measured at time of collection on March 1, 1993.

The cuttings were planted on March 1 in a MSU nursery containing six rows spaced 90 cm (3 feet) apart, with rows raised to an average height of 30 cm above the furrows. Spacing between cuttings within rows was 30 cm, and overhead irrigation was provided daily. The nursery experimental design was a RCB with four replications and 12 cuttings (one per clone) in each replication. Replications ran across rows, so each replication consisted of two cuttings per row on each of the six rows. Measurements of number and length of sprouts per cutting were taken on May 1 and June 16, 1993, and sprout diameter was added to these measurements on March 10, 1994. The cutting measurements taken at time of collection were subdivided into classes and examined for effects on percent propagation success for the March 10 data.

1994 Nursery Cutting Study

Four origins of cuttings and 24 clones were used as sources of 25-cm cuttings, which were planted on March 10-14, 1994, in the same MSU nursery as the 1993 study. The four origins were: (a) one-year sprouts from the rootstocks in the 1993 study [12 clones]; (b) lower limbs (lower 1/3 of crown) of the same ortets that were used for the 1993 study, but ortets were now three years old [same 12 clones as for (a)]; (c) lower limbs (lower 1/3 of crown) of 12 new selections in the three-year-old seedling progeny test [12 different clones from (a) and (b)]; and (d) upper limbs (upper 1/3 of crown) of the 12 new selections [same 12 clones as for (c)]. Cuttings were prepared, as in the previous studies, to have the first node within two cm above the basal cut, and basal diameters between 11 and 19 mm were sought. Cuttings were measured for diameter, distance from root collar of ortet to base of cutting, and number of nodes per cutting.

Cuttings were planted on the day of collection to the same number of rows and at the same spacing as used for the 1993 study. A RCB design was used with five replications, four treatments (origins), and 12 cuttings per treatment plot (one cutting per clone). Number of sprouts per cutting, height of tallest sprout, and cumulative length of all sprouts per cutting were measured on June 22, 1994, and February 10-21, 1995. Diameter of the largest sprout was also measured on the February date. Regression analysis was used to evaluate the effect of distance of cutting origin from root collar on three-month propagation success. Analysis of variance on an origin plot-mean basis was used to test differences among the four origin treatments.

1995 Cutting Field Trial

Three of the 12 clones used in both the 1993 and 1994 studies, and four rootstock (RS) treatments, were used as sources of cuttings planted to a non-irrigated field site at MSU on March 7, 1995. One RS treatment (TRT#1) provided cuttings from one-year-old sprouts on the two-year-old rootstocks of the 1993 nursery. These rootstocks had originally been taken from two-year-old seedling ortets. The second RS treatment (TRT#2) utilized cuttings from one-year-old sprouts on the one-year-old rootstocks in the 1994 nursery study. These rootstocks had come from one-year sprouts on the one-year-old rootstocks (at that time) in the 1993 nursery. Thus, the cuttings represented material from the second cycle of serial propagation, but the original rootstocks still came from two-year-old ortets. The third RS treatment (TRT#3) was represented by cuttings from one-year-old sprouts on one-year-old rootstocks in the 1994 nursery. These rootstocks came from the same ortets as TRT#1 and TRT#2, but were collected at ortet age three. The final RS treatment (TRT#4) used the original ortet as the rootstock. Cuttings came from limbs in the live crown (upper half of tree) of the 4-year-old trees. The same three clones were represented in all rootstock treatments, so the genetic makeup of the treatments was the same.

The field site was cultivated and subsoiled during the fall of 1994. The cuttings were collected, cut into 25-cm lengths with a bud node within two cm above the basal cut, measured for basal diameter, and planted on the same day. Spacing was 2.5 m (8 feet) between rows and 0.9 m (3 feet) within rows. The study has been cultivated monthly for weed control since planting, but no irrigation has been provided.

The experimental design consisted of a RCB with four replications, 12 clone-by-RS-treatment combinations, and three cuttings per combination plot within a replication. Cuttings were measured for number and length of sprouts on May 10 (two months) and May 31, 1995 (three months after planting). Analyses of variance to evaluate RS treatment differences were conducted on a plot-mean basis.

RESULTS AND DISCUSSION

The objective of the 1991 Nursery Cutting Study was to determine if serial propagation of cuttings from mature sycamore ortets would increase propagation success (i.e. "rejuvenate" mature material). Cuttings taken from one-year-old sprouts on the coppiced stecklings did sprout and survive significantly better than cuttings from either the upper limbs of non-coppiced stecklings or the three-year-old sprouts at the top of the 11-year-old ortets (Table 1). However, data from the original 1988 Cutting Study indicated that average survival for cuttings from one-year-old sprouts on the same six ortets at age eight was 32 percent at three months and 24 percent at nine months after being planted. The 29-percent survival obtained from the sprouts on the coppiced stecklings was essentially the same, indicating that the maturation process had only been arrested rather than reversed. Woody tissue from near the ground line (root collar) of the three-year-old steckling was still at the same level of maturation as the original cutting taken from the eight-year-old ortet, even though the top limbs on both the non-coppiced stecklings and the three-year-old sprouts of the ortets had continued to advance in maturation.

Subsequent objectives of the 1993-1995 studies have been centered on questions of what ages, what positions on the plant, and how many cycles of serial propagation are best for arresting maturation and maintaining rooting ability of selections in seedling progeny tests. Also important have been questions about when propagation success can be assessed after planting and about the height-growth pattern of sprouts.

Sprouted cuttings in June, three months after planting, provide a good assessment of success, since average results from the 1991, 1993, and 1994 studies all indicated only small declines in survival and no changes in rank from this time to the end of the growing season (Table 1). Ranks for sprout heights also did not change after the June measurement, and the average June height was approximately one fourth of the height at the end of the season. Land et *al.* (1995) have shown from monthly measurements in earlier tests that percentages of sprouted cuttings after two months (early May) are much higher than the percentages after three months, so conclusions should not be made before the first of June.

Table 1. Percent survival and length of tallest sprout at three months and one complete growing season after planting cuttings in four sycamore cutting studies at Mississippi State University.[a]

Study	Cutting Source	3-Mo. after Planted Survival (%)	3-Mo. after Planted Tallest Sprout (cm)	8-12 Mo. after Pltd Survival (%)	8-12 Mo. after Pltd Tallest Sprt (cm)
[1] 1991 Nursery	[a] 3-yr sprout on 11-yr-old ortet	6 a	5.8 B	4 b	49 A
	[b] upper limb on 3-yr-old steckling	15 a	6.2 B	10 b	55 A
	[c] 1-yr sprout on coppiced 3-yr steck.	29 a	9.5 A	29 a	64 A
[2] 1993 Nursery	[a] lower limb on 2-yr-old ortet	73	24.8	68	127
[3] 1994 Nursery	[a] 1-yr sprout on 1-yr rootstock from 2-yr-old ortet	87 A	55.7 a	87 A	165 a
	[b] lower limb on 3-yr-old ortet (same ortet as [a])	60 B	32.7 b	58 B	124 b
	[c] lower limb on 3-yr-old ortet (not same as [a])	68 a	41.0 A	67 a	147 A
	[d] upper limb on 3-yr-old ortet (same ortet as [c])	38 b	30.2 B	38 b	92 B
[4] 1995 Field	[a] TRT#1: 1-yr sprout on 2-yr rootstock#1 from 2-yr-old ortet	72 A	21.9 ab	--	--
	[b] TRT#2: 1-yr sprout on 1-yr rootstock#2 from 1-yr rootstk#1 from 2-yr-old ortet	89 A	23.5 a	--	--
	[c] TRT#3: 1-yr sprout on 1-yr rootstock#1 from 3-yr-old ortet	54 B	17.7 ab	--	--
	[d] TRT#4: upper limb on 4-yr-old ortet	14 C	16.0 b	--	--

[a] Means for the same trait (column) that are in the same study are not significantly different at the 0.05 probability level according to Duncan's Test if they are followed by the same letter.

Cuttings in the 1993 study from low limbs on two-year-old seedlings had higher propagation success than cuttings from low limbs on the same ortets at age three in the 1994 study ([2a] versus [3b] in Table 1). However, sprouts on the rooted cuttings of the 1993 study retained the higher rooting ability of the two-year-old ortets ([3a] versus [3b]). Furthermore, low limbs provided cuttings with higher propagation success than upper limbs from the same trees ([3c] versus [3d]). These results probably arise from effects of maturation and distance of cutting origin from the root collar of the ortet, as discussed below.

There was a significant decline in propagation success of the limb cuttings in the 1994 study associated with the distance from the base of the cutting's position on the limb to the root collar of the ortet (Figure 1). Most cuttings

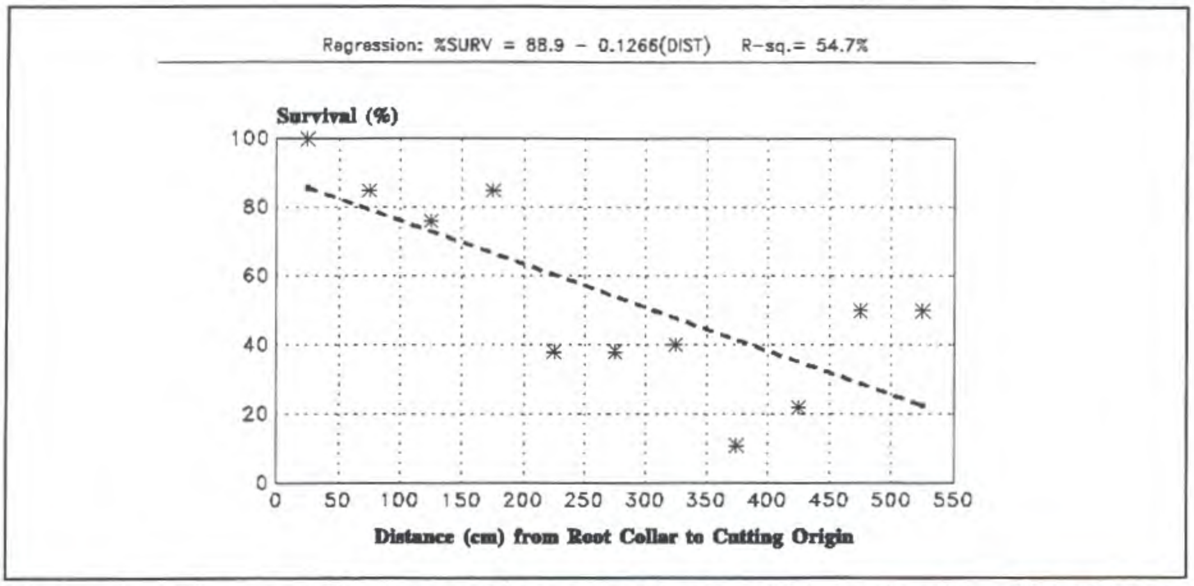

Figure 1. Three-month propagation success of cuttings in the 1994 Nursery Cutting Study from three-year-old trees as affected by distance of cutting origin from root collar.

from sprouts on the rootstocks in the 1993 study were close to the "root collar" and gave high survival with no effect of distance (Figure 2). The cuttings in the 1993 study also exhibited a decline in propagation success associated with increasing distance of source limb above root collar. Limbs originating within 40 cm of the root collar gave cuttings with 80-percent survival, while limbs from 41 to 80 cm above the root collar gave cuttings with only 55-percent success. Therefore, results for the 1994 study could be interpreted as follows. First, the better performance of treatment [3a] than [3b] in Table 1 for the same ortets arises because origins of [3a] were limbs closer to the root collar at age two than limbs of [3b] at age three. Lower limbs grew in diameter and length between ages two and three, and some died from shading. To get equivalent-sized cuttings from the two ages, collections had to be taken further from the root collar at age three, and survival declined. Serial propagation maintained the "closer-to-origin" effect of the collection at age two, thereby arresting the maturation effect associated with increased distance from root collar. Second, the better performance of [3c] than [3d] demonstrates the gradient in maturation within the plant. The lower limbs were more juvenile than the limbs from the upper crown, so that cuttings from the lower limbs were propagated with greater success.

The 1995 field trial was established to determine if cutting survival under non-irrigated field conditions would be consistent with nursery results, to examine how two cycles of serial propagation would affect cutting survival, and to evaluate performance of cuttings from the same clones when originally collected at ortet ages two, three, and four years. The three-month results in

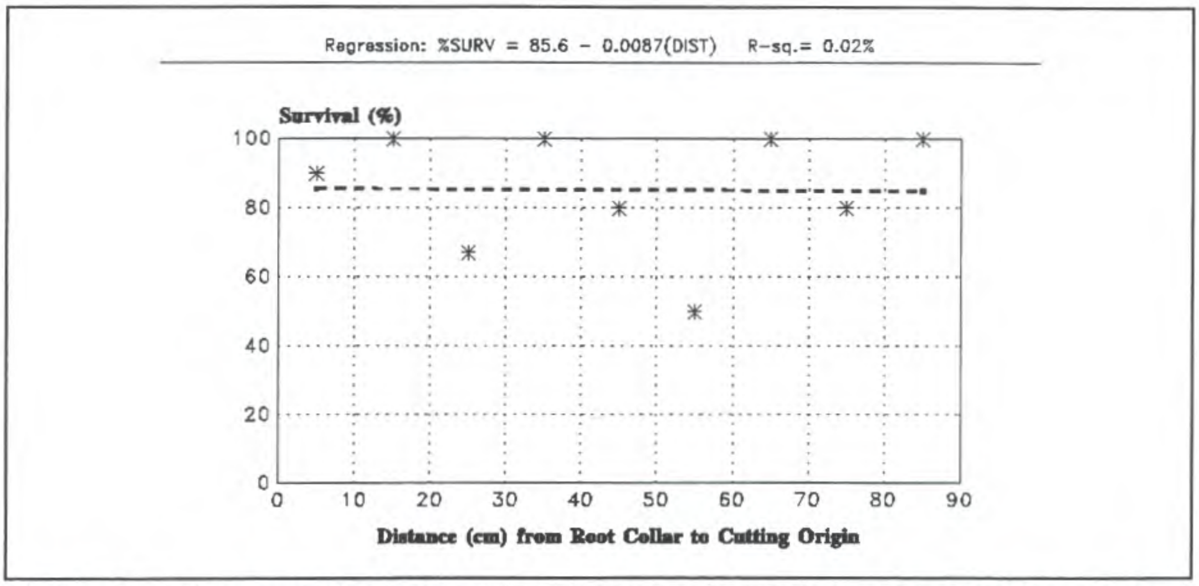

Figure 2. Three-month propagation success of cuttings from sprouts on one-year-old nursery rootstocks as affected by distance of cutting origin above the root collar.

Table 1 were reliable indicators of relative performance at the end of the growing season, as already noted for the first three studies, and survival results were consistent with nursery averages. Sprout heights were shorter than the nursery heights at three months, but this probably resulted from measurements being taken one to two weeks earlier than for the nursery studies. Cuttings from one-year-old sprouts on two-year-old rootstocks ([4a] in Table 1) did not differ significantly in propagation success from cuttings taken from one-year-old sprouts on the second serial cycle of rootstocks ([4b]), and survival was the same as obtained for one-year-old sprouts on the first serial cycle in the 1994 study ([3a]). Propagation success of cuttings from one-year-old sprouts on rootstocks taken from the three-year-old ortets [4c] was significantly poorer than for cuttings from rootstocks originating from the two-year-old ortets. This was clear evidence that serial propagation only arrested the maturation process, rather than reversing it. Thus, two serial cycles of propagation and up to two years of cutting harvest per rootstock cycle appear to maintain maturation at the stage of the original collection from the ortet. Cuttings from limbs of the four-year-old ortets [4d] had the poorest survival of all treatments. This probably resulted because lower limbs had died as crowns closed during the fourth year, so the only cutting sources were far from the root collar.

The overall effect of ortet maturation on propagation success of sycamore cuttings is summarized for all four studies in Figure 3. Sprouts maintained the maturity status of the limb source from which they were collected. Since most of the sprout-origin cuttings in the studies came from low limbs, sprouts and low limbs were combined in the figure. Also, propagation success was assumed to be 100 percent for a very young seedling (age zero). The figure illustrates (a) that rooting ability declined with increasing ortet age, (b) that the greatest decline occurred between ages two and four, when sources of cutting material (limbs) from near the root collar disappeared, and (c) that upper limbs were more mature than low limbs at the same ortet age, so that propagation success was less.

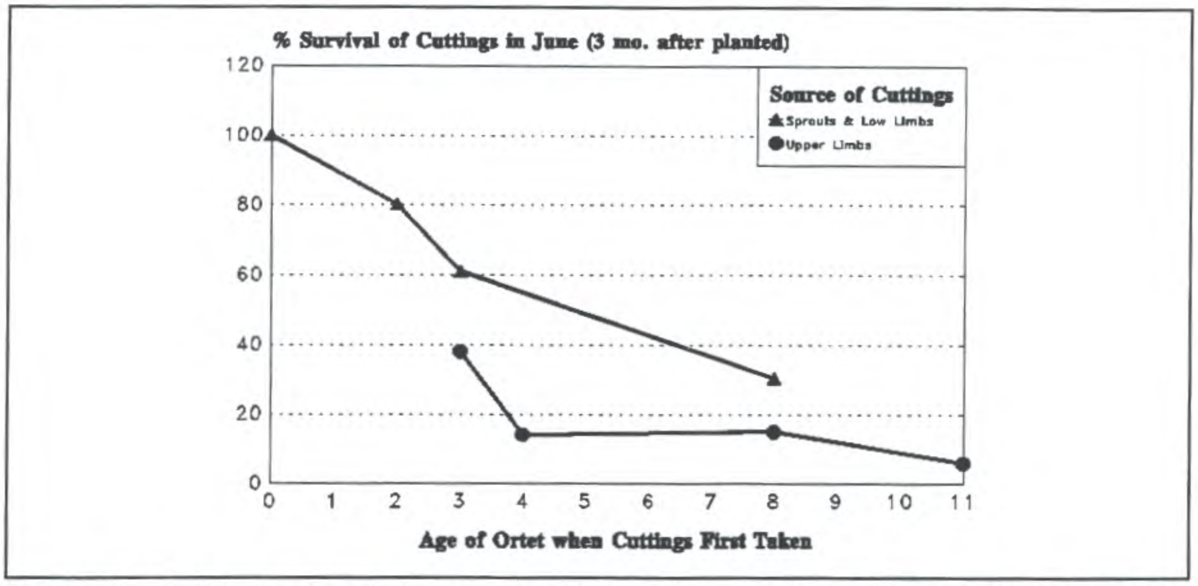

Figure 3. Effect of ortet age on propagation success of sycamore cuttings.

CONCLUSIONS

Serial propagation with cuttings arrests the maturation process of sycamore at the level of the original propagule taken from the ortet. The method does not rejuvenate the vegetative material. The maturation level varies within the ortet, being most juvenile for material (such as limbs) taken from near the root collar. As maturation increases, propagation success with conventional cuttings declines. Therefore, if it is undesirable to coppice selected individuals within progeny tests, serial propagation with cuttings from low limbs on young seedlings of no more than two years of age is required to maintain high rootability.

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LITERATURE CITED

Hackett, W.P. 1988. Donor plant maturation and adventitious root formation. pp. 11-28. In: T.D. Davis, B.E. Haissig, and N. Sankhla (eds.). Adventitious Root Formation in Cuttings. Advances in Plant Sciences Series--Volume 2. T.R. Dudley (gen.ed.). Dioscorides Press, Portland, OR.

Hare, R.C., and S.B. Land, Jr. 1982. Effect of cold storage and chemical treatment on rooting of hardwood sycamore cuttings. Can.J.For.Res. 12:417-419.

Land, S.B. Jr. 1983. Performance and G-E interactions of sycamore established from cuttings and seedlings. pp. 431-440. In: E.P. Jones (ed.). Proceedings of the Second Biennial Southern Silvicultural Research Conference, Atlanta, Georgia, November 4-5, 1982. USDA Forest Service. Gen. Tech. Rep. SE-24. Southeastern Forest Experiment Station, Asheville, NC.

Land, S.B. Jr., W.W. Elam, and M. Khan. 1995. Rejuvenated sycamore cuttings for energy plantations. Biomass and Bioenergy (In Press).