CLONAL PROPAGATION AND GENETIC TESTING OF VIRGINIA PINE

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Abstract. —Texas Christmas tree growers plant approximately 800,000 Virginia pine (pinus virginiana Mill.) seedlings each year. In 1981 a tree improvement cooperative was formed with the objective of providing genetically improved planting material to these growers. Clonal propagation could potentially be an integral component of this and other tree improvement programs through production of limited and/or proven genotypes. One technique of clonal propagation is tissue culture of cotyledon explants and the subsequent production of plantlets for operational plantings. Before this system of propagating Virginia pine can be considered successful, it is imperative that micropropagated plantlets are evaluated in field trials. Three trials consisting of both plantlets and seedlings were established in spring, 1990, to compare the performance of plantlets to genetically improved seedlings of similar genetic background. This is the first phase of our Virginia pine clonal field testing program. After one growing season, plantlets were shorter and had a slightly lower survival rate. Plantlets were smaller and more variable in size and age when planted compared to seedlings, due to the constraints of the tissue culture system.

Keywords: Pinus virginiana Mill., in vitro propagation, clonal field trials, plantlet.

INTRODUCTION

The estimated market for Christmas trees in Texas is over 3 million trees annually. Imports from the northern and western United States have, to date, captured most of this market (Chandler 1985). However, the Christmas tree industry has been steadily growing in Texas. In recent years, approximately 800,000 Virginia pine seedlings have been planted annually by Texas Christmas tree growers. In 1990, approximately 400,000 Texas—grown Christmas trees were sold, the large majority of which were Virginia pine. The value of the harvest was approximately $8.0 million, with a total economic impact of at least $17 million (J.W. Chandler, pers. comm. 1991).

In 1981 a cooperative of the Texas Christmas Tree Growers Association, the Texas Agricultural Extension Service, and the Texas Forest Service was initiated. The objective of the cooperative is to provide genetically improved planting

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stock for Christmas tree growers via the Virginia pine Christmas tree improvement program (McKinley 1989). In 1983 a seed orchard of approximately 4 ha was established at Magnolia Springs, Texas, with 50 families. Following evaluations from genetic tests, the poorest 25 families were removed leaving the best 600 trees for seed production (McKinley 1989). The first cone crop was collected at Magnolia Springs in 1987 and the first genetically improved Virginia pine seedlings became available in the fall of 1988. It is anticipated that the seed orchard will be in full production by 1995 and will produce approximately 50 kg of seed per year (C.R. McKinley, pers. comm. 1991).

Vegetative propagation could potentially be a valuable component of the Virginia pine tree improvement program. Some of the potential benefits are: the capturing of non-additive genetic variation and thus increased genetic gain (McKeand 1981, Libby and Rauter 1984, McKeand and Weir 1984, Ahuja and Muhs 1985, Johnson 1988); the reduction in the lag between selection and reforestation with select individuals (McKeand 1981); more information regarding genetic parameters (Libby 1969, Burdon and Shelbourne 1974); amplification of control-pollinated seed; in vitro selection (Mott and Amerson 1984); and propagation of transgenic plants (van Buijtenen and Lowe 1989).

Clonal propagation can easily be integrated into *a* classical tree improvement program. However, it is important to visualize the clonal option as *a* technique to maximize genetic gain at any point in time, rather than genetic improvement per se (Barnes and Burley 1987). It is vitally important to maintain genetic variability in broadly-based breeding populations. The rapid progress made in mass clonal propagation techniques can be applied to the conservation of this genetic variability and also allow for rapid exploitation of superior recombinants in each generation of the breeding program (McKeand 1981, Barnes and Burley 1987, Shelbourne 1988). In vitro, propagation systems appear to have the greatest potential for mass propagation (McKeand 1981, van Buijtenen and Lowe 1989)

There are three main approaches for in vitro culture of plants:

(1) Production of adventitious shoot buds directly from excised plant parts or from callus, then induction of rooting.
(2) Induced proliferation of the shoot apex, axillary or fascicular buds to produce multiple shoots which can then be rooted.
(3) Production of callus and suspension cultures from unorganized tissue explants, and then induction of somatic embryogenesis.

The most successful micropropagation systems to date for gymnosperms have involved induction of adventitious buds on explants of embryos or young seedlings (David 1982). Chang et al. (1991) successfully achieved organogenesis in cotyledon explants of Virginia pine. Approximately 5000 plantlets have recently been produced in our lab via this procedure.

Achieving the potential genetic gains from vegetative propagation depends entirely on the good performance of clonal material in the field (McKeand 1981). Information from clonal field trials indicates that vegetative propagules may initially grow significantly slower than seedlings (McKeand and Frampton 1984, Horgan 1987), have similar growth rates (Menzies and Klomp 1988), or grow faster (Bennett et al. 1986). Vegetative propagules sometimes show desirable "mature"

characteristics, such as resistance to rust (McKeand and Frampton 1984) and better form (McKeand 1985, **Bennett et** al. 1986, Menzies and Klomp 1988). Conversely, vegetative propagules have shown undesirable "mature" characteristics such as poor rooting, plagiotrophic growth, and decreased growth and survival in field plantings (McKeand 1985, St Clair et al. 1985, Ritchie and Long 1986).

The objective of this paper is to report on the first phase of our Virginia pine clonal propagation and genetic testing program. Height growth and survival of plantlets and seedlings from similar genetic sources are compared after one year of growth in the field.

METHODS

Open-pollinated seed from families 1-38 and 1-78 were obtained from the Virginia pine tree improvement program. Some of the seed was cold stratified and sown for containerized seedling production. Approximately 300 **seed** were retained for containerized plantlet production via methods developed by Chang et al. (1991) using embryonic cotyledon explants. After 4 **weeks** of acclimation in plastic tents, the plantlets were kept in the same greenhouse as the seedlings until field planting. Only the largest plantlets were selected for field planting.

Field Trial Design

Field trials for testing plantlet versus seedling performance were established in spring 1990. Three locations were chosen, as sites representative of Virginia pine Christmas tree growers land - in central Texas, southeast Texas and northeast Texas. The field trial design was *a* randomized block design with five blocks and 15 four-tree plots per block. Ten of these plots within each block were containerized seedlings (twenty seedlings each from Virginia pine families 1-78 and 1-38) and five of the plots per block were plantlets from families 1-78 and 1-38. Because of the small number of ramets per ortet, no within clone analyses was attempted.

Height and survival were measured monthly. The plantlets and seedlings are managed - in terms of weed control, clipping and shearing, and irrigation where needed - by the landowners, with the understanding that all the test trees are treated equally.

Statistical Analyses

Covariance analyses were performed using the general linear model (GLM) procedure in the Statistical Analysis System (SAS Institute, Cary, NC), with initial height used as the covariate. Data from each location was analyzed separately. Raw means for initial and final heights, and the least square means (adjusted for initial height) were determined using the MEANS and LSMEANS SAS procedures.

RESULTS

The covariance analyses are summarized in Table 1. Initial height is the only main effect showing a high level of significance (1%) all locations. Plant type was significant at the 10% level at the central Texas location, at the 1% level at the southeast location, and at the 5% level at the northeast location. The plant-type by initial-height interaction was significant (5% level) at the southeast Texas location only.

Table 1. Summary of the covariance analyses for Virginia pine plantlet and seedling heights after one growing season in the field, initial height being the covariate.

		LOCATION					
		CENTRAL TEXAS		SOUTH-EAST TEXAS		NORTH-EAST TEXAS	
SOURCE	d.f	Sum of Squares	F	Sum of Squares	F	Sum of Squares	F
REPLICATION	4	522.23	3.90***	2565.62	18.62***	312.61	1.12
PLANT TYPE (T)	1	110.17	3.29*	321.45	9.33***	461.78	6.63**
SOURCE (S)	1	11.10	0.33	7.04	0.20	11.42	0.16
INITIAL HEIGHT (I)	1	2731.77	81.59***	2636.73	76.53***	2646.57	37.98***
T * S	1	41.48	1.24	24.94	0.72	158.96	2.28
I * T	1	72.22	2.16	143.00	4.15**	143.47	2.06
I * S	1	26.74	0.80	12.21	0.35	0.23	0.00
ERROR	276	17154.00		9302.53		19231.10	

* Indicates significance at 0.10 level of probability
** Indicates significance at 0.05 level of probability
*** Indicates significance at 0.01 level of probability

At the end of their first growing season in the field, the plantlets were significantly shorter than the seedlings, but this was partly due to their lower initial height. Table 2 summarizes the raw means and least square means for plantlet and seedling height, respectively. The least square means have been adjusted for the variation due to initial heights. These adjusted means are graphically represented in Figure 1.

Percent survival for both plant types was good at all locations, ranging from 88% to 99% (Figure 2) and with *a mean* survival of 92% for the plantlets and 97% for the seedlings. At the central and northeast Texas locations seedling survival was better compared with plantlets. Considerable stress, in terms of drought at the central location and flooding at the northeast location, were **encountered soon** after planting. In contrast, plantlet survival was comparable or better at the southeast Texas location where stress occurred later in the season with little rainfall in September and October.

Raw means and least square means for plantlet and seedling heights

LOCATION	PLANT TYPE	SOURCE	RAW MEANS Initial Height (cm)	RAW MEANS Final Height (cm)	ADJUSTED MEANS Final Height (cm)	ADJUSTED MEANS Std. Error
	Plantlet	1-38	13.4	28.6	27.2	0.84
CENTRAL	Plantlet	1-78	9.5	24.3	27.4	1.44
TEXAS	Seedling	1-38	15.4	32.6	29.1	0.71
	Seedling	1-78	13.2	28.3	27.2	0.69
	Plantlet	1-38	11.0	28.3	29.8	0.81
S.E.	Plantlet	1-78	7.6	25.4	30.1	1.42
TEXAS	Seedling	1-38	11.3	31.9	32.9	0.70
	Seedling	1-78	11.5	32.0	32.7	0.72
	Plantlet	1-38	10.1	30.1	32.5	0.85
N.E.	Plantlet	1-78	10.8	31.0	32.7	1.36
TEXAS	Seedling	1-38	13.0	39.4	38.7	0.60
	Seedling	1-78	12.3	35.2	35.1	0.96

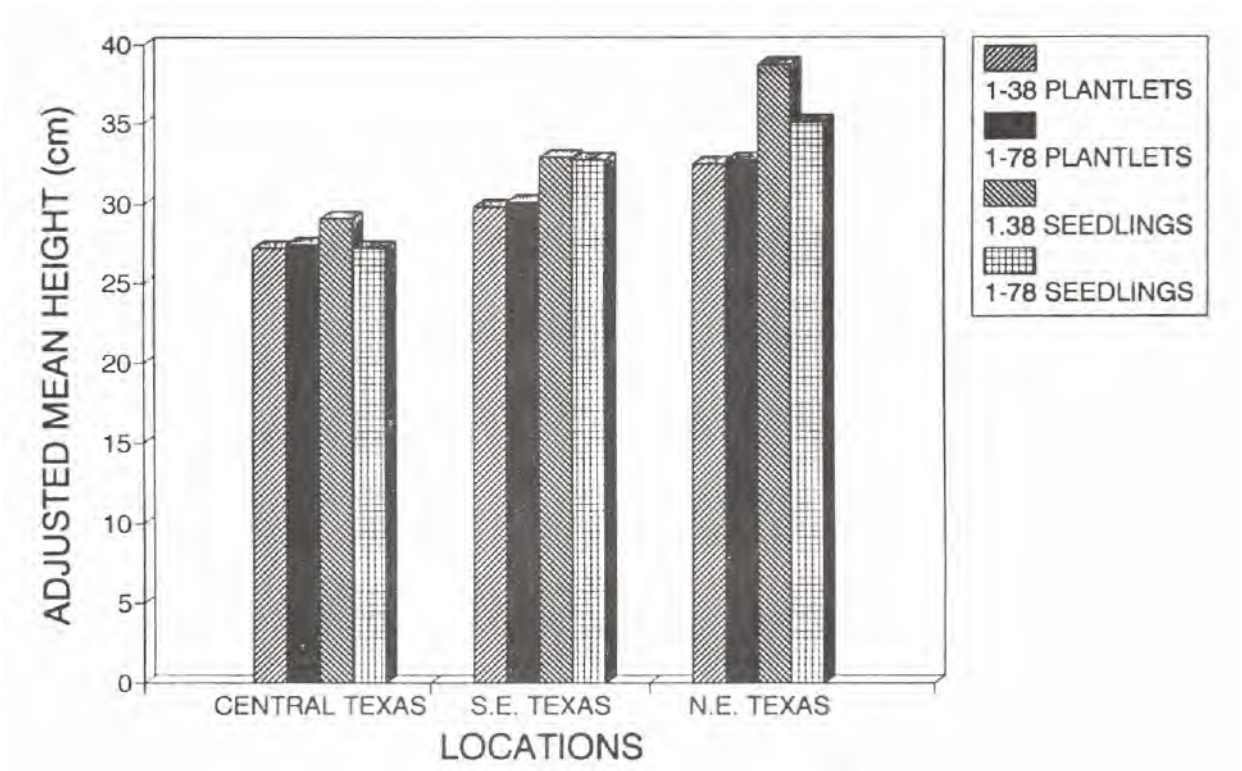

Figure 1. Least square means for height of plantlets and seedlings from two genetic sources after one growing season at three different field locations. The means are adjusted for initial height.

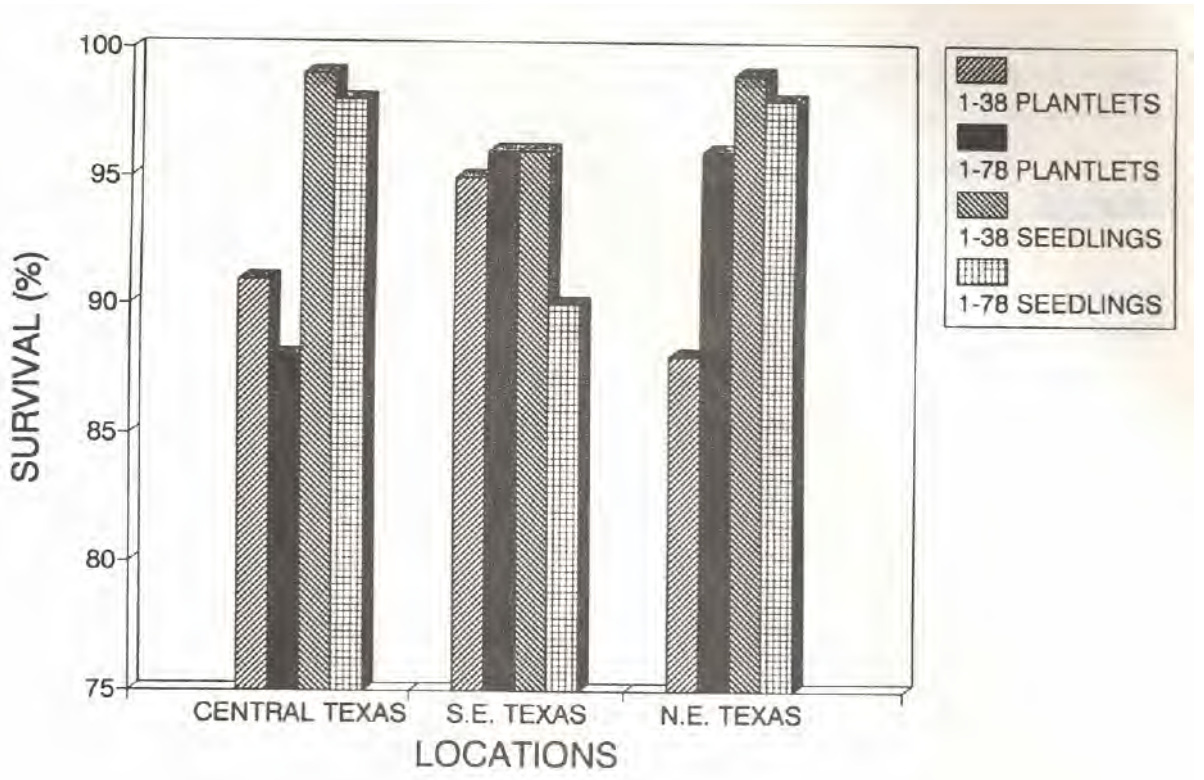

Figure 2. Percent survival of plantlets and seedlings from two different genetic sources after one growing season at three different field locations.

DISCUSSION

This paper presents the initial results on the relative performance of plantlets and seedlings from our first phase of clonal field trials. After one year in the field, plantlets have a 5% lower survival rate and were significantly shorter compared with seedlings. This has been reported in other similar studies, but plantlets showed more comparable growth in subsequent years (McKeand and Frampton 1984, McKeand 1985, Ritchie and Long 1986). In our study, the plantlets may have been disadvantaged because they were significantly smaller than the seedlings when planted. It is difficult to produce planting stock via different methods and have them uniform in size at planting.

At the end of 1991, after two years in the field, the plantlets and seedlings will be ranked for form. Height and diameter measurements will be taken until the test trees are harvested as Christmas trees beginning in November 1992. In this study, both plantlets and seedlings were container grown in *a* greenhouse. Because of the strong preference for nursery grown bare—root planting stock, *a* pilot scheme with 300 plantlets was established at a commercial nursery in March 1991. The plantlets are being managed similar to bare—root planting stock.

The second phase of our clonal field trials was begun in the spring of 1991, with the establishment of clonal uniformity and clonal stability trials. The plantlets were derived in the same manner *as* the plantlets from the first phase of trials reported here. There is also strong interest in cloning Christmas trees

of sufficient age and size to indicate their superiority. We are currently developing a micropropagation procedure using fascicular bud proliferation techniques.

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