GENETIC IMPROVEMENT OF EUCALYPTUS GRANDIS FOR SOUTHERN FLORIDA

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Abstract.--Genetic gain potentials for a 5th-generation seedling seed orchard were 27 and 17% for 15-month height and survival, respectively. Larger gains may be expected from clonal selection and testing, and 232 clones now under test may have sufficient freeze resilience. Several clones averaged over 6 m tall in 1.25 years on a well-prepared site; three clones performed superbly in two tests. Laboratory freeze screening at -5°C rated clones similar to field results. Early plantlet development was like that of seedlings, and rooted cuttings and plantlets grew well in the field. Large numbers of rooted cuttings can be produced from selected clones, and direct micropropagation of certain clones has been accomplished commercially.

Keywords: Eucalyptus grandis Hill ex Maid., seedling seed orchard, clonal forestry, freeze resilience.

INTRODUCTION

Eucalyptus grandis was planted for pulpwood production in southern Florida from the 1960's to the early 1980's. Concurrently, a genetic improvement program conducted by the U. S. Forest Service increased tree vigor and quality considerably (Geary et al. 1983, Meskimen 1983). Severe freezes in January 1982, December 1983, and January 1985, extreme windborne/inversion freezes as low as -11°C and lasting for up to 18+ hours, provided unique opportunities to develop freeze-resilient, fast-growing trees. Recent genetic improvement activities have emphasized seed orchard development and clonal selection/propagation in support of a modest commercial planting program.

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METHODS

GP77, a 4th-generation genetic base population established July 1977 near LaBelle with 31,725 trees from 529 progenies (Meskimen 1983), was converted to seedling seed orchard G077 in 1986 (Reddy et al. 1986). Open-pollinated seed was collected in March 1987 from 21 of the best trees. Within a 25 ha test established near Palmdale in August 1982 with progenies from eight superior trees in the 3rd-generation orchard G073, 232 cloning candidates were located in December 1986 and propagated as rooted cuttings. These and other clones and progenies (Table 1) were outplanted in study ORNL-40 in August 1987 in the Palmdale-LaBelle area. Within a randomized complete block design with three replications, subplots were used for 1) clonal testing, 2) progeny testing, and 3) yield estimation. Clonal test subplots were subdivided into seven sets, each typically containing 4-tree row plots of 37 new clones and three clones 2798, 2814, and 2817 determined to be superior by Meskimen et al. (1987). Progeny test subplots consisted of 10-tree row plots of four progenies from G073, ramets and progenies of G077 ortets 2798, 2805, 2814, and 2817, and 17 other G077 progenies. Yield subplots were 16 24-tree blocks (four rows of six trees) consisting of pure plots of ramets or progenies of 2798, 2805, 2814, and 2817 and mixed plots of various clonal and progeny pairs. Within-row spacing was 1 m; between-row spacing was 2.85, 2, and 1 m, respectively, in the three replications. Height, DBH, and survival data were last taken in October 1988.

Narrow-sense and broad-sense heritabilities and genetic gains were calculated for 1.25-year height and survival using variance components derived by SAS. Survival analyses followed procedures outlined by Becker and Marsden (1972). Genetic gains for an open-pollinated seedling seed orchard with progeny testing and for clonal selection and testing were based on methods presented by Shelbourne (1969) assuming 10,000 trees were outplanted, e., 20 seedlings or ramets for each of 500 progenies or clones, respectively.

Table 1.--*Eucalyptus grandis* clones/progenies in ORNL-40 clonal test, progeny test, and yield test components by orchard origin and generation of selection.

Orchard Origin - Generation of Selection	ORNL-40 Clonal Test	ORNL-40 Progeny Test	ORNL-40 Yield Test
G073 - 1	1499,[1]		
G073 - 2	888, 1038	847, 1038, 1070	
G073 - 3	997, 1001, 1003 1010	1010	
G077 - 1		3674	
G077 - 2	2814, 2817	2814, 2817, 3336 3424, 3698, 4249 4292, 4305, 4361	2814, 2817 4305
G077 - 3		3516, 3521, 3706 4161, 4268, 4304	
G077 - 4	2798, 2805	2798, 2805, 4157 4160, 4360	2798, 2805

[1] Accession code of ortet/mother tree

Preliminary laboratory freeze screening of E. grandis clones 2786, 2788, 2798, 2805, 2814, and 2817 and E. camaldulensis clone 174 was conducted in October 1988. Up to five approximately six-month-old tissue culture plantlets, rooted cuttings, and seedlings (for E. grandis ortets 2798 and 2814 only) per clone were subjected to five hours of -3, -5, or -7°C during a 24-hour period beginning and ending at 4°C. Response was evaluated after two weeks as percent of leaves retained and after four weeks as percent of prefreeze stem height alive and as percent survival.

Growth and physiological response of plantlets produced by direct micropropagation of clones 2798, 2814, and 2817 (Rockwood et al. 1988) were compared to that of their half-sib seedlings through five months under greenhouse conditions. Hardened plantlets and germinated seedlings were moved from mist to the greenhouse at the same time, and base line dry matter data were collected. Over four harvests at 40 day intervals, plantlets were then compared to seedlings for: survival, net photosynthesis per dry weight, chlorophyll concentrations, total nitrogen percent, dry matter allocation and relative distribution, and relative growth rate.

Large-scale micropropagation of superior E. grandis clones was conducted in 1988 and 1989. In 1988, approximately 40,000 propagules of 11 clones including 2798, 2814, and 2817 were produced using media and procedures described by Rockwood et al. (1988). In 1989, similar procedures were used to produce 40,000 propagules of clones 174, 2798, 2805, 2814, 2817, and 5000. After three weeks in rooting, 20,000 plantlets were transferred to containers to develop and harden for 12 weeks prior to outplanting in July.

RESULTS AND DISCUSSION

Through 1.25 years, differences among the three reps (planting sites) in ORNL-40 vividly demonstrated the influence of site preparation (Table 2). On the site that had very thorough disking and high beds, clones 2798, 2814, and 2817, for example, averaged 93% survival and 5.4 m tall. On the site with low beds, their survival was also 93%, but their height was only 2.0 m, while on the third site, which had herbaceous competition, their survival dropped to 55% and height to 1.6 m. Good site preparation, specifically preplanting application of ground rock phosphate (Geary et al. 1983), bedding, and thorough vegetation control during the first year, is essential to realizing the growth potential of improved E. grandis.

At 1.25 years, progenies derived from 21 of the top-ranked trees in G077, the 4th-generation orchard, were similar to four of the best progenies from the preceeding orchard G073 (Table 2). As these G073 progenies have been evaluated in four to seven other tests, the comparatively good height and survival of the G077 progenies in their first test is encouraging. In terms of selection generation (Table 1), the 21 G077 progenies averaged 2.71 and the G073 progenies 2.25.

Table 2.--Average height, DBH, and survival of Eucalyptus grandis entries in ORNL-40 on the best site and across three sites at 1.25 years.

Study Component - Genetic Entry	Best Site			All Three Sites	
	Height (m)	DBH (cm)	Survival (%)	Height (m)	Survival (%)
Clonal Test-					
Clones 2798, 2814, 2817	5.4	3.8	93	3.2a[1]	81a
232 New Clones	4.3	3.3	65	2.6b*	55b*
Progeny Test-					
4 G073 progenies	3.0	2.3	98	2.1a	73a
21 G077 progenies	3.2	2.5	94	2.2a	66a
4 G077 progenies	4.0	3.1	88	2.6a	62a
4 Clones	3.4	2.3	85	2.3a	69a
Yield Test-					
4 Progenies - Pure Plots	3.4	2.4	79	1.4b	61a
- Mix Plots	4.2	2.8	88	1.6a	60a
4 Clones - Pure Plots	4.9	3.2	82	1.7a	63a
- Mix Plots	5.0	3.3	83	1.7a	68a

[1]Genetic entry means not sharing the same letter within a study component are significantly different at the 5% level.
*Variability among genetic entries significant at the 5% level.

Other comparisons based on generation of improvement suggest that G077 should produce fast-growing, somewhat frost-resilient trees. G077 has an average selection generation of 2.77 and contains 296 4th-generation selections. Meskimen (1983) reported large improvements with generation of selection. In GP77 at 64-months, differences between all 1st- and 2nd-, 2nd- and 3rd-, and 3rd- and 4th-generation trees averaged 206%, 20%, and 55%, respectively, for coppice stem volume, and 4th-generation progenies were best in frost resilience and coppice quality (Reddy et al. 1986). Estimated genetic gains in coppice stem volume of seedlings derived from G077 (compared to the GP77 mean) ranged from 54% if seed were collected from all 1309 orchard trees to as high as 179% if seed was obtainEd from only the best tree in each of the top 50 progenies (374 progenies retained).

A 5th-generation seedling seed orchard developed by the strategy espoused by Franklin (1986) may continue the improvement in growth that has accrued from this low-ccst, short-generation, wide genetic base program (Table 3). While differences among the G077 progenies were not significant at the 5% level, individual (Table 3) and progeny mean (h^2 = .332) heritabilities and levels of variation resulted in an estimated gain in tree height of 27% above the 4th-generation. These heritabilities, low compared to the estimates of .29 to .63 reported by Reddy (1985) for coppice height, may reflect a higher level of genotype x environment interaction in ORNL-40. Predicted gain in survival was modest. Consequently, commercial seed collection from the best tree in each of the top 50 progenies in a new orchard could be expected to produce faster-growing seedlings than can now be produced by G077.

Table 3.--Heritabilities and genetic gains in 1.25-year height and survival for seedling and clonal propagation options.

Propagation Option-Estimation Basis	Height		Survival	
	h^2	Gain[1] (%)	h^2	Gain (%)
Seedling - 21 G077 Progenies	.241	27.1	.092	17.1
Clonal- Six Clonal Sets	.096 to .368	22.2 to 67.6	.020 to .104	4.2 to 27.5

[1]Relative to mean for test component providing heritability estimate

Seedlings from G077 ortets were comparable to their corresponding clones in one test component but worse in another (Table 2). In 10-tree row plots, four G077 progenies were slightly taller with lower survival than the rooted cuttings. However, in 24-tree blocks in the yield test component, clones in pure plots had better height and somewhat higher survival than progenies in pure plots. There were also suggestions in the yield test that within plot variability for clones was less than that of seedlings.

Among clone variability was evident in contrasting proven clones with new selections and within the new candidates in the clonal test (Table 2). Clones 2798, 2814, and 2817 grew and survived well. In comparison to these proven clones, the 232 new cloning candidates were shorter and survived poorly overall, reflecting generally less extensive root systems, but varied widely, suggesting selection opportunities for vigor and survival. In comparison to the proven clones, 29 new clones were better in height, and 24 new clones had higher survival. Three clones averaged over 6 m on the well-prepared site after 15 months, and in May 1989 individual trees were over 8 m tall.

Clonal variation was heritable, and larger gains were predicted for propagation of the 50 best tested clones than for seedling production (Table 3). Generally, broad-sense heritablilities exceeded narrow-sense estimates for tree height, indicating that non-additive variation is important for growth improvement. Clonal testing following selection enhanced genetic gain in tree height but had little effect on survival improvement.

Heritabilities and gains calculated from ORNL-40 clones were less than those estimated by Meskimen et al. (1987). As with the seedling estimates, genotype x environment interaction, approximately 26% of phenotypic variance, is a likely cause for the lower values. As observed in ORNL-40, Meskimen et al. (1987) noted that increases in tree size, as well as in frost resilience, would be greater if clonal candidates were tested. These gains in volume were nearly double estimates developed for seedlings from G077 (Reddy et al. 1986).

Clonal selection and testing then is the best short-term alternative for increasing tree size and developing sufficient frost-resilience. Several options are available for expanding the number of cloning candidates from the current 340 to over 1,000 (Meskimen et al. 1987). The 232 new clones in ORNL-40, whose ortets had minimal stem damage and magnificent freeze-resilience after the

December 1983 and January 1985 freezes, were generally undamaged by a -6°C inversion freeze in February 1989. The uncertainty of testing clones by unpredictable natural freezes, however, necessitates the development of an artificial system of freeze evaluation as an adjunct to field testing.

Laboratory freeze screening results varied with temperature and clone (Table 4). The -3°C minimum was least damaging while the -7°C regime was most severe, but neither temperature caused much differential response among E. grandis clones. However, E. camaldulensis clone 174, excellent in field freeze resilience, surpassed all E. grandis clones in survival at -7°C. Five of six E. grandis clones survived -5°C, and the survival percentages of the six at -5°C paralleled (r = .65) field resilience observed by Meskimen et al. (1987). Notably, some seedlings from clones 2798 and 2814 were hardier than ramets from the same clones.

Table 4.--Responses of seven Eucalyptus clones to three temperature regimes in a laboratory freeze test.

Temperature-Clone	Freeze Response: Two-week Leaf Retention	Four-week Live Stem Height	Four-week Survival
		(%)	(%)
-3°C - All	95 A[1]	95.1 A	97.8 A
-5°C - 174	0 a	1.2 b	100.0 a
- 2786	0 a	1.0 b	50.0 ab
- 2788	0 a	0.0 b	0.0 b
- 2798	0 a	2.1 b	87.5 a
- 2805	0 a	0.6 b	20.0 b
- 2814	25 a	30.0 a	75.0 a
- 2817	0 a	0.6 b	18.2 b
- All	4 B	6.4 B	50.0 B
-7°C - All	0 B	.1 B	12.8 C

Overall means or clone means not sharing the same upper-case letter or lower-case letter, respectively, are significantly different at the 5% level

Micropropagation of proven clones can be an alternative to rooting of cuttings if plantlets grow normally. Development of plantlets produced by direct enforcement of buds from clones 2798, 2814, and 2817 was generally similar to that of seedlings in the greenhouse, with survivals of 98% and 95%, respectively. Plantlets had significantly lower net photosynthesis, chlorophyll a and b concentrations, and total nitrogen percent than seedlings after 40 days in the greenhouse; however, no differences were observed in later harvests. Photosynthesis was correlated more with foliar nitrogen than chlorophyll concentration. Plantlet dry matter accumulation, relative distribution, and relative growth rates differed significantly from seedling values over harvests, but the differences decreased sharply over time. Plantlets had higher shoot and root dry matter and higher root:shoot and relative growth rates than seedlings. The higher root:shoot ratio may have contributed to the high survival observed. Plantlet and seedling trends with harvests indicated similar basic responses to

changing environment.

Genetic differences, i. e., among plantlet clones and among seedling families, were only significant for root and shoot dry matter accumulation and their ratios. Rankings by genetic origin across plantlets and seedlings suggested genetic control independent of propagation method. Through four months in a field test, plantlets and seedlings from these same clones have shown similar trends.

The E. grandis clones used commercially also differed in their response to direct micropropagation. In 1988, clones 2798, 2805, 2814, and 2817 all had adequate multiplication rates. However, their relative sensitivity to cytokinin ranged from 2814 (most) to 2805 (least). Production of apical meristem callus in conjunction with tip necrosis was noted in 2798 and some minor clones. In the greenhouse, all clones were susceptible to an unexpected Cylindrocladium scoparium infection, which halved the number of plantlets available for transfer.

The problems encountered in 1988 were resolved in 1989. Tip necrosis was avoided by using a shorter transfer cycle and careful selection of material. Rooting was higher after altering nutrient concentrations in the rooting medium, and survival following transfer to the greenhouse increased. The container used for growing the outplantable tree was very suitable for good root system development. No infection was incurred.

These commercial efforts have suggested that the cost of a plantlet rooted for transfer to the greenhouse could be as low as $.15. Further progress with growth regulators in production and rooting and with methods for establishing propagules ex vitro will be needed to reach this goal, however. Additional cost reduction may be realized by using plantlets as greenhouse stock plants for hedging. Clonal micropropagation is currently more expensive than rooted cuttings, at an estimated cost of over $.11/cutting, and seedlings.

Genetic, silvicultural, and propagation potentials with E. grandis are promising for its production in southern Florida. Currently, its wood is in strong demand for mulch. It is one of several Eucalyptus species equal to native hardwoods for common pulping processes (Franklin 1977), is also suitable for hydrolysis and for the ester pulping process, and varies genetically in stemwood specific gravity (Rockwood et al. 1988, Wang et al. 1984). Under present economic and production scenarios, E. grandis seedlings and cuttings can be profitably grown in short rotations for energy wood (Rockwood and Dippon 1989).

CONCLUSIONS

Superior progeny-tested trees in G077 may provide commercial seed for vigorous, somewhat frost-resilient E. grandis. Testing of cloning candidates can result in greater increases in tree size, and clones presently under test may have excellent freeze-resilience. Three clones now recommended for commercial propagation will greatly reduce the freeze risks associated with E. grandis culture in southern Florida. Propagation by rooting and tissue culture is biologically feasible and economically promising. Plantlets survived well under greenhouse conditions and had growth and physiological trends similar to seedlings.

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