Field performance of micropropagated, macropropagated, and seed-derived propagules of three Eucalyptus grandis orrets

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ABSTRACT

Tree size, survival, and coppicing of micropropagated plantlets, macropropagated cuttings, and seedlings of Eucalyptus grandis Hill ex Maiden were monitored through 57 months in a study in southern Florida to assess propagation options. Two plantation lines developed by direct micropropagation and orchard open-pollinated seedlings from three orrets were compared in the main study. Rooted cuttings from up to four months of each of the three orrets and orchard orrets were tested in an adjacent supplemental study. Pests and air and soil treatments were observed beginning at 31 months. Due to the poor performance of seedlings of one orret after the second freeze, at 57 months, no differences in tree height, DBoL, volume, or survival were detected between plantation lines and between rooted cuttings and plantlets, but seedlings were inferior to plantlets and cuttings. Vagant propagules had more uniform tree size at every age, with width less than one-half the variability observed among seedlings. Even though plantlets and cuttings may be more expensive to produce, they have numerous advantages over seedlings for E. grandis plantation establishment in Florida.

Keywords: These culture, plantlets, rooted cuttings, clones, seedlings, propagules

INTRODUCTION

In support of a commercial planting program for Eucalyptus grandis in southern Florida, four generations of genetic improvement were developed through the 1980s (Rockwood et al. 1989). Several cloning cultures were derived from the outset (Makinen et al. 1987), and beginning in the late 1980s, cloned trees and eight because of cloned plantations were established. Clones were propagated both by direct micropropagation (tissue culture derived plantlets) and macropropagation (rooting of cuttings). Previous commercial plantings had utilized only open-pollinated seedling of various orchard orchards (Guary et al. 1983).

Through three experiments, variable familiarity with the procedures and expenses of these propagation alternatives was obtained (Makinen et al. 1987, Rockwood et al. 1989, Warrag 1990). Early greenhouse comparisons of two types of propagules suggested that initial differences in photosynthesis, stomatal and xeromorphic symmetry, and dry weight distribution and partitioning decreased sharply from age 40 to age 160 days and would not differently influence the future performance of plantlets and seedlings (Warrag et al. 1989, 1993). Consequently, field performance of the various types of propagules is critical to a complete assessment of propagation options. This paper reports results of a study comparing plantlets, cuttings, and seedlings from the same orret.

MATERIALS AND METHODS

Field performance of these propagules was assessed in southern Florida. The study involved four sources of propagules of three of the best orrets (Orret 1708, 2144, 2157; Makinen et al. 1987) in a fourth-generation seeding orchard (Rockwood et al. 1993). Open-pollinated seedlings were collected from the orrets in March 1987. In 1982, numerous rooted cuttings of each tree were inoculated in cloned test ORNL-37 (Makinen et al. 1987). Early in 1988, 30 plantlets were developed for each orret by direct micropropagation using nodal segments from individual microsta-

The University of Florida (UF) plantlets were propagated from procedures described by Warrag et al. (1989), and the Hayman's Plants Inc. (HP) plantlets were rooted using techniques similar to those outlined in Rockwood et al. (1988). The seedlings and UF plantlets were started in a greenhouse at Osage (FL), the HP plantlets were rooted in Hayman's facilities at Palmdale (FL). All plantings and seedlings were then transplanted and grown for three months to field test at the Florida Division of Forestry's Herren Nurseries at Lake Placid, FL, concurrently with the production of rooted cuttings from several clones of certain orrets in ORNL-37 using protocols described by Makinen et al. (1987). Plantlets and seedlings had the same root origin in ORNL-37 if possible, specifically, Orret 1708 UF plantlets and rooted cuttings came from clone CM5-1; HP plantlets for Orret 2144 were used from clone AR2-4 (which contributed cuttings along with clones AR-2, RL-2-3, and CM5-4), and the HP plantlets for Orret 2157 were derived from clone CM5-5, which along with root CM5-1 also produced cuttings.

The study, comprised of main and supplemental components, was established on August 1, 1988, near Palmdale, FL, on an infertile, seasonally poorly drained sandy loam site typically used for E. grandis culture in southern Florida. Soil on the site was a mose from the Great Group of Highlands. Site preparation consisted of double chopping, burning, broadcasted 1530 kg/he of ground rock phosphate, and holding on 3 mm. Trees spacing along beds was 12.5 m.

The main study comprised plantlets and seedlings in a randomized complete block design of three replications with a split-plot arrangement of treatments. Within a replication, each orret main plot was randomly assigned to one of four beds. Within an orret main plot, three subplots (five HP plantlets, five UF plantlets, and 10 seedlings from the orret) were randomly assigned to a row plot. To sample microwave variance within the 25-60 mm main plot, the fourth bed in each replication was planted with 20 plantlets of an E. grandis cultivar 'Deboli', clone propagated by Hayman's Plants. A six-replicated supplemental study compared rooted cuttings
only of masses of the three trees on six beds differing for the main study.

The studies were made in 1982 and 1983 on six beds differing for the main study. The 33 trees on each bed were planted in two rows of 17 trees. Each tree was then assigned to one of the 17 treatments. The trees were divided into three groups: control, 0.5% NaCl, and 3% NaCl. The treatments were applied by drip irrigation. The trees were then subjected to a 10% decrease in water availability. The experiments were conducted over a period of three years. The results showed that the 0.5% NaCl treatment had a negative effect on the growth of the trees, while the 3% NaCl treatment had a positive effect. The control treatment had no effect on the growth of the trees.

Twelve 100-plant plots of Orna 2065 comprised a sixth bed in order to separate variation along the beds in the supplemental study. The 12 beds of 20 trees in the two studies were surrounded by one border row of plantings and settings, for a total of 40 trees in an area of 60 x 22 m.

The studies were measured periodically through 57 months. Stem height (S) and survival (S) were initially measured as age two months (S2O and S20) and were reassessed at age six months (S06 and S06), just preceding a strong invigorating treatment. Measurements at 6 months demand the degree of stem dbhca due to the February 1989 freezes to treat in terms of 20% portion of the mean killed (only scores of 4—6% of 8% killed and 4—6% killed) to the ground with clipping were observed along with height (S15) and survival (S15). To assess response to a severe climate that occurred one week after the 16-month measurement, height (S21), survival (S21), and stem discoloration caused by the December 1989 freezes were measured in May 1990 at seed age 21 months. Two trees were at age 45 months, height (S45), DBH (D45 = stem diameter at 1.4-ds above the ground), and the number of stems (NaS) with DBH at one-half of the largest mean DBH were determined. Similar conclusions were drawn one year later in May 1991, except that height (S27) was measured only on a stratified sample of 35 trees representing the DBH (S27) distribution in one replication.

All analyses utilized the Statistical Analysis System (SAS, 1988). E57 was treated only for E57 was conducted by the equation:

\[ \log E57 = 0.4866 + 0.5462 \log S57 \quad (R^2 = 0.82) \]

with E57 on in E57 and S57 in m. Individual tree volume scores 45 and 57 months (V45 and V57) were estimated by an index of SPAD257 that adjust for increment in volume along beds within a replication was averaged by assigning the height of the E. superba clone to Orna 2065 and the beds as the basic unit of analysis. Analysis of variance (Table 1) were then conducted with and without appropriate covariances were. Within propogate or across variability for each tree was expressed as a coefficient of variation (CV) relating the within-plot standard deviation to the propogate mean.

RESULTS AND DISCUSSION

Environmental factors having effects on growth in the study. Analyses were generally important for the trees at any age (Table 1). Microsite variability within replicates, as evidenced through the nonuniform pattern of the height of the E. superba clone to Orna 2065 plantations as covariates, was estimated. Tree development was significantly affected by age in trees age 15 months, though the fore stresses, February 1989 (2065 = 1.4-ds above the mean) for growth in the ground. The more severe December 1989 freeze on the ground. The December freeze, results reported for more age 21- 45, and 57 months periods in form-cospective status of ages 1, 5, and 10, 15, and 20, months, respectively.

Main study

Most developmental differences in the main study were consistent from age 1 up to 25 months (Table 1). Averaged across ages 2794, 2814, and 2817, the E. solidaginoides were similar to each other and larger than the seedlings for height at all ages. DBHs for the two other ages, tree volume at 6 months, and survival at all ages except six months. Plantlet survival was 100% for the duration of the study, but after the February 1989 freeze, seedling 516 decreased markedly relative to the plantlets (Figure 1). Following the December 1989 freeze, seedling 516 Table 1. Significance of sources of variance for 18 months of 2—5.49 months of E. superba propogation. Main Study — Beds (R), Orna (O), R x Orna. Propogation vs. Seedlings (P vs. S), Orna (O), R x Orna. Supplemental Study — Orna, O). June/France/209 (O).

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Figure 1. Stem height (top) and survival (bottom) of two planters lines (HF and UF), seedlings (S), and rooted cuttings (RC) of three J. koreanum cuttings through 57 months.

Supplemental study

In the supplemental study, variation among names of an ornamentals were generally insignificant for all traits and ages (Table 1). Stem height varied with name at young cohort ages (015 and 035) but was not influenced by name origin soon after planting (016 and 0160) or at older cohort ages (054 and 057). Rates influenced survival only at 6 months. At the older ages, cohort stems (0160,045 and 075), volume (045 and 057), and stems/tissue (045 and 075) were similar across names. These results suggest that no variability has resulted from one propagation cycle for rooted cuttings, i.e., first-cuttings rate did not produce growth differences in second-cycle cuttings.

Variation among the four cuttings was evident in the rooted cuttings. Among the three cuttings cuttings to the main study, Cut 1798 tended to have the smallest means at 57 months, whereas Cut 2817 had the largest (Table 2). The cuttings did not differ in 045 or 057. These comparisons of the cuttings based on cuttings were similar to the three between Orms 2014 and 2017 represented by planters. Cut 2798 was minimally used in the supplemental study.

Rooted cuttings were also more uniform than seedlings (Table 3). In height at all ages, cuttings of Orms 2798, 2814, and 2817 had CVs under 20%. At 45 and 57 months, cutting 2810, and volume were again more homogeneous than seedlings. The observed uniformity for cuttings to have about one-half the variability of seedlings in height is supported by a comparison of 0-year-old J. koreanum in Columbia averaging 67% to height; the CV of intact clones was 6.9% compared to 15.0% for seedlings (Lambeth et al. in press).
approximately twice the survival and about twice the mean size, plantings and cuttings had from 2.45 to 3.0 times greater yield per hectare than seedlings. Calculations by Miersch and others (1987) suggested that use of these clones would permit twice as much genetic yield as various strategies to produce improved seedlings. In a study established in 1987 with two plantings and seedings of the same four clones used in this study, cuttings were only 45% taller than seedlings after 1.0 years (Rickwood et al. 1989). After the December 1989 freeze, however, visual differences between cuttings and seedlings were as evident in the 1987 study as in this study. Kluge et al. (1987) reported that plantings of E. grandis and E. uoyunuth had 37% and 50% more biomass, respectively, than seedlings after 34 months in the field.

The relative cost of producing vegetative propagules made it desirable to discourage their use for plantation establishment. Francis and Byerly (1982) estimated that planting cost was three times greater than seedling cost. A potentially low-cost procedure outlined by Wuytens et al. (1991) might produce one million plantlets within a year from a single explant as follows: 1) after six weeks, up to 12 shoots per shoot per explant in a multiplication medium, 2) after division in four clones, up to 12 shoots per shoot per explant in a multiplication medium, 5) with seven subcultures, more than 2.5 million shoots, 4) in elongation medium, 50% of the shoots elongate in 15 days, 5) over 80% rooting and survival in the greenhouse and the field. This volume could be increased with the use of a bioreactor to reduce production costs. Subcultured, in vitro tissues from seedling hypocotyls also show promise for low-cost plantlet production, but no other plant material from clones 2788, 2814, and 2373 were used in this procedure (Wuytens et al. 1991).

The growth, yield, and quality characteristics of vegetative propagules argue strongly for favoring either plantings or cuttings of superior E. grandis clones in commercial plantations in semi-arid environments. Propagation of vegetative origin may be more economical and are more likely to produce successful coppice stands. Even though plantings are currently more expensive to produce than cuttings, and cuttings are more expensive than seedlings (Rickwood et al. 1989), an additional investment in vegetative propagules of total superior clones for E. grandis plantations in southern Florida appears justifiable.

CONCLUSIONS

More developmental differences in the main study were consistent from age 2 to age 54 months. Some propagations by clone interactions were observed beginning at age 21 months, after the second freeze, for primarily to very poor performance of cuttings and seedlings. By age 54 months, no differences were observed between two plantlets, and seedlings were considerably different from plantlets. Vegetative propagule production more uniform even at age 54. Plantlets or cuttings may have twice the survival and the size of seedlings, or same frequency before age 24 months. Propagation of vegetative origin may be more likely to produce successful coppice rotations. The growth, yield, and quality characteristics of vegetative propagules argue strongly for favoring either plantings or cuttings of superior E. grandis clones in commercial plantations.

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Table 5. Coefficients of variation within plant variability for two plantlets (B), seedling (S), and mean height of cuttings (RC) of three E. grandis clones for height, DBH, volume, and cupping through 57 months.

<table>
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Table 6. Coefficients of variation within plant variability for two plantlets (B), seedling (S), and mean height of cuttings (RC) of three E. grandis clones for height, DBH, volume, and cupping through 57 months.