TIMOTHY PRESS: In theory you can always cool the air down to what is known as the "wet bulb" temperature, which varies considerably in different areas. In a very humid area, as southern Florida in the summer, the wet bulb temperature is about 80°F, so cooling only to 80°F can be obtained, but if ambient temperature is 90°F, 10° of cooling can be obtained.

TISSUE CULTURE PROPAGATION OF SELECTED MATURE CLONES OF *LIQUIDAMBAR STYRACIFLUA*

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Liquidambar styraciflua (sweetgum) is a desirable tree for the urban landscape. It possesses several qualities such as striking fall color, a pleasant form, attractively shaped leaves, and an ability to provide shade, which have made it increasingly popular as a street tree. However, sweetgum has several disadvantages which must be considered when selecting it for the urban landscape.

When sweetgum is grown from seedlings, the trees exhibit great variability in form and color. It also has an invasive root system that necessitates extensive and costly sidewalk repairs. Finally, grafting clonal scions onto seedling rootstocks as a means of overcoming variability is an expensive procedure, and results in higher costs for the growers, and consequently for the consumer.

Considering the popularity of sweetgum, it would be desirable to obain superior selections and propagate them clonally. However, sweetgum cuttings do not root easily (1) and thus must be produced by budding to maintain clonal selections. Not only is budding expensive, when scions are budded onto seedling rootstocks the rootstocks continue to impart some variability to the entire tree. Nonetheless there would be a value to budding, even if expensive, if a valuable rootstock could be identified and if it could be clonally produced.

Several trees in the San Francisco Bay area of California have recently been identified that possess superior fall color, delayed leaf drop, and non-invasive root systems, all of which would contribute favorably to an improved clone of *Liquidambar sytraciflua*.

Since these trees are all mature, ranging from 20 to 30 years old, they are difficult to root from cuttings. A possibility was to try micropropagation, a technique which has been used to propagate trees that are difficult to propagate by conventional methods such as cuttings and layering (2). For many tree crops, micropropagation has been used most successfully on explants in the juvenile stages of growth, since mature woody plants often do not respond favorably in culture (5,7).

The objective of this study was to propagate selected, mature clones of *Liquidambar styraciflua* by micropropagation. In this way clonally propagated material could be developed from superior mature individuals without the necessity of grafting.

MATERIALS AND METHODS

Both seedlings and mature trees were used in this study. One-year-old seedlings were donated by Saratoga Horticultural Foundation, Saratoga, California. Material from mature trees was collected from selected trees in the San Francisco Bay area.

Lateral buds were used as explant sources. Explants were surface-sterilized by a series of steps. Stems were washed in a dilute soap solution, and then dipped for 30 seconds in 5% Amphyl. A 20-minute wash in 20% laundry bleach to which 0.1% Tween 20 had been added followed, with a second wash (5 minutes) in 20% laundry bleach. The shoots were then rinsed four times in sterile, distilled water. Buds were excised from the shoots and placed in culture.

For the nutrient medium, both Linsmaier-Skoog (LS) (3) and Woody Plant Medium (WPM) (4) were used, without additional hormones, or with benzyladenine (BA) at 0.2 or 1.0 mg/l. Cultures were incubated at 25°C for a 16-hour photoperiod under 60 $\mu Em^{-2}sec^{-1}$.

RESULTS

Growth of seedling explants in vitro. Initial work was performed on explants from seedlings because we found it difficult to adequately disinfest mature material during the autumn when the study began. Excessive rains had resulted in large amounts of bacterial contamination on explants.

Explants from seedlings grew well on WPM. Those on LS, at all hormone concentrations, turned brown and became necrotic. Buds on WPM elongated after 4 to 6 weeks in culture. Multiple shoots appeared on plants in WPM medium containing both 0.2 mg/l BA and 1.0 mg/l BA, but those on the higher concentration of BA were rosetted and did not elongate even

after 2 months. Consequently the medium used to propagate shoots initially was WPM with 0.2 mg/l BA.

An experiment was conducted to determine the optimum salt concentrations of the various components of WPM. All stocks were prepared at ⅓ and 3 times the original concentration. Shoots that had been produced on the original WPM formula were placed on these different media for 4 weeks. There was no improvement in growth of the shoots on most of the different media compared to controls. The only change in salts that resulted in more vigorous growth than the control was ⅓ the concentration of $CaCl_2$. Consequently the revised medium consisted of WPM with ⅓ concentration of $CaCl_2$ stock and 0.2 mg/l BA.

Single shoots, approximately 0.5 cm tall, produced on the modified WPM, were removed and placed on different media for rooting. Of the 5 treatments used (Table 1), WPM with 0.5 mg/l indolebutyric acid (IBA) and 1.0 mg/l IBA were the most effective in producing high percentages of rooting (Table 1). At higher concentrations of IBA, many shoots turned brown and died. WPM with 0.5 mg/l IBA was selected as the medium of choice for root induction since 1.0 mg/l IBA produced excessive amounts of callus. The plants were vigorous and were transplanted to flats in humidity tents in the greenhouse without difficulty. After 1 month in the greenhouse, plants were transferred to pots (Figure 2).

Table 1. Effect of different IBA concentrations on root formation of *Liquidambar styraciflua* shoots regenerated *in vitro*.

	Root formation (percent of plants forming roots)	
Treatment	After 3 weeks	After 6 weeks
0.2 mg/l IBA	31	77
0.5 mg/l IBA	22	78
1.0 mg/l IBA	40	78
2.5 mg/l IBA	0	25
No hormone	0	0

Figure 1. Tissue culture derived plant of *Liquidambar styraciflua* 2 months after transfer to greenhouse. Explant source was from a 1-year-old seedling.

Growth of explants from mature plants in vitro The best medium selected using seedlings as source plants was used for incubating explants from mature plants of sweetgum. Shoots were taken in early spring just before bud break. The shoots were forced indoors and all explants were taken from actively growing buds on the upper 3 to 4 in. of the shoot. The buds were excised from the stem before being placed in culture.

It was critical that the buds be transferred every 3 to 4 days during the first 2 weeks *in vitro*. Buds that were transferred weekly and then at 3 to 4 weeks intervals did not grow as vigorously as those transferred more frequently.

Most shoots grew well but there were obvious differences in response of different genotypes. One genotype remained stunted throughout the study, whereas the other 3 genotypes grew rapidly and formed multiple shoots.

Single shoots were detached from the cultures and incubated on rooting medium determined previously. The rooting percentages for shoots derived from mature trees were lower than for those derived from seedlings and were related to genotype (Table 2). The 3 genotypes that grew vigorously also produced the most prolific roots (Figure 2).

Table 2. Effect of genotype in mature trees on rooting of *Liquidambar styraciflua* shoots regenerated in vitro after 4 weeks on rooting medium.

Genotype	Rooting (percent of plants forming roots)
A	20
C	40
D	40
E	100

DISCUSSION

It is possible to propagate complete plants from excised buds of mature specimens of *Liquidambar styraciflua* by micropropagation. The plant selections used in this study had not been able to be propagated by conventional methods previously. It was advantageous to determine the basal medium using seedling material grown in pots in a greenhouse because the buds were easily surface-disinfested and could be obtained in active growth when mature plants in the field were becoming dormant. The medium developed using seedling material was applicable to mature material although differences in responses among genotypes were noted. It has been shown for a variety of other material that there can be significant differences among cultivars in response to media (6).

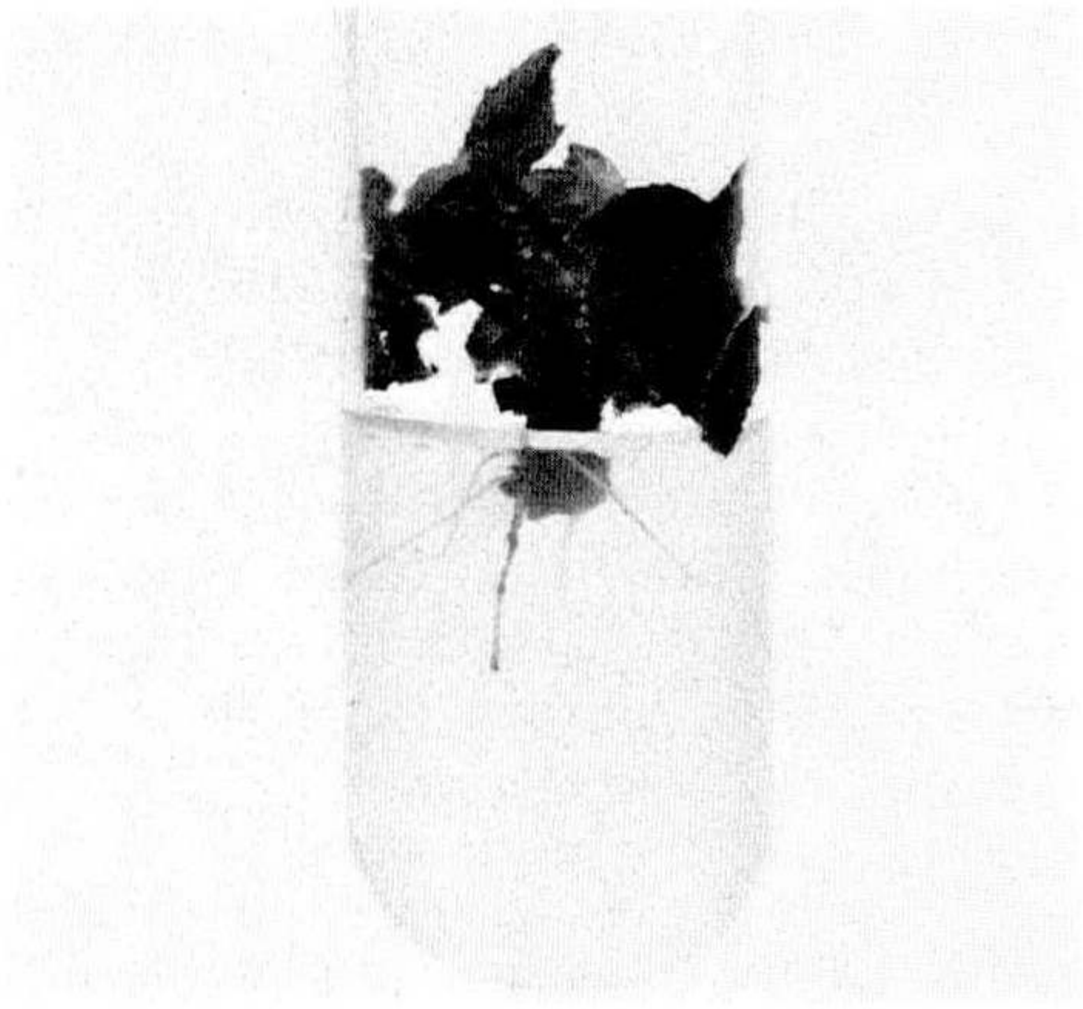

Figure 2. Complete plant of *Liquidambar styraciflua in vitro* derived from a mature plant.

The rooting percentage of mature material was lower than that from juvenile material but all genotypes could be rooted. Preliminary experiments with seedling material indicated that complete plants can be transferred to the greenhouse without difficulty.

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