GROWTH OF TISSUE CULTURE PLANTLETS OF LOBLOLLY PINE IN A NURSERY AND GREENHOUSE

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Abstract.--Tissue culture plantlets of loblolly pine (Pinus taeda L.) were grown in a nursery and a greenhouse in the summer of 1982 to compare the growth and developmental patterns of the trees in the two environments. The plantlets were compared to seedlings, excised embryos (embryos removed from the female gametophyte and grown in tissue culture medium), and rooted hypocotyls (embryos cut at the mid hypocotyl region and rooted "in vitro").

The initial growth rate of all four plant types was slower in the nursery than in the greenhouse. By the fourth month, nursery performance equalled or exceeded greenhouse performance with regards to height growth in all four plant types. However, the plantlets and excised embryos grew significantly slower in the greenhouse. The reduction in growth of the plantlets and excised embryos in the greenhouse may result from restriction of root growth in containers.

INTRODUCTION

The use of tissue culture as a tool in reforestation programs has become very promising in recent years. With the improvement of laboratory and greenhouse techniques, it is now possible for plantlets to be produced from several important forest tree species (Mott 1981). Among these species, loblolly pine (Pinus taeda L.) has been studied extensively by researchers at North Carolina State University. Over 4000 plantlets from 30 different familes have been produced and established in various greenhouse, nursery and field experiments.

Growth of loblolly pine plantlets in the greenhouse has been reported in a few experiments (Leach 1979, Amerson et al. 1981, McKeand and Wisniewski 1982, McKeand 1983). The growth patterns of the plantlets appear to be different than seedlings grown under similar conditions. Plantlets have a slower growth rate initially when compared to seedlings (McKeand and Wisniewski 1982, McKeand 1983). This lag period arises while the plantlets are becoming acclimated to the greenhouse environment. Once the plantlets become acclimated, the relative growth rates between the plantlets and the seedlings are about equal (McKeand 1983).

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Although there have been reports of plantlet growth in the greenhouse, there have been no reports of growth in an outdoor nursery. The ability for plantlets to survive and grow in a pine seedling nursery would allow for the operational production of plantlets in a nursery in future years.

This experiment was initiated to study the survival and growth of the loblolly pine tissue culture plantlets in a nursery bed and to compare growth between the nursery and the greenhouse environments.

MATERIAL AND METHODS

A range of plant types were produced to form a gradient from a tissue culture plantlet composed of both an adventitious shoot and root to a normal seedling with epicotyl, hypocotyl, cotyledons and radicle. The tissue culture plantlets and intermediate plant types (i.e. rooted hypocotyls and excised embryos) were produced in the laboratory from a mix of five open-pollinated families as follows:

Tissue Culture Plantlet -Multiple shoot were initiated on an excised cotyledon. After the shoot elongated, it was the rooted on agar medium. Both the shoot and the root were adventitious (Mott and Amerson 1981).

Rooted Hypocotyl -Seeds were germinated in 1% H_2O_2 and when the radicle emerged about 5 mm, the embryo was removed. Embryos were grown on a sterile agar nutrient medium until the epicotyl started to elongate. The embryo was then cut at mid-hypocotyl (about 5 mm below the cotyledons) and placed on root initiation medium. Only the roots were adventitious.

Excised embryo -The same procedure was followed as for the rooted hypocotyls except the radicle was not removed. This procedure produced a "normal" seedling grown in tissue culture medium.

All plant types were transplanted to the soil once the shoot was 1-2 cm long and the roots were approxiamtely 5 mm long (Figure 1). Seeds were sown 1 to 3 weeks after the plantlets were transferred from the lab. Seeds germinated and grew in 164 ml (10 cu. in.) RL Super Cells® in a greenhouse. The plantlets, rooted hypocotyls, and excised embryo were grown along with the seedlings using methods of Amerson et al. (1981). During the first 4 weeks in the greenhouse mist bench, a solution of Peters® 15-30-15 at 30 ppm N was applied 3-5 days per week to wet the upper soil surface. When the trees were removed from the mist and placed on a standard greenhouse bench, Peters® 20-19-18 at 39 ppm N was applied 3-6 days per week. The fertilizer solution was added until it dripped from the container. The trees were watered heavily once a week to prevent excessive fertilizer salts accumulation.

After approximately six weeks in the greenhouse, half of the trees were transplanted to Federal Paper Board's nursery in Lumberton, North Carolina. They were planted bare root using a dibble at a 7.6 cm x 15.2 cm (3" x 6") spacing. Root length and dry weight and shoot length and dry weight were measured in a 20 tree subsample of each type at the time of transplant to the nursery.

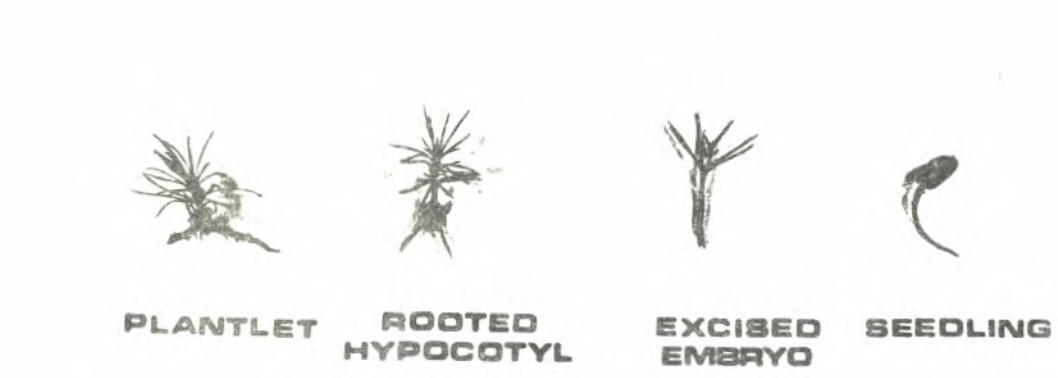


Figure 1.--Loblolly pine tissue culture plantlet, rooted hypocotyl and excised embryo when transplanted to greenhouse compared to newly germinated seed.

Conventional nursery practices were used to grow the trees through the nursery growing season. Five hundred-sixty kilograms per hectare of 5-10-30 fertilizer were applied in February. Ammonium nitrate (112 kg/ha) was applie five times through the growing season. One application of 20-20-20 (11.2 kg/ha) in early August and one application of muriate of potash (78.5 kg/ha) in late August was also added. Trees were irrigated as necessary to provide one inch of water per week. Weeds were controlled by hand.

The remaining trees were left in their original containers in the greenhouse on the North Carolina State University campus in Raleigh, N. C.. The trees were watered every day and fertilized with Peters® 20-19-18 at 39 ppm N three days per week. They were watered and fertilized until the solution dripped from the bottom of the container.

The experimental design was a randomized complete block in two locations (i.e. greenhouse and nursery). Within each location, the four plant types where randomized in four blocks with 7 plants/type/block/location for a total of 224 trees.

Height was measured every thirty days from May to October, 1982 in both the nursery and the greenhouse. Analyses of variance was performed to determine differences between treatments and plant types for shoot growth.

RESULTS AND DISCUSSION

Survival of all four plant types was excellent in both the greenhouse and nursery, with an average of 100 and 96 percent, respectively (Table 1). The rooted hypocotyls had the lowest percent survival (91%) in the nursery because of their very small initial size. The tissue culture plantlets had 97% survival in the nursery.

Table 1.--Characteristics of different plant types used in the study.

Plant Type	When Transplanted in May			
	Stem Height (cm)	Main Root Length (cm)	Root Dry Weight (gr.)	Survival in August
Tissue Culture Plantlets	1.4	8.8	0.013	97%
Rooted Hypocotyls	0.7	6.0	0.003	91%
Excised Embryos	1.6	15.6	0.006	98%
Seedlings	4.5	-	0.014	99%

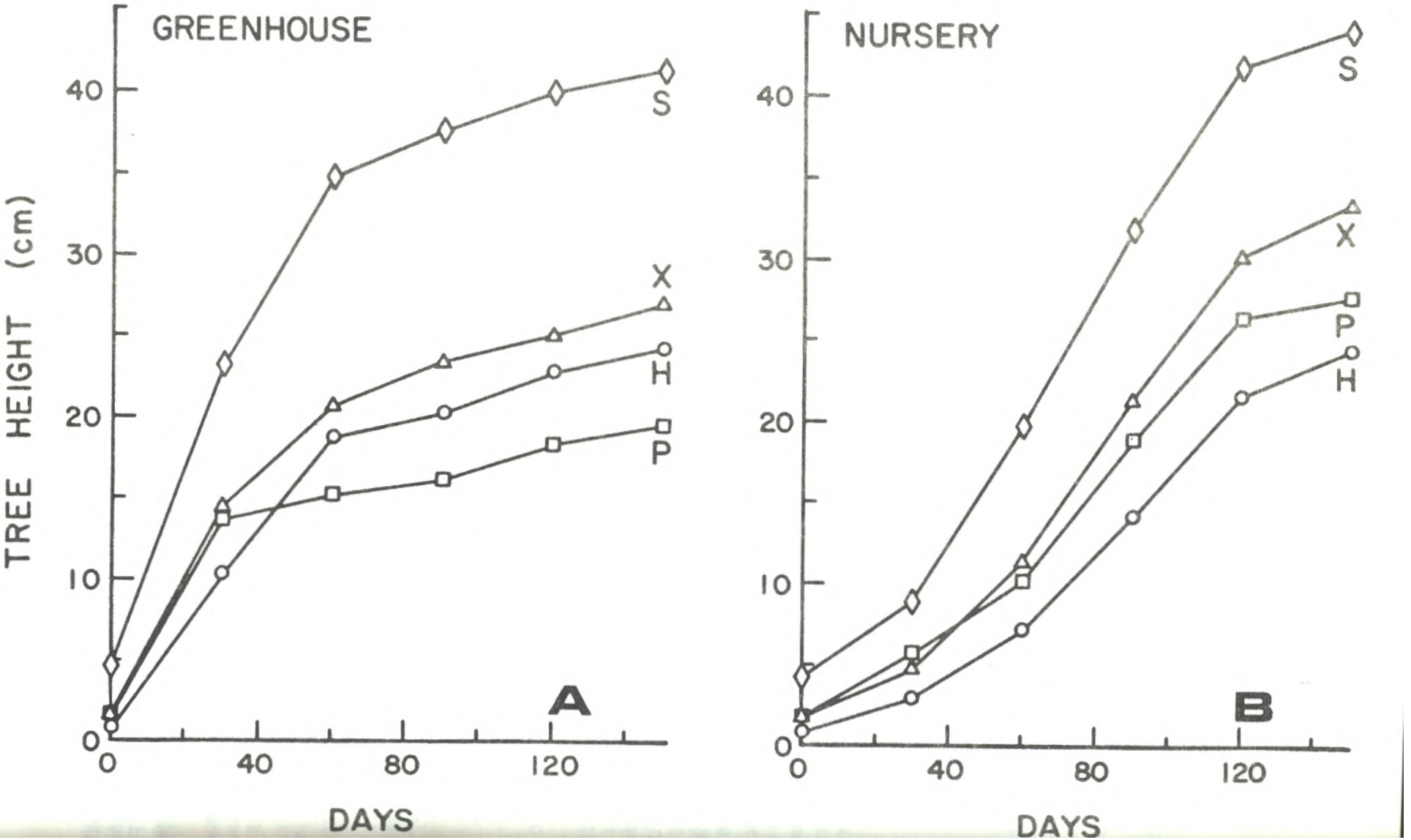
Early growth in the nursery was slower than in the greenhouse for all plant types (Figure 2). This was expected since there was a lag period while the trees became acclimated to the nursery. In the nursery, plantlets had the highest percent growth for the first 30 days, at 320 percent compared to 140 percent for the seedlings (Table 2). Although percent growth of plantlets in the nursery was slower in the second month, it had surpassed the percent growth in the greenhouse. From this time on the seedlings and the plantlets grew about the same, suggesting that the plantlets would have been the same height as the seedlings if their initial sizes had been equal (Figure 2b).

Growth in the greenhouse followed the same pattern as growth in the nursery (Figure 2a.) The plantlets had a much higher percent growth initially than the seedlings. After the first month, both the plantlets and the seedlings had approximately the same percent growth.

Table 2.--Percent growth in the nursery and greenhouse for the different plant types throughout the growing season.

	0-30 Days		30-60 Days		60-90 Days		90-120 Days		120-150 Days	
	N	G	N	G	N	G	N	G	N	G
Seedlings	140	413	126	56	63	9	32	7	5	3
Plantlets	320	934	78	11	93	6	42	16	5	7
Excised Embryos	166	794	144	43	99	10	43	7	10	8
Rooted Hypoctyls	267	1374	143	86	104	9	61	13	13	8

N = nursery
G = greenhouse


GREENHOUSE
TREE HEIGHT (cm)
0
10
20
30
40
0
40
80
120
DAYS
S
X
H
P
A
NURSERY
B

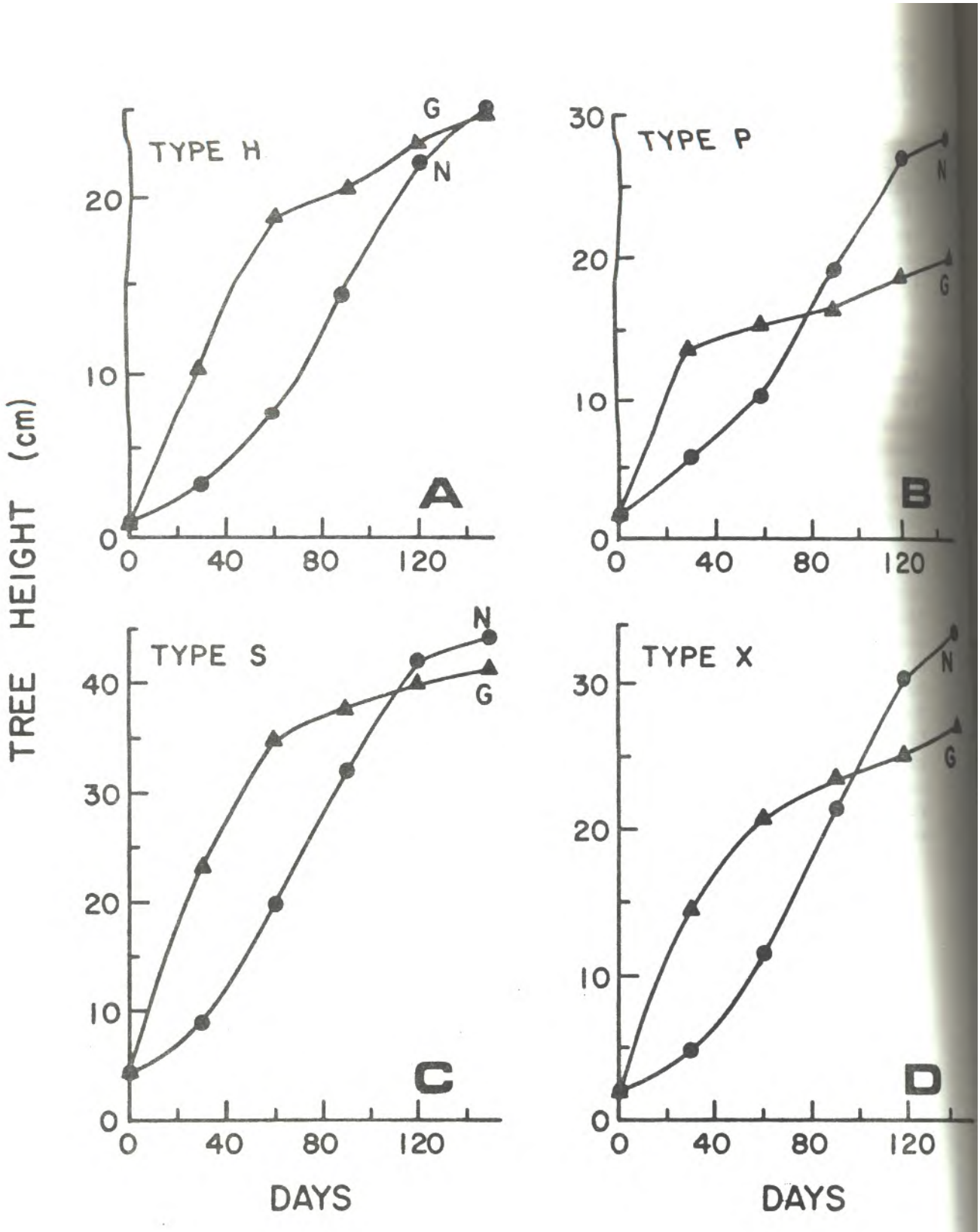


Figure 3.--Height growth in the nursery and greenhouse of loblolly pine (A) rooted hypocotyls, (B) tissue culture plantlets, (C) seedlings and (D) excised embryos.

CONCLUSIONS

The excellent survival and good growth of the tissue culture plantlets in the nursery showed that conventional seedling nursery practices can be used to grow plantlets to a size suitable for field planting. Future propagation systems for plantlets may need to utilize nurseries to make tissue culture economically feasible.

The growth reduction of the plantlets in the greenhouse suggests the need for further study of the root systems of the plantlets. The trees used in this study are being destructively sampled to measure several root and shoot characteristics. The results will be reported in a later paper.

ACKNOWLEDGMENTS

The use of trade name does not imply endorsement of products named nor criticism of products not named.

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