FIELD PERFORMANCE OF MICROPROPAGATED DOUGLAS FIR

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ABSTRACT

In March 1981, Weyerhaeuser Company established a field trial across five sites in coastal Oregon to test performance of Douglas fir (**Pseudotsuga menziesii** (Mirb.) Franco plantlings derived from cotyledon explants through tissue culture. Main objectives of the trial were to (1) compare survival and growth of plants produced by different propagation techniques (stock types) from the same genetic material, (2) compare field performance of plantlings derived from select orchard families with that of plantlings derived from local wild seed, and (3) assess the effects of size and form at planting time on subsequent plantling performance.

After 5 years, survival in the stock-type trials was 91% for plantlings, 99% for seedlings, and 49% for rooted cuttings. In the larger family comparisons, plantlings exhibited 74% survival. Owing to smaller initial size and 1 early year of reduced growth, plantlings are now 11% shorter than seedlings but both have had the same height increment during the past 3 years. Rooted cuttings are only 66% as tall as seedlings. Plantlings derived from select families are significantly taller on all sites than those derived from wild seed, the best family being 27% taller.

Early plagiotropism in some plantlings reduced survival but had no effect on height growth. All plagiotropic tendencies disappeared by the third season in plantlings but continued to persist in the rooted cuttings. Poor rooted cutting performance probably reflected ortet age (8 years) and/or unsatisfactory culture environment.

Keywords: plantlings; rooted cuttings; survival; height growth; plagiotropism; **Pseudotsuga menziesii**.

INTRODUCTION

Much has been written about the potential value of tissue culture as a technique for producing forest planting stock (Durzan & Campbell 1974; McKeand & Weir 1984; Mott 1981; Sommer & Brown 1979; Timmis 1987; Timmis *et al.* 1987). However,

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relatively little published information is yet available on field performance of micropropagated forest trees (McKeand 1985). Therefore, the projected benefits to be derived through tissue culture remain largely hypothetical.

In the early 1980s, having invested a significant effort into developing technology for micropropagating coastal Douglas fir by tissue culture (Timmis & Ritchie 1984), Weyerhaeuser Company established a trial for field testing such propagules in southwestern Oregon. The trial had three broad objectives:

(1) To compare field performance of Douglas fir propagules derived from the same select half-sib families but propagated by three different methods – seed, rooted cuttings, and tissue culture (plantlings);

(2) To compare field performance of plantlings derived from several families with that of plantlings derived from wild seed;

(3) To evaluate the effects of size and form at planting time on subsequent performance of plantlings.

Here we report on survival and growth of the three stock types over five seasons in the field.

METHODS

Propagation and Outplanting

Propagation of seedlings

Seeds from four top-performing half-sib families of a coastal Oregon provenance were collected from the Weyerhaeuser Seed Orchard, Turner, Oregon, in winter 1976. They were extracted, cleaned, dried, and stored according to normal operational procedures (Tanaka 1984). These seeds were stratified, along with seeds from a wild local source*, for 8 weeks at 4°C and sown in Leach cells (Ray Leach, Canby, Oregon; approximate volume = 60 cm^3) in early spring 1980.

The resulting seedlings were then grown for 1 year in a commercial container greenhouse. In fall 1980 they were placed into an unheated greenhouse under natural photoperiod to attain dormancy and to overwinter until planting time the following spring.

Propagation of rooted cuttings

Cuttings were collected from 8-year-old ortets derived from the same four half-sib families and same wild seedlot used to propagate the test seedlings. Ortets were located on Weyerhaeuser progeny test sites near Coos Bay, Oregon. Collections were made during winter 1980. Cuttings about 10 cm long were dipped in rooting hormone (Rootone-F) and placed into horticultural flats containing a mix of peat and vermiculite (1 : 1 vol.). They were rooted in a warm (c. 20°C) greenhouse under an extended 16-h photoperiod and intermittent mist. After 16 weeks, rooted cuttings were transferred to Leach cells and placed in a warm greenhouse to grow until autumn. They were then moved to the unheated greenhouse for overwintering as above.

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* Wild source was bulk seed collection, from the same seed zone, that is used as a standard in our regular progeny tests.

Propagation of plantlings

Seed from the same four orchard families mentioned above and the same wild seedlot (control) were surface sterilised with 10% Clorox solution and sown in sterile vermiculite medium. When the cotyledons had fully emerged and become horizontal they were excised and transferred intact to an agar medium. Shoots were cultured *in vitro* from the cotyledons using the juvenile pathway procedures described elsewhere (Cheng & Voqui 1977; Abo El-Nil 1987). The shoots were transferred to trays containing peat and vermiculite (1 : 1 vol.) and rooted in a greenhouse which was covered with a 47% shade cloth. For 2 weeks after transfer the rooting flats were covered with clear plastic tents. After removal of the tents, shoots were misted intermittently from above. After 18 weeks those shoots which had rooted were removed and transferred to Leach cells and placed into the greenhouse with the seedlings and cuttings.

Up to six shoots were derived from some cotyledon explants. These clones were distributed at random among experiments and across test sites. Because of the small number of ramets involved, no effort was made to track clones.

Establishment procedures

Some of the plantlings and many of the rooted cuttings exhibited plagiotropic growth so all stock was graded for size and form prior to planting. With the exception of the size and form comparisons (described later) grading standards were 2.0 mm stem caliper at the root collar, 150 mm height, and orthotropic form. Despite the latter criterion, a small percentage of plantlings and many rooted cuttings with plagiotropic form were included in the tests to maintain balanced field designs. Stock was outplanted on five logged sites in coastal Oregon (Table 1). The sites were scarified in preparation for planting and fenced to exclude browsing mammals and large rodents. Planting was carried out during 6 days spanning 17 to 31 March 1981. Weather conditions were generally cool and cloudy except for one warm (15°C) partly sunny day. Weeds have subsequently been controlled by herbicide treatment as needed. Large invading brush has periodically been removed by hand.

TABLE 1—Douglas fir seedlings, rooted cuttings, and tissue culture-produced plantlings were field tested on five sites in south-western Oregon

Site	Elev. (m)	Aspect	Slope (%)	Soil series	Topsoil texture	Parent material
2176	300	NE	10	Doerner	Clay	Eocene siltsone
4230	480	SE	25	Millicoma	Clay loam	Eocene siltstone
3252	210	NW	5	Bessee	Clay loam	Alluvium
212	180	W	15	Yonker	Clay loam	Eocene shale & siltstone
Doe Creek	320	SW	10	Willakenzie	Granular loam	Eocene sandstone

Experimental Design

Stock-type comparison

This test was established to compare field performance of plants from the same four half-sib families propagated by the three methods (three stock types). It also contributed information to the family comparisons described below. A split plot design was used with stock types as main plots and families as subplots. Subplots were five-tree rows and there were 10 replications of the main plots for a total of 50 trees per family per stock-type combination. Trees selected for this test were the most vigorous and orthotropic of the available stock. The test was planted on the 2176 site (Table 1) at a spacing of 2 × 2 m.

Family comparison

This unreplicated test was designed to compare field performance of plantlings derived from four half-sib families with that of plantlings derived from the wild seed. It was planted across four sites (4230, 3252, 212, Doe Creek) and comprised block plantings of plantlings from each of the four families. On three of the four sites, one block per family was paired with a similar block of plantlings derived from wild seed. On the 4230 site, eight blocks of wild plantlings were planted with two blocks of each family. Each block contained 49 trees at approximately 2 × 2 m spacing and trees were randomly selected from available remaining stock. At one location (4230 site), several blocks of seedlings were added.

Size and form comparison

Two smaller tests were designed to ascertain what impacts initial size and form might have on field performance of plantlings. Plantlings were graded for size (large $\geqq$ 12.1 cm height; small $\leqq$ 12 cm height) or form (orthotropic = 0° to 30° angle from vertical; plagiotropic = > 30° angle from vertical) before planting. The size test included the same families used in the other comparisons. It was planted with one 20-tree row plot for each family × size combination, with families serving as replicates of the size effect. All plantlings in the form test were from Family No. 1. They were planted in a randomised complete block design with five replications of the 20-tree row plots. Both tests were planted at a 2 × 2 m spacing on the 2176 site.

A summary of the three comparisons is given in Table 2.

Measurements

At the end of each growing season every plant was visually assessed for survival, and its height from the ground line was determined to the nearest centimetre. Data were analysed separately for each comparison using analysis of variance with Duncan's Multiple Range Test for mean separation at $p = 0.05$.

RESULTS

Stock-type comparison

At the end of the fifth growing season (October 1985) survival of seedlings and plantlings was 98% and 91% respectively. Rooted cutting survival was only 49%. The highest mortality among rooted cuttings occurred during the first and second years

TABLE 2—The present study embodied three different comparisons involving Douglas fir seedlings, rooted cuttings and tissue culture-produced plantlings

	Comparisons		
	Stock type	Family	Size and form
Propagules	200 plantlings 200 seedlings 200 rooted cuttings	2000 plantlings	400 plantlings
Genetic structure	4 half-sib families	4 orchard families 1 wild control	4 half-sib families and wild control (in size test), 1 family (in form test)
Site (number of plants)	2176 (600)	4230 (800) 3252 (400) 212 (400) Doe Creek (400)	2176 (500)
Conditions			Large (> 12 cm) v. small (< 12 cm) Orthotropic v. plagiotropic
Design	Split plot: stock types = main families = subplots Main plots = 20 trees Subplots = 5 trees	Unreplicated (on each site) 49-tree blocks. Sites are replicates in ANOVA 10 reps	20-tree row plot, 5 reps in form test, families are reps in size test.

after planting, with little subsequent mortality. In addition, several of the rooted cuttings continued to exhibit some degree of plagiotropic growth while none was evident among the plantlings.

Seedlings and plantlings followed generally similar growth curves during the first 5 years (Fig. 1). However, owing partially to different sizes at planting time, seedlings were significantly taller than plantlings at 5 years. Rooted cuttings lagged considerably behind the other two stock types throughout the trial period.

All three stock types exhibited similar height increment during the first growing season (Fig. 2). During the second growing season, seedling height increment exceeded that of plantlings and rooted cuttings. By the third growing season, plantling height increment had caught up with seedling height increment but rooted cuttings remained substantially behind. This pattern persisted through the fifth year.

A significant family × stock-type interaction for total height was indicated by the analysis of variance (Table 3). This resulted from both a change in variance among families for the different stock types (with plantlings more variable than seedlings)

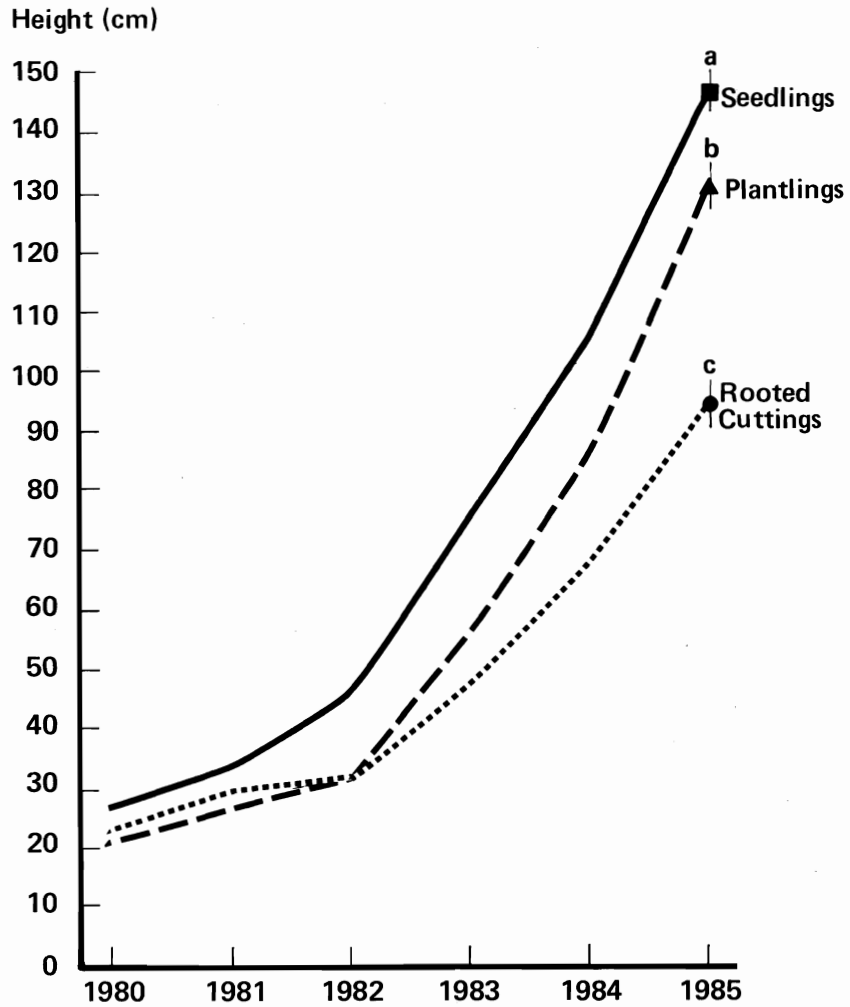

FIG. 1—Height of Douglas fir seedlings, micropropagated plantlings, and rooted cuttings in southern Oregon; 1980 is initial height at time of planting. Each point is a mean (± S.E.) of about 200 (seedlings and plantlings) or 100 (rooted cuttings) individuals. Mean separation ($p = 0.05$) by Duncan's Multiple Range Test.

and a change in family rank within stock types (Table 4). However, family ranks for total height were the same in the third as in the fifth year. This suggests that differences in family ranks in the fifth year were probably still reflecting family differences at establishment rather than a field interaction. Meaningful evaluation of family × stock-type interaction would require more families than were used in this trial.

Family comparison

Over-all plantling survival was considerably lower (74%) in the family comparisons than in the stock-type comparisons (91%). This was partially because the stock-type

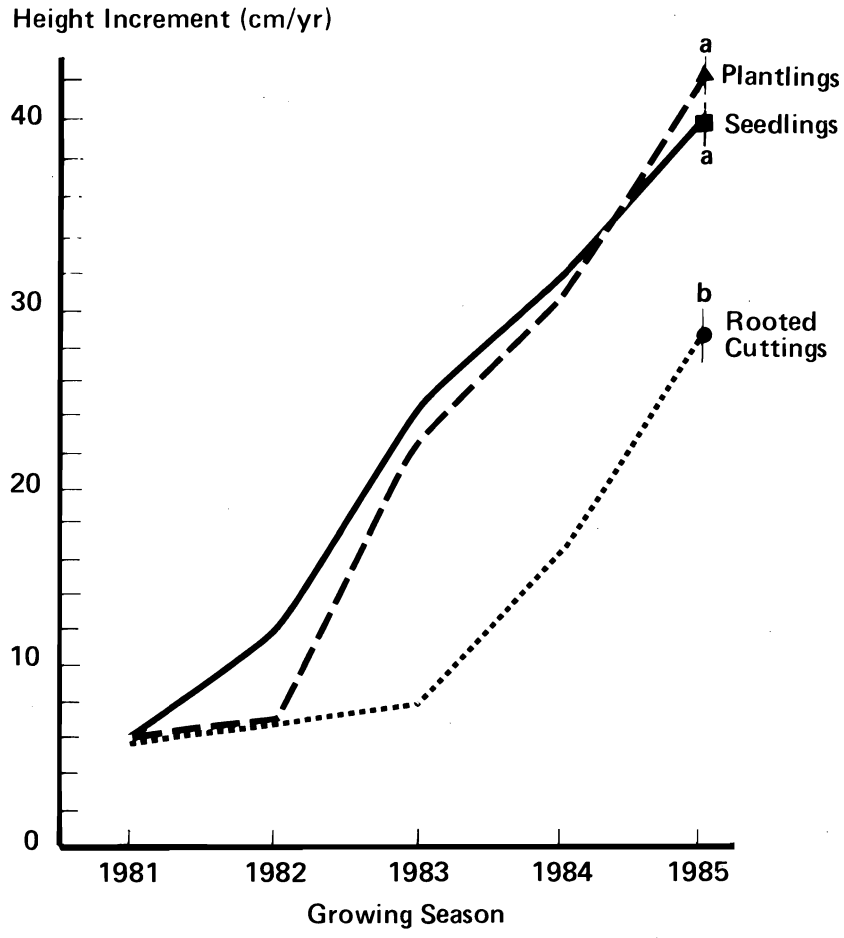

FIG. 2—Height increment of Douglas fir seedlings, micropropagated plantlings, and rooted cuttings on a site in southern Oregon. Statistics are as in Fig. 1.

TABLE 3—Stock-type comparisons. ANOVA on height, age 5 years

Source of variance	DF	MS	F*	p
Replicate	9	3023	2.17	0.02
Stock type	2	84638	7.94	0.0001
R × ST	18	4402	3.16	0.0001
Family	3	7358	5.28	0.002
F × ST	6	6431	4.61	0.0001
Error	439	1394		

* F calculated by MS/MS for all but ST, with required a synthesised F test of $\frac{MS_{ST} + MS_{E}}{MS_{R \times ST} + MS_{F \times ST}}$ because all variables except ST were fixed.

TABLE 4—Fifth-year height (cm) of plantlings, seedlings, and rooted cuttings derived from Douglas fir families planted on the 2176 site. No wild controls were planted on this site

Stock type	Family				Mean
	1	34	42	47	
Plantling	111(6)*	128(6)	149(6)	133(5)	131(3)
Seedling	153(6)	132(6)	144(6)	152(5)	146(3)
Cutting	106(7)	79(7)	89(6)	101(6)	95(4)

* S.E. in parentheses

comparison was planted on a relatively favourable site while the family comparisons were planted across several sites – some of them apparently quite harsh (3252 and Doe Creek). Also, the stock-type comparison received some irrigation during a record heat wave in August 1981. Family 47 had generally highest survival across all sites, while the other families and the wild control averaged about 10 percentage points lower. Nearly all mortality occurred within the first year after planting.

Family differences significantly ($p = 0.0001$) affected height across all five sites used in the family and stock-type comparisons (Fig. 3; Table 5). Variability in height among families was greatest at Doe Creek and least on Site 4230. Family rankings with respect to height were fairly consistent across sites, with Families 42 and 47 tending to be taller than the others. When data were averaged by family across all sites, plantlings from select families were consistently taller and showed greater height increment than those of the wild control (Table 6). Representative individuals from Family 42 on Site 212 are shown in Fig. 4.

Size and form comparison

Five years after establishment, plantlings which were less than 12 cm tall when planted were equal in height to those which were taller than 12 cm (Table 7). Survival was only slightly lower in the smaller stock. Orthotropic plantlings had much higher survival than plagiotropic plantlings but had not attained significantly greater height nor height increment. All indications of plagiotropism had disappeared by the end of the third growing season.

DISCUSSION

At the time these tests were established Douglas fir tissue culture was in its infancy. There was serious concern then about the ability of the cultured trees to survive, grow, and behave "normally" in comparison to seedlings when planted in a forest environment. Therefore, a key element in this trial was the stock-type comparison in which trees propagated by tissue culture were compared in the field growing side-by-side with seedlings of the same half-sib families.

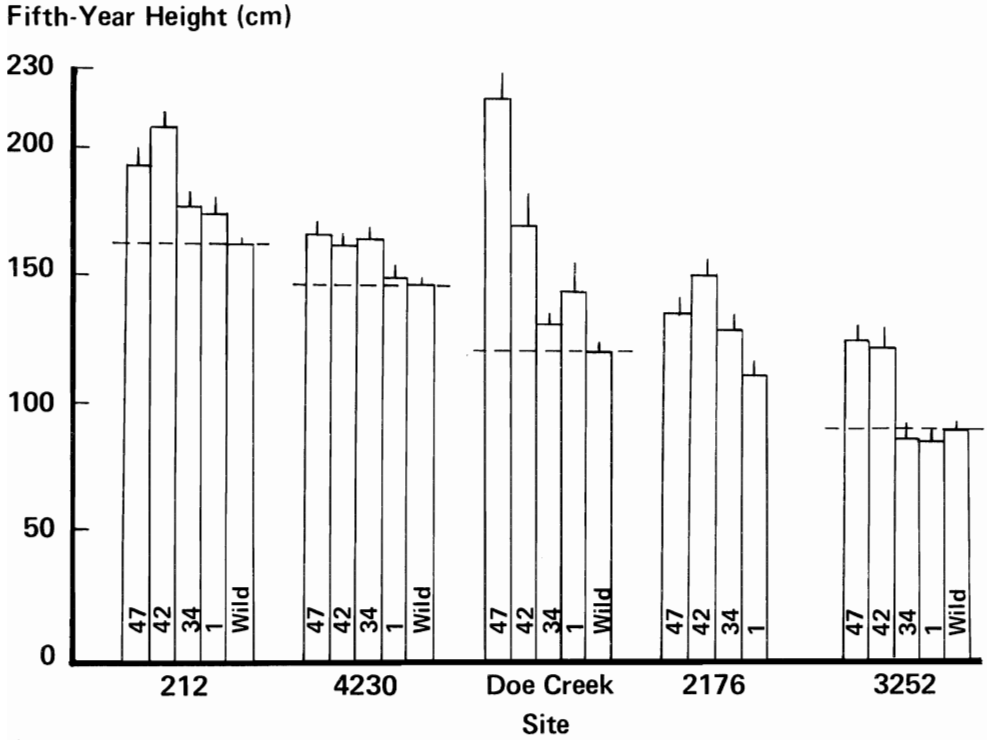

FIG. 3—Fifth-year height of micropropagated Douglas fir on five sites in southern Oregon. Each bar represents a mean (S.E.) of about 50 plants (100 plants at site 4230). Plants were propagated from seed of four half-sib families (1, 34, 42, 47) and one wild local seedlot.

TABLE 5—Family comparison. ANOVA of height, age 5 years

Source of variance	DF	MS	F	p
Replicate (site)	4	220423	120.7	0.0001
Family + wild control	4	77309	42.3	0.0001
R × F	16	10116	5.5	0.0001
Error	1356	1827		

TABLE 6—Family comparison. Family and "wild" mean height age 5

Family	Total height in 1985 (cm)	1985 height increment (cm)
1	145.1 b	45.2 cd
34	149.2 b	47.6 c
42	171.4 a	52.6 b
47	173.9 a	56.6 a
Wild*	136.5 c	42.4 d

* No wild controls were planted on Site 2176.

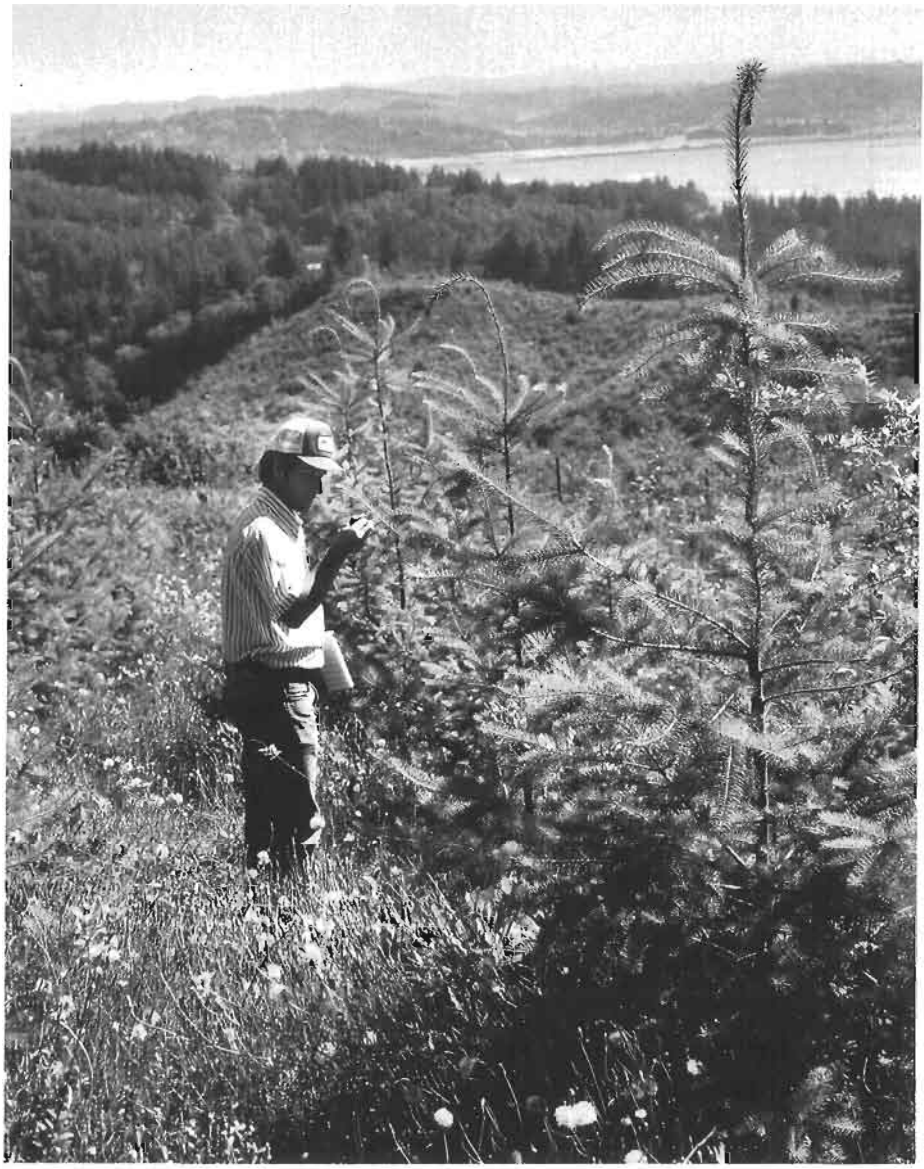

FIG. 4—Micropropagated Douglas fir plantlings in their fifth year of growth on a site in southern Oregon.

Survival of plantlings and seedlings in the stock-type comparison was exceptionally high (91% and 98%, respectively). Operational regeneration activities with bare-rooted and transplant stock in this area achieve 80% to 90% survival in an average year. In contrast, rooted cuttings dropped to about 54% during the first growing season and are currently at 49%.

TABLE 7—Size and form comparison. Means of large **v.** small stock, and plagiotropic **v.** orthotropic stock - survival, height, and height increment (S.E. in parentheses)

Size and form	Survival (%)	1985 height (cm)	1985 height increment (cm/yr)
Large*	97	138 (13)	38 (4)
Small	92	138 (10)	37 (3)
Orthotropic†	88	140 (3)	45 (2)
Plagiotropic	62	131 (6)	41 (2)

* Large = height greater than 12 cm; small = height 12 cm or less.
† Orthotropic = angle of stem from vertical 30° or less; plagiotropic = angle of stem from vertical 31° or more.

The seedlings in this test are now taller than the plantlings (Fig. 1). However, the height increment of these two stock types has been identical for the past 3 years (Fig. 2). So the size difference apparently does not reflect some inherent inability of plantlings to grow once established, but rather some temporary impediment to establishment. Likewise, the tendency of plantlings of *Pinus taeda* L. to make slow growth in the first year and then more normal growth in subsequent years has also been reported (McKeand & Frampton 1984).

In the present test, the two stock types were of different size when planted. It is nearly impossible to produce planting stock via two entirely different cultural pathways and have them come out identical in size at planting time. So an initial height disadvantage may have accounted for part of the current height difference. In addition, we now know that a container production system, as was used to produce all the planting stock for this study, may not be the optimum system in which to grow plantlings. In several (unpublished) trials we have observed that plantlings which are transferred to a nursery soon after *in vivo* rooting and then grown out as bare-rooted stock, are larger and have more rapid initial growth than those grown in containers. Wisniewski *et al.* (1983) have reported similar results with *P. taeda* plantlings.

Poor performance of the rooted cuttings in this trial probably reflects ortet age (8 years from seed). It is well known that Douglas fir cuttings show progressively poorer rooting (Brix 1974; Roberts & Moeller 1978), more plagiotropism (Starbuck & Roberts 1983), and perhaps slower growth as ortets increase in physiological age. Although 8 years is not "old" in the life of a Douglas fir tree, the phase change from juvenile to mature appears to begin at age 2 or 3. This effect probably explains why the rooted cuttings in this trial had poor form and slower growth than the seedlings and plantlings. The poor form in turn resulted in heat damage in many cuttings during the 1981 summer because of proximity of their prostrate stems to the hot soil surface. It is probable that, had the cuttings been taken from juvenile ortets, their performance would have been similar to the other stock types.

The second most important element of this trial was the family comparison. In theory, plants propagated vegetatively, since they have not undergone sexual recombina-

tion of genes, are genetically identical to their ortets. If ortets are drawn randomly from a population of high-performing individuals, then the ramets should also, on average, exhibit high performance. If this theory were to prove invalid, due to "c" effects, somaclonal variation, or other reasons, then the utility of tissue culture for mass-propagating genetically improved forest planting stock would come into serious question.

Five-year height growth data from the family comparisons indicate that plantlings derived from ortets selected from progeny-tested, high-performing, half-sib families are showing significantly greater height growth than those propagated from wild seed of the same geographic provenance (Table 6). This trend appears across the four family test sites (Fig. 3). Family rankings were also generally consistent across sites. Families 42 and 47 were highest in rank while Families 1 and 34 were lowest on four of the five sites. These results are, of course, preliminary in the sense that all genetic test results are preliminary until final harvest. However, it has been our experience that height rank changes in Douglas fir tests are not common after 5 or 6 years in the field.

One unexpected characteristic of Douglas fir plantlings propagated for this and subsequent tests was that many exhibited plagiotropic growth habit at time of planting. Plagiotropism is a manifestation of topophysis (Olesen 1978) wherein cuttings, for some time after rooting, retain the branch-like form and growth habit they exhibited as shoots on the ortet. It is well known that rooted cuttings of Douglas fir may exhibit strong plagiotropic tendencies (Starbuck & Roberts 1983) as was observed with rooted cuttings in the present study. It had been speculated that one way around this problem would be through juvenile micropropagation. The reasoning was that since the explant was from a cotyledon no "branch-message" would be present and growth would, therefore, be orthotropic.

Unfortunately, this has not happened with Douglas fir. We have observed in this, and many subsequent studies, that plagiotropic growth in micropropagated Douglas fir from cotyledon culture is the norm. It apparently has a strong clonal component. In a recent evaluation of 56 clones derived from seven full-sib families, 23 were strongly plagiotropic and only six were orthotropic about 6 months after rooting. Variance associated with clone was highly significant while family variance was not significant (G. A. Ritchie, unpubl. data).

Circumstantial evidence from other experiments at Weyerhaeuser suggests that plagiotropism is related, in plantlings, to some property of the root system. For example, plantlets* that were transferred to different-sized containers immediately after rooting developed plagiotropism more rapidly the smaller the container. Also, shoot tips from plagiotropic plantlings grew orthotropically when grafted to containerised seedling root systems.

By the end of the first field season in the present study new growth on all plantlings was orthotropic and by the end of the third season all evidence of early plagiotropism, including lower stem sweep, had completely disappeared. It was also encouraging to

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* In our terminology a "plantlet" is a rooted micropropagated shoot not physiologically ready for field planting, in contrast to a "plantling", which would meet planting-stock grading standards.

observe that early plagiotropism did not apparently affect height growth (Table 7). It did, however, reduce survival, probably owing to early heat damage incurred by plagiotropic individuals during the August 1981 heat wave.

Another curious property of some tissue-cultured conifers is the early appearance of mature characteristics. Maturation in some pines is marked by measurable morphological and growth traits. Some of these are slow initial growth, increased needle length and diameter, and reduced number of lateral branches (Greenwood 1984). These can be used as indicators of early maturation in the field. Based upon such indicators, tissue-cultured *P. taeda* in the south-eastern United States (McKeand 1985; McKeand & Frampton 1984) and *Pinus radiata* D. Don in New Zealand (D. R. Smith, Forest Research Institute, Rotorua, pers. comm.) are showing apparent early maturation. Why this is occurring is not understood. The explants in both cases were very juvenile tissues (excised embryos) hence one would expect the propagules also to be juvenile. It is speculated that this may be related to root system abnormalities such as inability to take up nutrients or to synthesise growth regulators (McKeand 1985).

It is not yet possible to ascertain whether early maturation is occurring in Douglas fir plantlings because we have no characteristics or indexes, short of early flowering, by which to assess maturation state in this species. A study is now under way to develop such criteria. Casual observation of seedlings and plantlings growing side-by-side does provide clues to this question, however. There appears to be a tendency for some plantlings to exhibit shorter and fewer branches, narrower branch angle, darker foliage, and coarser needles than do seedlings. In addition, seedlings tend to break buds earlier in spring than do plantlings. The tendency for early bud-break appears to be a juvenile attribute in Douglas fir.

Taken together, the results of these trials to date are very encouraging for the operational use of micropropagated Douglas fir in the not-too-distant future. Two major uses envisioned at this time are (1) multiplication of seed of improved genotypes, and (2) field evaluation of large quantities of material toward identification of elite clones.

It should also be kept in mind that the technology used to produce propagules for this study is now nearly 10 years old, and in the past decade large improvements have been made. These include improved subculturing, rooting, acclimatisation, and planting stock production techniques – all of which result in more vigorous, faster-growing plantlings. These advances have also enabled us to propagate large numbers of individuals for field clonal testing. Such tests are now in progress.

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