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PROJECT NO. I-204-80

Vegetative Propagation of Selected Clones of *Eucalyptus Camaldulensis* Dehn

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Abstract

A technique was developed to mass produce rooted cuttings of superior trees of Eucalyptus camaldulensis Dehn. Major findings were as follows.

Best coppice production is achieved in Israel by felling trees during March-May. In Florida, March is recommended for girdling with a chain saw at 100 cm above ground. Grafting to rejuvenate mature tissue is useful for producing cuttings and to start a clonal line without damaging an elite tree. Offspring of stockplants younger than 8 weeks is too shortlived for the cuttings to root. Shading of 50% consistently lowered quantities of cuttings by approximately 30%. Open growing of stockplants will provide the largest number of cuttings at the lowest expense and effort. 10,000 stockplants per ha spaced 1 x 1 m can produce > 1,000,000 cuttings per year yielding some 500,000 rooted plants. Seedling growth is directly related to size of container, but neither container nor spacing apparently affect root-collar diameter. Root and shoot dry-weights also increased with increasing tube size. Shoot dry-weight appears to be inversely related to seedling density. Shoot-root ratios on a dry-weight basis were directly related to tube diameter, and indirectly related to tube length. Cuttings can be rooted in a wide range of media, preferably those with high water-holding capacity. Heating of the rooting medium improves and accelerates rooting of cuttings with intermediate or high rooting capability but does not improve rooting of difficult-to-root clones. Bottom heat should cease after rooting has taken place to increase root and shoot growth. The positive effect of frequent mist application is confirmed provided that the leaves are treated with Benlate and Captan ($0.8 \text{ g/m}^2/\text{week}$) added to the spray system. Large-diameter cuttings (3-7 mm) had higher percentages of rooting and flushing. Bleaching the base of cuttings accelerates rooting but does not raise final rooting percentage after longer period under mist. Etiolation of the lower end of the cutting at the early stage of shoot growth is most effective to improve rooting. There was no difference in rooting between two - and four - leaved cuttings. Rooting capability among clones varies considerably (4-93%). Seed source appears to affect markedly the rooting percentage. In in vitro culture a polystyrene plug system (polyurethane) for rooting and hardening of explants is recommended. External contamination was highest in explant derived from mature trees and very low in explants derived from cut-back stockplants.

Explants derived from coppice shoots occupied an intermediate position. The ratio between the number of new cultures, obtained from explants cut into new segments, and the initial number of cultures, increased until it reached a steady state typical for each clone. Cytokinin BA suppresses rooting even if auxin is added; the auxin IBA is more effective than NAA in inducing rooting; both cytokinin and auxin are needed to achieve maximum growth. Rates of response and growth are not the same in the 4 clones compared. Of the different concentrations tried, 0.25 mg/l seems adequate in most cases; higher concentrations induced callus production and some depression in shoot growth. Maximum shoot production was achieved with a combination of both cytokinin BA and auxin NAA each at the rate of 0.25 - 0.5 mg/l. Induction of root formation in all clones studied was easy. Propagation of clone ILA-IV from explant was easy but difficult from cuttings under mist.

A. PRODUCTION OF CUTTINGS

Cuttings from seedlings and basal stem shoots of many Eucalyptus species often root, but those from branches rarely do (Hartney, 1980). Therefore, to clone silviculturally superior Eucalyptus trees by cuttings, the trees are girdled near the base to stimulate epicormic shoot growth below the girdle, or the trees are felled to produce coppice shoots. An alternate method for trees that fail to produce basal shoots, or which cannot be felled or girdled, is to rejuvenate branch tissue by grafting it on seedling rootstocks (Cauvin, 1982). Cuttings from scion shoots that are produced after grafting often root. The rooted cuttings produced from epicormic, coppice, and scion shoots can be used as stockplants for multiplying cutting production. We tried the three basic methods of initial cutting production on E. camaldulensis Dehn. and also studied the effects of cultural treatment on the maintenance of stockplants and cutting production from them.

1. Girdling - T. Geary, U.S.A.

Objective.: To determine the effects of season, girdling tool, and girdle height on epicormic shoot production.

Methods: A fresh group of 55 trees that were randomly scattered through a seven-year-old plantation were girdled each month for a year at 15 cm above ground with an electric router that cut a 12 mm wide opening down to sapwood. In March 1982, towards the end of the seasonal study, three additional girdling treatments were tried: router girdle at 100 cm above ground, and chain-saw girdles at 15 and 100 cm. The chain-sawed girdles were also 12 cm-wide, and cut to sapwood.

Results: No conclusions were made on the effect of season, because girdles made with a router at 15 cm did not cause many shoots to be produced in any month. Girdling with a chain saw was much more effective than with a router, and girdles made at 100 cm produced many more epicormic shoots than at 15 cm, as shown in Table A.1. None of the girdling treatments killed any trees.

Discussion: The greater production of shoots from chain-saw girdles was not obviously due to greater depth or width of girdle over that of the router,

because both tools produced girdles of similar dimensions (7 mm-wide). A chain saw is then a preferred tool for girdling, because of its portability, and the speed at which girdles can be made. The fear that chain-saw girdles would kill trees was unfounded. While the best month for girdling is still to be determined experimentally, March was a good month for girdling with a chain saw at 100 cm; and because it would be expected that spring would be the most effective time for inducing epicormic shoot production, March is likely to be one of the best months.

2. Coppice Production - D. Heth and L. Fanger-Vexler, Israel.

Objective: To study the effect of cutting season on coppice production.

Methods: A provenance trial at Ein Teo, 8 km south of Qiryat Shmona, Hula Valley, was selected for study. A description of the experimental plot is given in Table A.2. Eight trees (two each of four provenances) 15-years-old, spaced at 3 x 3 m apart, were felled monthly from December 1980 until December 1981. The number of shoot clumps (epicormic knobs or bursting centers) and the height of the tallest coppice shoot on each stump were measured monthly for a year from date of felling.

Results: Coppice emerged most rapidly from March to May fellings (Fig. A.1). Felling at other times resulted in an emergence delay of up to four months. In no felling season was stump mortality pronounced and only 5% of the stumps failed to coppice within one year. The mean number of shoot clumps per stump after 12 months was 5.1 (Fig. A.2). After a spring felling, the maximum number of shoot clumps was reached within four months and then the number dropped; some died and some broke off. In contrast, felling in other seasons required at least six months to reach the maximum number.

Height growth of the tallest shoot per stump, followed a strongly seasonal pattern (Fig. A.3). Growth surged at the end of spring, reaching approximately 0.5 m per month until September, then dropped to less than 10 cm per month throughout winter. The mean height of the tallest coppice shoot per stump after 12 months ranged between 2 and 3 m, regardless of month of felling.

Discussion: Coppice emergence and height growth varied greatly according to season of felling. A spring felling period (March - May) is best for quickly achieving maximum coppice production of E. camaldulensis. Similar results were received in France with other Eucalyptus species (Cauvin, 1982) when late spring (May 15 - June 15) was found to be the best time for coppicing. However, in Italy, Ciancio and Morandini (1971) did not find a significant relation between season of felling and coppice growth of eucalypts. In Israel, coppice emergence of E. camaldulensis continues at a lower rate during summer and early autumn (July - September) and nearly stops during winter (November - February). Height growth of coppice shoots was very energetic during summer and nearly stopped during winter. In conclusion, best coppice for subsequent vegetative propagation in Israel can be achieved by tree felling during the months of March - May.

3. Production of Stockplants by Grafting on Seedlings - O. Reuveni, Israel

Objective: To rejuvenate mature tissue so that rootable cuttings can be produced.

Methods: Three different seed sources were used for seedlings. The seedlings were grown in a 750-ml container filled with peat:polystyrene flakes (1:1). Daily drip irrigation was done from 5 to 15 h with 400 mg NPK fertilizer (3:1:4) per liter. Temperatures in the greenhouse in summer were 15° - 28°C and in winter 14° - 25°C. Three-to-five-month-old seedlings were top cleft grafted with scions taken from four different mature selected trees at Ilanot and labelled as ILA I to ILA IV. The grafts were enclosed in plastic bags and shaded to prevent desiccation until graft union. Three to four months later, the first batch of cuttings was harvested for rooting under intermittent mist in another greenhouse. Then the grafted plants were transferred to 4-l containers to enhance growth and cutting production.

Results: Initial grafting success was high and was not affected by scion or seedling origin. Leaving a few leaves on the rootstocks was essential, because rootstocks without leaves died. Shortly after stock and scion tissue united, incompatibility between scions and rootstocks was noted. The early sign of incompatibility was a swelling at the graft union of both the rootstock and the scion, or the rootstock alone. Later signs were new shoots below the graft.

Most incompatible grafts eventually died. The incompatibility and desiccation of grafts were characterized by variability in frequency and time of occurrence. The take of grafts and survival with time are given for clone ILA IV in Fig. A.4. One month after grafting, 81% of the grafts were alive but one year later only 13% of the grafts survived.

Discussion: The disadvantage of grafted plants as a permanent source of cuttings is the eventual death of scions from incompatibility between scions and the rootstocks. However, grafts are useful for producing initial cuttings to start a clonal line without damaging an elite tree. In general, the incompatibility in this study was not very serious right after grafting, which is in contrast with other eucalypts (Davidson, 1978; Burgess, 1974). Death of the scions was delayed to some extent if new rootstock shoots were removed. It pays to harvest 1 to 2 cuttings from a grafted plant before it dies. Another advantage of grafts over epicormic shoots and coppice is the proximity of the cutting source to the rooting facilities. If only a few cuttings from one grafted stockplant are wanted, then there is no need to maintain the stockplants in big containers. The grafted plants can be grown in 750-ml containers from the time of sowing of the rootstock until the first cuttings are harvested from the graft scions.

4. Production of Cuttings from Pruned Stockplants - O. Reuveni, D. Heth and L. Fanger-Vexler, Israel

Objective: To study the effect of shade and fertilizer on cutting production of stockplants.

Methods: Cuttings taken from grafts and rooted cuttings were planted in mid-July 1982 at Ilanot's semi-commercial pilot plant, in the Central Coastal Plain (Table A.2.). The semi-commercial pilot plant was designed to provide space for 480 stockplants, or 120 plants from each of four selected Ilanot clones, when planted at a spacing of 0.5 m within rows and 1.4 m between rows, in the four growth areas (Fig. A.5). The four growth areas (A-D) allowed manipulations for studying the effects of light and heat. During summer, areas A and B were covered with 50% shade netting and C and D were open to full sunlight. From October to June, A and C had a 0.1 mm plastic (polyethylene) cover. In each area, 30 stockplants of each clone were planted along three separate drip irrigation lines

(10 stockplants per clone) to which different levels of a fertilizer were injected. A 1-m-deep plastic soil barrier prevented crossover of roots between treatments. Each plant was provided with one dripper (2 l/h). The plants were irrigated with 1 to 2 liters of water per day depending on the season. Three fertilization treatments: approximately 100, 200, or 400 ppm of (20 N: 20 P: 20 K + microelements) were injected into the irrigation water. Later, the lowest level of fertilizer was omitted. Several pruning methods were tried on clones ILA I, II, and III to maximize cutting harvests. In the first three harvests, stockplants were pruned to no more than 20 cm height, and only the lowest side branch remained. This branch was removed when new growth elongated to 10 cm. Two major disadvantages become evident with this method. Firstly, the severe pruning made plants susceptible to fungal attacks, especially on new succulent shoots. The fungi controlled by spraying the plants with Captan 0.25% + Benlate 0.15%. Secondly, many shoots were cut but not used, because they were too small in diameter to be used as cuttings. The method was improved at 4th harvest, stage I, by pruning only cuttings with a minimum basal diameter (i.e., 3 mm) and then returning to cut three weeks later (stage II). This method gave healthy stockplants, vigorous growth and steady supply of cuttings. The harvesting potential was determined by counting the number of six-leaf cuttings (an eight-leaf stem segment with the bottom two leaves removed to provide insertion depth) that were taken from a stockplant.

Results: Table A.3 shows the quantities of six-leaf cuttings harvested per stockplant for each growth area throughout the experiment. The type of covering had an important effect on growth, and therefore cutting numbers. Shade netting of 50% consistently lowered quantities by approximately 30%. The addition of a plastic layer over the netting during the winter months did not appear to promote growth. Winter plastic alone did increase quantities by 7-38% for the spring harvesting periods; however, this does not appear to justify the expense of covering. Open growing of stockplants will provide the largest number of cuttings at the least expense and effort.

Increased levels of fertilizer showed an initial response in growth, which later disappeared (Table A.4). This may be explained by the location of the growth areas on a previously abandoned field of red sandy loam with a hardpan at approximately one meter. The early applications were highly beneficial, whereas

with time the soil fertility was restored and even the lowest fertilizer levels brought about good growth. Reduction of the lowest level to zero was done late to reverse this effect.

Discussion: Stockplants required about six months growth and pruning to develop sturdy stems and begin production of uniform shoots from the basal portion. During 1983, five harvests were made from the stockplants every six weeks from about April to November. In France (Cauvin, 1982), harvesting of eucalypt stockplants during spring showed an optimum shoot age of 8- to 12-weeks for the production of cuttings that rooted best. Cuttings younger than 8 weeks were too fragile to live the four weeks necessary for rooting. Using open conditions (Growth Area D, Table A.3), the mean number of six-leaf cuttings available from a stockplant per year was between 70 and 120. Numbers could have been increased substantially by utilizing cuttings of smaller diameters and lengths. If four-leaf cuttings were used then potential harvests would increase by at least 25%. At a planting density of one stockplant per square meter, one hectare could produce well over a million cuttings per year. If 50% of these million cuttings rooted and these were planted in plantations at a spacing of 3 x 3 m, an area of 455 ha can be planted. The ratio between stockplant nursery and plantation is 1:450. Even if this ratio were larger, it would still be smaller than the 1:100 ratio found in Aracruz, Brazil (Campinhos and Ikemori, 1983), and the 1:83 ratio in the Congo (Martin and Quillet, 1974). The better ratio obtained in Israel is due to the more intensive cultural practices and closer spacing in stockplant management.

Table A.1 Trees that sprouted and bursting centers/tree as related to girdle height and girdling tool

Girdle height and girdling tool	Trees that sprouted (%)	Bursting centers/tree (No.)
15 cm		
Router	20	2.7
Chain saw	68	4.8
100 cm		
Router	51	7.6
Chain saw	89	9.1

Table A.2 Description of the experimental plots

	Ein Teo (Hula Valley)	Ilanot (Central Coastal Plain)
Latitude N	33°08'	32°18'
Longitude E	35°34'	34°54'
Altitude	70 m	40 m
Mean max. temp. hottest month	34.4°C	31.1°C
Mean min. temp. coldest month	6.2°C	7.0°C
Mean annual rainfall	559 mm	603 mm
Geology	Quaternary alluvium	Quaternary sands
Soil	Grumusol	Red sandy loam
pH	8.0	6.5
Vegetation	Previously under field crops	<u>Desmostachya bipinnata</u> - <u>Centaurea procurrens</u>

Table A.3 Mean number of six-leaved cuttings harvested per stockplant grown under various area coverings

Clone	Growth area	First harvest		Second harvest		Third harvest		Fourth harvest				Fifth harvest		Total			
		Cov.*	no.	harvest	Cov.	no.	harvest	Cov.	no.	Stage I		Stage II		Cov.	no.	per stock plant no.	% control
										(24.IX-14.X.82)	(11.IV-20.IV.83)	(9.VI-19.VI.83)	(8.VIII-14.VIII.83)				
I	A	Ne	12.1	Np	12.4	Np	8.5	Ne	12.5	Ne	7.9	Ne	10.4	Ne	10.4	63.4	76
	B	Ne	13.4	Ne	13.5	Ne	8.3	Ne	14.0	Ne	5.3	Ne	9.3	Ne	9.3	63.8	77
	C	Op	17.2	PE	24.9	PE	16.4	Op	20.9	Op	8.0	Op	14.3	Op	14.3	101.7	122
	D	Co	16.5	Co	20.0	Co	15.3	Co	14.6	Co	6.2	Co	10.6	Co	10.6	83.2	100
II	A	Ne	19.9	Np	15.4	Np	14.5	Ne	7.2	Ne	8.9	Ne	8.4	Ne	8.4	74.3	60
	B	Ne	24.9	Ne	12.2	Ne	11.0	Ne	8.5	Ne	9.4	Ne	9.4	Ne	9.4	75.4	61
	C	Op	33.2	PE	26.6	PE	23.7	Op	9.7	Op	15.4	Op	10.8	Op	10.8	119.4	97
	D	Co	47.7	Co	19.6	Co	20.9	Co	10.2	Co	13.0	Co	12.2	Co	12.2	123.6	100
III	A	Ne	18.2	-	-	Np	13.0	Ne	7.9	Ne	7.0	Ne	7.2	Ne	7.2	55.3	74
	B	Ne	20.7	-	-	Ne	8.5	Ne	6.1	Ne	6.5	Ne	7.9	Ne	7.9	49.7	70
	C	Op	27.8	-	-	PE	18.0	Op	6.3	Op	8.6	Op	10.3	Op	10.3	71.0	99
	D	Co	29.1	-	-	Co	13.0	Co	7.6	Co	10.4	Co	11.4	Co	11.4	71.5	100

*Covering code: Ne = 50% shade netting; Np = 50% shade netting plus polyethylene; PE = polyethylene; Op = open, no covering in this season; Co = control, never covered.

Table A.4 Mean number of six-leaved cuttings harvested per stockplant grown with various fertilizer levels.

Clone	Fertilizer levels	First harvest ppm	First harvest no.	Second harvest ppm	Second harvest no.	Third harvest ppm	Third harvest no.	Fourth harvest-I ppm	Fourth harvest-II ppm	Fourth harvest-II no.	Fifth harvest ppm	Fifth harvest no.	Total no.
I	1	85	14.0	90	15.8	90	13.4	0	14.5	0	7.2	0	10.1
	2	170	12.2	180	16.9	180	9.6	200	14.2	200	5.9	200	10.9
	3	340	19.0	360	20.9	360	13.2	400	16.5	400	7.3	400	12.2
II	1	85	26.8	90	20.6	90	18.7	0	11.0	0	12.1	200	10.3
	2	170	29.2	180	17.5	180	15.3	200	6.5	200	11.9	200	9.1
	3	340	38.2	360	17.3	360	18.8	400	9.3	400	10.9	400	11.3
III	1	85	18.2	-	-	-	-	0	6.5	0	8.3	0	8.9
	2	170	27.7	-	-	-	-	200	6.4	200	9.0	200	8.3
	3	340	27.1	-	-	-	-	400	8.1	400	6.8	400	10.2
Total	1	85	59.0	90	36.4	90	32.1	0	32.0	0	27.6	0	29.3
	2	170	69.1	180	34.4	180	24.9	200	27.1	200	26.8	200	28.3
	3	340	84.3	360	38.2	360	32.0	400	33.9	400	25.0	400	33.7

ppm = fertilizer concentration in irrigation water.

Fig. A.1. Percentage of stools sprouted as related to month of felling and number of months until coppice emergence.

Fig. A.2. Variation with time of cumulative mean number of sprout clumps per stump .

Fig. A.3. Mean height increment of the tallest coppice shoot per stool as related to month.

Fig. A.4. Percent survival with time of 274 grafts of Eucalyptus camaldulensis.

Fig. A.5. Scheme of a semi-commercial pilot plant at Ilanot.

B. ROOTING OF CUTTINGS

Four aspects influencing rooting were studied:

- (1) Environmental conditions during rooting.
- (2) Cutting source and pretreatments of the stockplants.
- (3) Leaf quantity and leaf retention.
- (4) Clonal differences.

1. Effect of Environmental Conditions During Rooting

Rooting media constituents, media temperature and humidity influence speed and percentage of rooting of Eucalyptus (Campinhos and Ikemori 1983, Cauvin 1982, Cauvin and Marien 1979, Chaperon and Quillet 1977, Giordano 1961, Hartney 1980). The environmental factors studied were media constituents, media temperature, mist intensity, and fungicides.

a) Media - O. Reuveni and L. Fanger-Vexler, Israel.

Objective: To study the effect of media constituents on speed and percentage of rooting.

Methods: The commonly used medium in rooting trials conducted in Israel was peat moss and polystyrene flakes (1:1/v:v). This medium was compared to others in three trials. In the first one, 14 media were compared (Table B.1.). For each medium, 48 cuttings derived from young seedlings were planted in six randomized blocks under mist, and with 30°C bottom heat. Rooting was recorded after 30 and 50 days. In the second trial, the common medium was compared with vermiculite and polystyrene flakes (1:1/v:v). Twenty cuttings of clone BH (selected at the Univ. of Melbourne, Australia) were planted in each medium under mist in May. Bottom heat was not used. Rooting was recorded after 30 days. In the third trial, a mixture of peat and perlite (1:1/v:v) was compared to No.2 vermiculite and perlite (1:1/v:v). In each medium 130 cuttings were planted under mist in July. The cuttings came from coppice shoots of six randomly selected stumps. No bottom heat was used and rooting was recorded after 21 days.

Results: The percentage rooting did not differ significantly among the media used in the first trial (Table B.1.). In the second trial 70% of the cuttings in the peat: polystyrene (1:1) rooted, while only 46% in the vermiculite: polystyrene (1:1) did so. In the third trial, rooting in peat: perlite (1:1) was 50% and in vermiculite: perlite (1:1), 51%.

Discussion:

It was reported that peat might cause rotting (Campinhos and Ikemori 1983) and inhibit rooting (Hartney, personal communication). Our results do not confirm this. It was shown that eucalypt cuttings can be rooted in a wide range of media. This means that medium is not the main limiting factor in rooting. Choice of medium constituents can be decided by availability and price.

b.1.) Bottom heat - O. Reuveni, Israel.

Objective: Study the effect of bottom heating on rooting and its interaction with mist intensity.

Methods: Cuttings of the four ILA clones were used in a trial where the effect of bottom heating on rooting was studied together with the effect of mist frequency. Cuttings were derived from young (ILA-II and ILA-IV) or old grafted (ILA-I) stockplants. In one clone (ILA-I) cuttings were taken also from stockplants grown on their own roots. The rooting conditions and medium were as described in section A.4. Cutting bases were dipped in talcum powder containing IBA (0.8%) + Benlate (1%). Leaves were sprayed with Benlate and Captan (0.8 g/m², each) introduced once a week into the mist nozzles system. Half of the rooting beds were bottom heated (30°C) and half were not heated (25°C during day time). Half of the heated and non-heated beds were mist sprayed for 6 sec., every 1 min. (high intensity) and the other half - every 2 min. (low intensity), during day time. There was a difference in number of cuttings of each clone and source, but they were apportioned equally among the four combinations of medium temperatures and mist frequencies. The trial started in August and ended after 50 days.

Results: Though the main factor which determined rooting was the genotype of the cuttings, increasing the temperature of the rooting medium and increasing mist frequency increased rooting (Table B.2.).

Discussion: Heating of the rooting medium might increase and accelerate rooting of cuttings having a relatively medium or high rooting capability (ILA-II, ILA-III), but it does not improve rooting of difficult-to-root cuttings (ILA-I, ILA-IV). Heating is expensive and will probably only be economical for special situations, such as a need for the rapid multiplication of stockplants. Without bottom heat, cuttings can not be rooted in winter, therefore rooting of cuttings under subtropical conditions is best done in summer. The positive effect of high mist frequency found in this trial is in agreement with the findings of Giordano (1961), Martin and Quillet (1974), Poggiani and Filho (1974), Campinhos and Ikemori (1983), but we have not found the inconveniences of misting mentioned by Cauvin and Marien (1979) and Cauvin (1982).

b.2.) Effect of bottom heating on rooting and growth of rooted cuttings -
M. Cunningham, U.S.A.

Objective: To determine the effect of bottom heating on rooting success and growth of rooted cuttings.

Methods: The study was done from December 5, 1983 to March 16, 1984 in a heated greenhouse in Raleigh, North Carolina. The greenhouse temperature was kept at $25^{\circ}\text{C} \pm 3^{\circ}\text{C}$, and lighting was extended to 18 hr. Each tube containers were placed on holding racks over a styrofoam tank. The tank was filled with water to a level just below the bottom of the tubes and an aquarium heater was placed in the tank. The heater was adjusted to raise the media temperature to approximately 29°C for half of the containers. Temperatures average 20°C in the unheated tanks. Five clones were used and the cuttings were kept under mist for six weeks.

The study was continued past the six weeks under mist to examine the influence of bottom heat on the growth of rooted cuttings. Some cuttings were removed from bottom heat at six weeks, while others remained for either nine or twelve weeks. Cuttings for all four treatments (0, 6, 9 or 12 weeks bottom heat) were grown for a total of fourteen weeks, at which time measurements were made of height, root-collar diameter, number of primary roots and shoot and root dry weight.

Results: Average rooting percentage by clones for the two heat treatments is shown in Table B.3. Bottom heating caused a substantial increase in rooting within six weeks. However, a graph of the cumulative number of cuttings rooted by days since sticking, showed that the number of cuttings rooting with bottom heat began to level off around the fifth week after planting, while rooting of the control cuttings continued to increase steadily through week six. Bottom heating had slight effect on height growth or the number of primary roots initiated as shown in Table B.4. However, there was a 30% reduction in root dry weight from the control to the twelve weeks in bottom heat and a 13% reduction in shoot dry weight. The greater reduction in root dry weight than shoot dry weight also resulted in an increase in the shoot/root ratio.

Discussion: The increase in percentage of cuttings with roots at six weeks in the heat treatment (Table B.3.) probably represents an increase in the rate of rooting more than an increase in the number of cuttings that would potentially root (Table B.4.). Heating by shortening the rooting time by two or three weeks, increases the number of crops that could be placed under mist in a year. The reduction in dry weight (Table B.4.) may be a result of increased metabolism, particularly root respiration, and/or changes in levels of endogenous growth hormones with higher media temperatures. These data suggest that, while bottom heat can speed up rooting, once it occurs the cuttings should be removed from bottom heat to obtain greater accumulations of biomass in both the roots and shoots.

c.) Mist intensity - T. Geary and M. Cunningham, U.S.A.

Objective: To determine how much mist should be applied to cuttings, and if the amount of mist applied effects the amount of fungicide and fertilizer needed.

Methods: Two experiments tested the amount of mist to be applied to cuttings. In the first experiment, mist was applied to cuttings for either 4, 8, or 12 seconds every 2 minutes, and fungicide (0.15% Benlate wettable 50% powder) was applied either once or twice a week or not at all. There were, then, 9 treatment combinations, and these were replicated. The experiment was done from May 15 to July 19, 1982.

In the second experiment, mist was applied either every 5, 10, or 15 seconds every minute, and the peat-vermiculite rooting medium contained either 0, 2.5, or 5.0 g/l of Osmocote 14-14-14 controlled release fertilizers. The 9 treatments were replicated, and the experiment was done from July 29 to October 7, 1982.

Results: Percentage of cuttings rooted under different mist and fungicide treatments is shown in Table B.5.

Percentage of cuttings rooted under different mist and fertilizer treatments is shown in Table B.6.

Discussion: No significant treatment differences were found for either experiment but, when judging how much mist to apply, it appears better to err in overmisting rather than undermisting. A once-a-week application of fungicide was ineffective in helping rooting but twice a week was effective. The cuttings fungicided twice a week appeared to have healthier leaves than the other cuttings. Adding controlled release fertilizer to the rooting medium did not affect rooting.

2. Effect of Cutting Source and Pretreatments of Stockplants

It is well known for plants (Hartman and Kester 1975), including eucalypts (Giordano 1961. Franclet 1970, Davidson 1974) that rooting capability changes with the ontogenetic development of a plant from the juvenile to the adult phase. Cuttings taken from young seedlings, root more readily than those from an adult tree. Consequently, cuttings taken from different parts of an individual plant, at various stages of development, differ considerably in their rooting ability. In many tree species juvenility tends to persist in the lower part of the plant. Cuttings taken from vigorously growing epicormic shoots near the base of a tree (Hartney 1980) than shoots higher on the tree must be preserved in the stockplants. One obvious method is to take cuttings from the lower parts of the plants. But, there are several treatments that can be given before harvesting of cuttings in order to alter their physiological condition to a juvenile stage. These include practices such as grafting on young seedlings and etiolation. This chapter describes results obtained in cuttings as effected by the different factors mentioned above.

a) Effect of height of epicormic shoots on rooting - L. Fanger - Vexler, Israel.

Objective: To compare the rooting capabilities of cuttings taken from epicormic shoots developed on different heights on the trunk.

Methods: One of the selected trees (ILA-1) developed young epicormic shoots from the base upward at different heights along the bole. Four-leaf-cuttings were taken from the mature crown and from epicormic shoots developed at the base or at a height of 1 to 4m. Temperature of the medium was uncontrolled and it consisted of peat and polystyrene flakes (1:1/v:v). Cuttings were planted under mist in June and rooting was recorded after 45 days.

Results: Cuttings taken from the basal epicormic shoots rooted at a higher percentage than those taken from epicormic shoots at a height of 1 to 4 meters on the trunk. None of the cuttings taken from the mature crown rooted as shown in Table B.7.

Discussion: The results indicate the greater rooting ability of cuttings taken from the basal epicormic shoots as compared with epicormic shoots from higher points on the stem. At least for difficult to root or test, such as ILA-1 (see Table B.2.), cuttings should be taken from as low an epicormic shoot as possible.

b) Effect of height of epicormic shoot in rooting ability of cuttings -
T. Geary, U.S.A.

Objective: To determine the height to which cuttings should be collected in tree boles.

Methods: Advantage was taken of a severe freeze that caused epicormic shoots to grow along the entire length of tree boles. The boles were demarcated into sections with widths starting at 0.5 m at the base of the trees and expanding to 2 m. Ten trees were selected, and within each section in each tree, 10 cuttings were collected. The cuttings were subjected to standard rooting procedures.

Results: The results are shown in Table B.8.

Discussion: Rooting success at all levels was less than normal for E. camaldulensis in the U.S.A. facilities. However, a similar experiment with another eucalypt species, for which rooting success was greater, had cuttings produced from shoots produced as high as 4 m that rooted, easily as shoots from lower on the bole. A question unanswered is whether rooted cuttings produced from shoots at different heights on the boles are similar in vigor, form and other

silvicultural characteristics when planted. Samples of rooted cuttings from all heights tested have been planted in the U.S.A. to determine this. On the basis of this study and the girdling study (Section A. 1), we recommend that a standard height for girdles be 1m above ground for E. camaldulensis, which should result in an optimum production of rootable cuttings.

c) Stem position on stockplants - L. Fanger - Vexler, Israel.

Objective: To study the effect on rooting of the position of cuttings on the stem.

Methods: Four-leaf cuttings from field grown stockplants, of two clones (BH and ILA-IV) derived from in-vitro culture were grouped as follows: originating from the upper stem just below the tip (U) and from the stem base and middle (L). The rooting trial began on May and rooting was recorded after 30 or 45 days. Medium constituents were peat: polystyrene flakes (1:1/v:v) without controlling the temperature.

Results: In both clones lower stem cuttings rooted in much greater numbers than those from the upper stem as shown in Table B.9.

Discussion: In addition to the difference in speed and rate of rooting between the two clones, the upper succulent cuttings of both clones were inferior to the lower ones. These results are in agreement with those of Bachelard and Stowe (1963). In most of the rooting experiments in Israel described in this report, the upper part of the shoots of stockplant were not used for cuttings.

d) Stem position on stockplants - T. Geary, U.S.A.

Objective: To determine what parts of stockplants should be used for cuttings.

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Methods: The methods described here are taken from Geary and Harding (1984). Cuttings were taken from 0.5 to 1.0 m tall clonal mother plants that were maintained in an unheated plant house in southwestern Florida, U.S.A. The mother plants were established as rooted cuttings from coppice shoots of phenotypically superior trees of Eucalyptus camaldulensis Dehn. The mother plants had single stems with up to 36 alternate, foliar nodes and few side branches. Sectional cuts were made 5mm above every fourth node, starting at the base of the plant and working upwards. Sections that had lost leaves, (basal sections mainly) and those with succulent leaves (usually the terminal) were discarded.

Immediately after a cutting was severed from the mother plant, it received a leaf treatment or was further sectioned. The cutting's basal 5 cm was soaked in 0.12% aqueous solution of benomyl fungicide (50% active ingredient) for at least 14 minutes. Next, the wet base was lightly blotted and rolled in a root promoting powder (0.8% IBA in talc), and the cutting was stuck to a point just below the bottom leaf in a preformed hole in a Leach Container tube (Geary et. al., 1983) that was filled with a 1:1:2 mixture of peat, vermiculite, and perlite. The hole was pressed closed and 0.14 g Osmocote 14-14-14 controlled release fertilizer was placed on the rooting medium. The tubes were suspended through holes in an insulation cover over a tank of water kept at 28°C in an unheated plant house. The tube bottoms were above the water, but were bathed by warm moist air. The cuttings were misted intermittently during daylight hours. The experiments were done in winter and early spring, when night temperatures were always lower than that in the rooting tanks, and day temperatures were often lower. Sections cut from plant shoots were numbers from 1 to 2, starting with the basal section. Sections 1 and 2 were not used because many had lost, or were losing their leaves. Experimental sections were numbers 3 to 6, each section being a 4-leaf-cutting. The cuttings came from 8 clones, and there were 6 cuttings for each section from each clone, or a total of 48 cuttings per section.

Results: Percentage of rooting and flushing of cuttings taken from different distances from the shoot base, is shown in Table B.10.

Discussion: Flushing did not differ significantly among segments. The percentage of cuttings that rooted and the percentage of cuttings that both rooted and flushed was least from segment 6 which had the youngest tissue. For practical purposes, all cuttings with healthy leaves should be used.

e) Cutting diameter - L. Fanger-Vexler and O. Reuveni, Israel.

Objective: To evaluate the effect of cutting diameter on its health and rooting ability.

Methods: The effect of cutting diameter on rooting was studied in two experiments. In the first, (see section B.1.b.1.), cuttings of 3 different diameters of ILA-II clone were randomly planted under the different conditions of rooting.

In the second experiment cuttings of three clones (ILA-I, ILA-II, ILA-III) were taken from field grown stockplants (see section A.4.). Semi-hard to hard cuttings with six healthy full-sized leaves were made from the base upwards from the long shoots harvested from the stockplants. Upper small diameter and succulent parts were discarded. All axillary branches and the two bottom leaves were removed. Cutting bases were dipped in talcum powder containing IBA (1%) + Benlate (1%) or IBA (0.8%) + TBZ (5%). The rooting medium was peat: polystyrene (1:1/v:v) without bottom heat. Harvesting and inserting of the cuttings under intermittent mist was done from the end of September to the middle of October. Results of rooting after two months are brought for all clones according to cuttings diameter

Results: In the first experiment medium to large diameter cuttings rooted better than the small size as shown in Table B.11.

In the second experiment, in spite of low rooting percentage, there was increasing rooting success with an improved cutting condition with increased diameter size (Table B.12).

The larger the diameter of the cuttings the more they produced new growth. The newly flushed branches on the layer cutting were larger and present in more leaf axils along the cuttings than on the smaller cuttings.

Discussion: The large shoots from which cuttings were derived (especially in the second experiment) were still green and were not yet lignified. In both experiments the medium to large cutting diameter were best. The better rooting of the larger cuttings might be explained by their greater carbohydrate reserves, which influence rooting rate, and at later stages, flushing and growth of the rooted cuttings.

f) Grafting - O. Reuveni, Israel.

Objective: Study of rooting capability of cuttings taken from grafted plants as compared to cuttings from stockplants grown on their own roots.

Methods: Cuttings were harvested from stockplants that were grafted on seedlings or from plants grown on their own roots. Semi-hardwood cuttings were used with 8 leaves left after removal of the upper succulent growth. After removing the basal 2 leaves their bases were dipped in a talcum powder containing IBA (1%) + Benlate (1%). Growing and rooting conditions of the stockplants and cuttings were as described in section A.3. As a result of repeated cutting harvesting, the stockplants remained small and compact, a procedure which brought to branching and production of new growth, enabling harvesting of cuttings every 4-6 weeks. It was found important to leave leaves on the stockplants in order to keep them alive. The rooted cuttings were used to establish new stockplants grown on their own roots. Since grafting of mature material was done at different dates, it was possible to compare the effect of stockplants age on the rooting of cuttings. Such a comparison was done with one clone (ILA-1) where 48 cuttings were used for each stock and age plant source. Cuttings were inserted in the medium in 4 randomized blocks. Rooting was recorded after 40 days under mist.

Results: Though the rooting percentage of the clone used was not high an advantage was found with cuttings taken from young grafted plants (Table B.13.).

Discussion: As found by other researchers (Davidson 1974, Martin and Quillet 1974, Cauvin and Marien 1979, Franclet 1981, Cauvin 1982) grafting mature material onto young seedlings brings rejuvenation and to an improvement in rooting. In another experiment with the same clone, 40% out of the 140 cuttings, rooted when cuttings were taken from young grafts. The effect of

rooting improvement brought by grafting appears to be limited and it drops with aging of the grafted stockplants. In another experiment (see section B.1.b.) rooting of cutting of two clones taken from different sources was compared. In the ILA-I clone which have a low rooting capability there was no difference in rooting between cuttings taken from a coppice of a mature tree or from old grafted stockplants. Cutting taken from stockplants grown on their own roots, rooted at a higher rate (Table B.14.).

The difference in rooting rate of ILA-I cuttings in the two experiments (Tables B.13. and B.14.) may be explained by the date of rooting which was late November in the first experiment and late of May in the second one.

The procedure of grafting mature material onto seedlings in order to produce stockplants for getting a permanent source of cuttings, does not seem practical for the following reasons: (i) as a result of incompatibility many of the plants die with time (section A.3.); (ii) reduction with time of rooting ability; (iii) sprouts of the rootstocks may lead to mistakes where by there are harvested instead of the grafts. On the other hand it is a good way to rejuvenate mature material and the rooted cuttings obtained might serve as permanent stockplants. The older grafted plants were variable in vigor due to incompatibility of grafts; this might influence rooting capability. If such a connection between vigor and rooting capability does exist, then it would be worthwhile to take cuttings from compatible grafts only. Further study is needed to determine whether to continue growing these grafted plants, or to do one harvesting of cuttings from vigorous plants in order to obtain a high rooting percentage. The practical justification for one harvesting lies in the fact that the take of grafts is high and the grafted plant can be grown in a small container.

g) Etiolation - O. Reuveni, Israel.

Objective: To study etiolation of the cutting base while on the stockplant and its effect on rooting capability.

Methods: Etiolation as practiced in various horticultural crops in order to improve rooting, seems to be too expensive for forest trees. Therefore the effect of partial etiolation was tried by employing it during different stages of shoot growth which was used later as cuttings. Two different methods were tried as follows: In the first one shoot bases while still on the stockplants, were smeared with a black plastic paste 2-3 weeks before being harvested as

cuttings. As a result the tissue below the paste lost its green color and turned white. This method is referred to as "bleaching". Cuttings were prepared in such a way that their bases were "bleached" while still on the stockplants. This method was tried with old grafted plants and with self-rooted stockplants. The second method was based on inducing new growth of shoots under leaf shade of the stockplants so that the initial growth and the base of the shoots used as cuttings would not receive full light. To achieve this, old stockplants grown on their own roots, were cut back many times in order to obtain many branches at the base of the stock plants. By cutting these plants very frequently, small bushy plants were obtained. Before inducing shoot production used at a later stage as cuttings, all the new and succulent growth was pinched. As a result new shoots started to grow under the canopy of the leaves. After these shoots grew above the lower leaves of the stockplants and became semi-hard they were harvested and used as cuttings. The base of these cuttings was not fully green and is referred to as "etiolated". The environmental conditions of growing the stockplants and rooting the cuttings were as described in section A.3. Many preliminary experiments were done, and the results of two experiments with each of the methods, are presented below.

In the first experiment basal bleaching of the cuttings was compared with non-bleaching of old stockplants of ILA-I clones grown on their own roots (Table B.15.). Cuttings were harvested in two different months after having been smeared 3-4 weeks previously. On each date 80 cuttings for each treatment were inserted in the rooting medium, in four randomized blocks. Rooting percentage was recorded after 30 days. In the second experiment cuttings of two clones (ILA-I and ILA-II) were taken from old grafted stockplants and from stockplants grown on their own roots. Some of the cuttings were bleached before harvesting. For both clones a fifth source of cuttings was used for comparison; these cuttings were taken from coppices of the original trees (Table B.14.). For each source there were 45 cuttings arranged in five randomized blocks under the mist. The bases of the cuttings were dipped in talcum powder containing IBA (%) + Benlate (1%) before being inserted in the rooting medium. The other conditions for the stockplants and the rooting of cuttings were as described in section A.3. In the two experiments where etiolated cuttings were compared with nonetiolated ones (referred to as "green") the cuttings were taken from stockplants of four different clones grown on their own roots (Figs. B.1. and B.2.). The "green"

cuttings were those produced on the same stockplants as the etiolated ones but at the time of harvesting their bases were fully green as compared with the etiolated ones which had white to pale green bases. Cuttings were arranged under mist in four randomized blocks, with 36 per treatment in the first experiment (planted in December) and 28 per treatment in the second experiment (planted in April). The rooting conditions and base treatment were the same as those for the bleached cuttings. There was only one exception from other rooting experiments, in that only four instead of six leaves, were left on each cutting.

Results: In the first experiment bleached cuttings of the ILA-I clone rooted at a higher percentage than the non-bleached ones. There was a low rooting rate during the winter, although the medium was bottom heated (Table B.15.). In the second experiment (Table B.14.) which started at the end of May, a higher rooting percentage was obtained. There was a clear difference in rooting rate of the two clones as well as in the response to the bleaching treatment. Cuttings of the ILA-I clone which have a low rooting capability differed in their response according to their source. There was no difference in rooting between cuttings - bleached or not bleached - taken from coppices or from grafted (old) stockplants. Cuttings which derived from stockplants grown on their own roots, generally ~~at~~ rooted at a higher rate; bleaching accelerated ~~of~~ rooting but did not raise the final rooting percentage after a longer period under the mist (Table B.14.).

With the relatively easy-to-root ILA-II clone, an advantage was found with those cuttings taken from stockplants as compared to those taken from coppices. In grafted (old) stockplants an advantage was found to bleaching as expressed in speed and rate of rooting. In stockplants grown on their own roots there was no advantage to bleaching in respect to speed or rate of rooting.

Cuttings base etiolation performed at the early stage of shoot growth was found to be most efficient (Figs. B.1. and B.2.), leading to a faster and higher rate of rooting in all the clones studied. The ILA-I clone cuttings, which were found to be difficult-to-root in many other experiments, responded to the etiolation treatment in both experiments. Cuttings of the ILA-II clone, which root the most easily of the four clones compared, responded also to the treatment in the same way and especially when rooting conditions were unfavorable for the non-etiolated cuttings (Fig.B.2.). Rooting of cuttings of other clones which are relatively moderate or difficult-to-root, was also, improved by the etiolation treatment (Figs.B.1 and B.2.).

Discussion: Of the two partial etiolation methods tried, bleaching was found less effective than etiolation. This result is in agreement with Delargy and Wright (1978) who showed that in order to improve rooting of difficult-to-root apple cuttings, etiolation of the cuttings' bases has to be done between the early stages of shoot development till the time of harvesting. In Eucalyptus cuttings the response to etiolation seems to ~~work~~ be the same: the earlier and longer it is performed, the more efficient it is. In clones which are relatively difficult-to-root the bleaching method is limited in its effect, while in those which root more easily it improves rooting but no more than the etiolation method.

In order to avoid performing the base etiolation of cuttings as is done with horticultural crops (e.g. the stool beds method, Hartman and Kester, 1975), another version was tried - successfully - in this study. This method is based on repeated cutting back and pinching of the stockplants in order to produce a bushy plant. At a later stage bases of shoots that are produced below the leaf canopy remain etiolated and are used as cuttings. The method was tried on a small scale with field grown stockplants and was found to be very promising. In order to run it on a large commercial scale, there is a need to study further mechanical pruning and the use of pinching agents.

3. Effect of Number of Leaves, and of Leaf Retention

Bachelard and Stowe (1963) and Franclet (1970) found that leaves were essential for rooting of Eucalyptus camaldulensis cuttings. As with other evergreen cuttings (Reuveni and Raviv, 1980), the retention of leaves on cuttings is a prerequisite for rooting. In any case when leaves shed during rooting or were removed artificially, no rooting was obtained. On the other hand, in many cases, although leaves were retained on the cuttings no rooting occurred. It was found that older leaves of eucalypts contain rooting inhibitors

(Paton et al., 1970; Crow et al., 1971, 1976, 1977; Nicholls, 1972; Nicholls et al., 1972). The decline in rooting ability with ontogenetic age was correlated with an increase in concentration of these rooting inhibitors. In contrast to the above results there was no close correlation between rooting ability and the position of the node on the parent plant in organ culture of nodes from Eucalyptus grandis (Durand-Cresswell and Nitsch, 1977). It was found that the age of the leaf on the node was the most important factor determining rooting ability. In most cases in our rooting experiments, young cuttings bearing young leaves were used. Therefore, our attention was given to studying the effect of number of leaves retained on the cutting during the rooting process or after being removed artificially. In some cases we studied the interaction between leaf retention and rooting ability of cuttings as determined by genotypic characters and pretreatments given to stockplants. Experiments on this line were conducted in detail in both the U.S. and Israel.

a) Effects of leaf quantity and trimming on rooting success- T. Geary, U.S.A.

Objective: To determine if the standard practice of trimming leaves in rooting affected rooting success, and if shorter cuttings than the standard four node cutting could be used.

Methods: We conducted three experiments on leaf trimming and leaf number. Flushing of new foliar growth was recorded as a response to treatment besides rooting, because a cutting that roots but does not flush is useless. Trimming leaves stimulated rooting and flushing in the first experiment. The second experiment checked the repeatability of the first, studied a wider range of leaf treatments, and confirmed the benefit of leaf trimming. In the third experiment, cuttings with four foliar nodes were compared to cuttings with two, and four were best by far. Rooting methods are described in section B.2.d. The first experiment had three treatments: (1) a four-leaf cutting

with leaves untrimmed (4-whole-leaves); (2) leaves cut in half by cross-sectioning (4-half-leaves), and (3) all leaves cut off at the petioles (4-leaves-removed). Each treatment was applied to 64 cuttings and these were distributed among 4 replications. Eight clones and cuttings of different developmental ages were used and these factors were observed for 67 days.

The second experiment had 6 treatments. Two were repeats of the first experiment: (1) 4-whole-leaves and (2) 4-half-leaves. The new treatments were: (3) the first and third leaf from the base were cut off at their petioles (two-whole-leaves); (4) four leaves were cut to one quarter length by cross-sectioning (4-quarter-leaves), (5) the three upper leaves were cut off at their petioles (1-whole-leaf), and (6) the one leaf was cut in half by cross-sectioning (1-half-leaf). Each treatment was applied to 32 cuttings, which were distributed between 2 replications. The effects of 6 clones and different tissue ages were controlled by a factorial experimental design. Rooting and flushing were observed for 75 days. In the third experiment 32 of 64 four-leaf cuttings (4 foliar nodes) were sectioned into 2 two-leaf cuttings (2 foliar nodes) to produce three treatments: cuttings with (1) four leaves, or (2) two upper leaves, or (3) two lower leaves. Leaves were cut in half. The effects of four clones and different tissue ages were controlled by a factorial experimental design. The 32 cuttings of each treatment were distributed between 2 replications. Rooting and flushing were observed for 75 days.

Data were collected every few days. Rooting was recorded when roots first protruded through the tube drain holes. Flushing was recorded when axillary buds opened. A cutting that had rooted was removed from the heating tank and the mist system, and was maintained by routine nursery practices in the plant house. Observations for flushing of rooted cuttings were continued. Arc-sin transformed percentages of rooting and flushing were examined by analyses of variance to detect statistically significant differences. The speeds of rooting and flushing were the ratios of the numbers of cuttings to have rooted or flushed halfway through an experimental period to the numbers that had done so by the end of an experiment. Results of the effects of clones (pronounced) and of tissue ages (slight) are not given in this report.

Results: In the first experiment trimming leaves in half increased rooting and flushing and resulted in a 50% increase in the number of usable cuttings, i.e., those that flushed as well as rooted (Table B.16.). Removing leaves prevented rooting, and decreased flushing. Trimming leaves did not speed rooting, but it did hasten flushing (Table B.17.).

In the second experiment, trimming four leaves to half length increased rooting and flushing as in the first experiment (Table B.18.). Trimming to one-quarter length caused a greater increase, although the differences between half and quarter length treatments were not statistically significant. Removing two leaves increased the number of usable cuttings as much as leaf trimming did. Removing all but one leaf, did not effect rooting, and it increased flushing. However trimming a single leaf in half decreased rooting significantly. The greater the reduction in leaf area the faster the speed of flushing (Table B.19.). Speed of rooting was greater with some leaf treatments than others, but because there was no statistical test for the ratios, caution is needed in interpreting these results. Two-leaf cuttings in the third experiment rooted and flushed so much more poorly than four-leaf cuttings that the sum of the parts did not equal the whole (Table B.20.). This response was the opposite of that of 2-leaf cuttings with 4 nodes in experiment 2.

Discussion: Cuttings of E. camaldulensis root better when leaves are trimmed and this has several economic benefits. Bed area is saved because cuttings can be packed closer together. Rooting and flushing are hastened, which saves propagation time. Unit costs decrease as the percentage of usable cuttings produced increase. Trimmed leaves might help cuttings survive if the mist system fails. A large leaf area can be removed. We speculate that trimming increases the percentage of usable plants by a chain reaction: trimming stimulates foliar bud development and this stimulates rooting. Detailed histological and biochemical studies are needed to determine the causal mechanism.

Removing some leaves instead of trimming also is stimulatory but the response is not as great. Reducing leaf area 50% by removing 2 of 4 four leaves gave a response similar to trimming off half of each of the 4 leaves. However, reducing leaf area 75% by removing 3 leaves did not stimulate rooting, while a similare reduction by leaf trimming greatly increased rooting. So more than leaf area is involved. The number of useful cuttings from mother plants can not be increased by shortening cutting length, because rooting and flushing of 2-node cuttings is dismally poor. Stem length might be a factor, because

4-node cuttings with only 2 leaves rooted and flushed as well as cuttings with 4 leaves. Left unanswered is whether cuttings with 5 or more nodes will root better than those with 4 nodes.

b) Effect of number of leaves on rooting of cuttings taken from coppice shoots
- L. Fanger-Vexler, Israel

Objective: To study the effect of leaving two half-leaves as compared with four half-leaves on cuttings from coppice shoots of different ortets.

Methods: Trees of four provenances were felled to investigate coppice production. Two coppice shoots of two trees of each provenance which had attained a large volume, were cut after about one and a half year from the first felling, in April, to 8 cm above the original stump. The regrowth of coppice shoots was harvested after 12 weeks (June). The shoots were divided at random into two groups and sectioned into cuttings as follows: with two half-leaves and with four half-leaves; the lowest two leaves of all cuttings were removed. The upper quarter of each shoot was discarded. The cutting bases were dipped into talcum powder containing IBA (0.8%) + TBZ (5%). All cuttings were set into a peat and polystyrene flakes (1:1/v:v) medium, without bottom heat control. From three to six weeks after being placed under mist, the cuttings were examined for rooting and flushing rates.

Results: Differences in speed and rooting percentage were found mainly between cuttings taken from different trees (see section B.4.). The results of rooting and flushing percents with time, for all the eight clones are given in Table B.21. Leaf number did not affect ~~rooting~~ flushing. Two-leaved cuttings flushed at a significantly higher percentage than four-leaved cuttings.

Discussion: The size of cuttings or number of leaves determines how much material is available from a given mother plant. Compinhos and Ikemori (1983) suggested that the decision to use two or four-leaved cuttings be dependent upon internode length. Martin and Quillet (1974) found that for vigorous

rooting of Teminalis superba and Eucalyptus Platyphylla (= E. urophylla x E. alba), sufficiently large foliar surface area is necessary. Definite choice of leaf surface area is a compromise between the largest dimension acceptable (for maximum photosynthesis) and the minimal vigor necessary for the production of root system (for maximum profitability of 1 m² under mist). In most cases four leaved cuttings (partially cut or not) gave the best results. However, for E. deglupa two partially cut leaves were enough. In our study there was no difference in rooting between the two and four leaved cuttings but there was a significant effect on flushing. Most of the four-leaved cuttings produced new growth with time, and were easier to outplant than two-leaved cuttings. Therefore, the decision remains a matter of quantity vs. size.

c) Rooting as affected by number of leaves retained on cuttings under mist -
O. Reuveni, Israel

Objective: To study the effect of leaf retention on cuttings during rooting under mist of different clones with and without etiolation of cutting bases.

Methods: A follow-up of leaf retention on cuttings during their stay under mist and its effect on rooting, was conducted in two rooting experiments in which etiolated and non etiolated cuttings of different clones were compared. The design of the experiments, cutting source, environmental rooting conditions, and rooting percentages were described in section B.2.g.

Results: In both experiments the number of leaves retained on rooted cuttings was higher than on non-rooted ones (Figs. B.3. and B.4.). This was the case regardless of clone and whether the cutting bases were etiolated or not.

Discussion: In the many rooting experiments conducted in this study it was noticed that cuttings which shed their leaves under the mist at early stages, did not root. The rate and speed of shedding was in some cases a result of environmental conditions (failure of the mist spray, season of the year, etc.) and in other cases differences in leaf shedding were observed between clones and differences of leaf shedding were observed between clones and differing

in rooting ability, as determined by the genotype and their physiological condition. The results obtained demonstrated the importance of leaf retention on cuttings whether they were of different clones or pretreated (etiolated) in order to increase rooting. In the more difficult-to-root cuttings (ILA-IV and ILA-I) leaf shedding started at an earlier stage and was at a higher rate (Figs. B.3 and B.4). This is in agreement with other evergreens where difficult-to-root cuttings tend to shed their leaves at a higher speed and rate (Reuveni and Raviv 1980). As mentioned above, leaf retention is not enough to ensure a high rooting percentage. Such leaves might produce rooting inhibitors if they are old (Paton *et al.*, 1970; Crow, *et al.*, 1971, 1976, 1977; Nicholls 1972; Nicholls *et al.*, 1972). It has been shown recently in avocado, that rooting promoters which are not of an auxin type, are produced in the leaves and are essential for rooting (Raviv and Reuveni 1984a). The physiological condition of the cutting base where the rooting process occurs, is the final determinant of the success of the rooting procedure. In etiolated cuttings which root more readily, delayed leaf shedding did not influence rooting (Fig. B.4.). It can be concluded that in order to obtain a rapid and high percentage of rooting, leaves have to be retained and the cutting base has to be in a physiological condition enabling rooting. The last condition is determined by many factors such as genotype, ontogenetic age, growing condition and pretreatments of the shoots while on the stockplants. Of the pretreatments, etiolation is the most efficient.

d) A test for predicting rooting capability of Eucalyptus cuttings based on leaf retention - O. Reuveni, Israel

Objective: Since testing rooting capability of cuttings taken from different clones or stockplants, consumes much time, work and space, it was deemed worthwhile to find a short and a simple laboratory assay aimed at predicting rooting ability as affected by leaf retention.

Methods: The method used was basically the same as that described for avocado by Raviv and Reuveni (1984 b). It is based on the fact that speed of leaf shedding is related to two distinct processes: abscission layer formation in the petiole and blade senescence. For this purpose abscission layer formation with time in petioles from detached internodes, was studied. Leaf senescence was assayed by incubating (at 28°C) leaf discs and measuring chlorophyll degradation with time. Studies were carried out in different periods of the year. The absolute figures were not the same at different dates of sampling, but the trend in clone behavior was the same.

Results: Leaves were taken from four different clones which were used in many rooting experiments. The relative rooting capability of these clones (ILA-II = 100%), as calculated from different comparable experiments, was found to be as follows: ILA-III/66%, ILA-I/44%, and ILA-IV/9%. The results of chlorophyll retention after incubation were inconsistent in the different assays and were not correlated with the rooting ability of the clones. The results of petiole retention were consistent: relative easy-to-root clones, retained their petiole on the node for a longer time (Fig. B.5.).

Discussion: Contrary to the results obtained when using the same assay for avocado clones (Raviv and Reuveni, 1984 b), no correlation was found between rooting capability of certain clones and chlorophyll retention in incubated leaf discs. It seems that in Eucalyptus the petiole retention assay is more valid, as the results obtained were consistent for the four clones. This assay may give some information about the leaf retention potential of cuttings under mist. Since leaf retention, however important, is not the only factor which determines rooting (see Discussion in section B.3.c), the petiole retention assay might be useful as a partial screening method for rooting capability of Eucalyptus ortets.

e) Relationship of leaf senescence and dehiscence to rooting and flushing success - T. Geary, U.S.A.

Objective: To learn signs and symptoms that could assist in recognition of cuttings that will not root.

Methods: Observations were made on leaf retention and leaf color of the cuttings used in the first experiment described in section B.3.a. These observations were made on the 43rd day of the experiment, at which time 38% of all cuttings that were to root had rooted, and 44% of all cuttings that were to flush had flushed. Rooting and flushing results are reported for the 67th day of the experiment.

Results: Senescence and dehiscence of leaves were related to rooting and flushing success as shown in Table B.22.

Discussion: Leaf color and leaf retention give useful clues in whether cuttings are going to root.

4. Effect of Clonal Differences

As mentioned above, differences in rooting capability of different clones were detected, both in the experiments in the U.S.A. and in Israel. The four clones (ILA-I, II, III, IV) that were used in different rooting studies in Israel were found to be difficult-to-root. The results obtained with rooting of these cuttings in an inexpensive building as compared with those of other sources of cuttings rooted under the same conditions are presented in this chapter. The differences in rooting ability as found in U.S. material are given too.

a) Semi-commercial mass propagation of selected clones - L. Fanger-Vexler, D. Heth, and O. Reuveni, Israel

Objective: To carry out mass propagation of selected clones in the semi-commercial pilot plant at Ilanot.

Methods: Stockplants of four selected clones were grown on their own roots in the field. Management and growing conditions were as described above (Section A.4.). Most of the cuttings harvested from the stockplants grown under different conditions were put under intermittent mist on different dates. The mist propagation house was constructed of from inexpensive materials without any bottom heat installation. The medium was peat and polystyrene flakes (1:1/v:v). Cutting bases were dipped before planting in talc containing IBA (0.8%) with Benlate (1%) or TBZ (5%). Data are given for three clones since there were not enough stockplants of ~~one of the~~ clone ILA-IV which was found to be very difficult-to-root even when stockplants were grown in a green house and rooting was done with bottom heating.

Results: The number of cuttings used and the rooting percentage obtained with clones ILA-I, II and III are given in Table B.23. Since there was no difference in rooting between cuttings taken from different fertilization regimes or degrees of shading of the stockplants, results are presented for all the cuttings of one clone. The final rooting percentage was poor (Table B.23.).

Discussion: It could be concluded that the low rate of rooting of the three clones used in the semi-commercial pilot plant at Ilanot does not justify mass vegetative propagation of Eucalyptus by rooting of cuttings. However, such a conclusion would be too hasty, if two aspects are taken into account: (i) Cuttings of the clones used were difficult-to-root, as was found in a comparison trial where cuttings of other clones rooted at a high rate under the same conditions (see below). (ii) The method of producing based etiolated cuttings which was found to be the most promising in increasing rooting capability, was not applied to the field grown stockplants. Therefore, before reaching a final conclusion, it is necessary to adapt the principles of etiolation worked out in this project, for container-grown stockplants (in a greenhouse), to field grown stockplants.

b) Differences in rooting capability of cuttings from the same provenance -
L. Fanger-Vexler, Israel

Objective: Trees of the "local" E. camaldulensis population (originated from South Australia) were felled in 1971. Their coppice shoots reached 1-2 m in height by June 1983 at which time six trees were randomly selected for study. The coppice shoots were cut, sectioned into four leaved cuttings (leaves were clipped to half-length), and placed under mist on July 27, 1983. The medium was peat and polystyrene flakes (1:1/v:v) without bottom heating. Except for five trees, for which no knowledge was available about their rooting capability, cuttings were taken also from field-grown stockplants of ILA-11 (found to be the relatively highest inrooting capability of the four clones studied). Rooting percentage was recorded after 3 weeks under the mist.

Results: A large inter-clonal variation (4-93%) was found in rooting capability (Table B.24.). The clonal source is therefore of primary importance in determining the degree of rooting success.

Discussion: Although a small number of trees of the "local" Israeli type were checked for rooting capability a very large difference was found between the individual trees tested. Common practice for mass propagation system is to select clones which root at level of 70% and higher (campinhos and Ikemori 1983). Poorer rooting clones are discarded, unless they have some extraordinarily desirable characteristic (s). In such cases there is a need to apply pretreatments to the stockplants in order to raise rooting capability, or use another method of propagation, such as by in vitro culture, which is dealt with later on. In general, it seems to be necessary to select for ease of rooting, early in the propagation process.

c) Differences in rooting capability of cuttings from different provenances -
L. Fanger-Vexler, Israel

Objective: To carry out a preliminary study of the rooting ability of cuttings taken from trees of different seed sources (provenances).

Methods: Eight trees, two from each of four provenances growing successfully in Israel, were selected for study and felled in 1981. Coppice shoots having developed on each stool were cut back in April 1983 and the subsequent regrowth was harvested after 12 weeks and again after an additional 6 weeks. The coppice shoots were sectioned into two or four half-leaved cuttings and placed under mist on June 30 (first experiment) and on August 14 (second experiment), respectively. The rooting medium was peat and polystyrene flakes (1:1/v:v) without bottom heating. In the first experiment, from 3 to 6 weeks after being placed under mist, the cuttings were examined weekly for rooting. The results of rooting of two and four half-leaved cuttings are presented in section B.3.b. for all the seed sources together, while more details on each tree and seed source, separately, are presented here. In the second experiment, rooting was recorded after 4 weeks under mist.

Results: The rate of rooting in both experiments after 4 weeks under mist is given in Table B.25. Rooting after different times under mist of the eight clones in the first experiment is recorded in Table B.27. The speed of rooting was different in the different clones but already after 3 to 5 weeks (during the summer) it is possible to judge whether the cuttings can a root.

Discussion: Although each provenance was represented by only two clones, the influence of seed source appears to be important. The high rooting percents of the tropical Katherine clones is contrary to the findings of Giordano (1967). An extensive study of rooting ability as related to seed source is necessary to determine whether any provenance patterns exist.

d) Clonal Differences in rooting - T. Geary, U.S.A.

Objective: To observe the magnitude of differences in rooting success among clones.

Methods: Records were kept of rooting and flushing success for the clones used in the first experiment described in section B.3.a.

Results: Differences in rooting among cuttings from different clones was pronounced. Interpretation of the results as strictly due to genetical differences must be viewed with caution, because tissue ages differed between clones, although not dramatically. The results are given in Table B.26. Clone 1.2 was propagated from Clone 1.1. Cuttings taken from clone hedge 1.1. for the experiment were the second harvest from that hedge. No loss in rooting capacity has occurred from repropagation.

Discussion: Some clones root much better than others, and it is the better rooting clones that should be selected for mass propagation, provided they have good silvicultural value, in order to keep down propagation costs.

Table B.1 Rooting of cuttings of Eucalyptus seedlings in different rooting media, after 30 and 50 days under mist.

	Constituents of the Medium (V:V)	Rooting (%)	
		30 days	50 days*
1	Peat:Polystyrene** (1:1)	56	69
2	Peat:Perlite (1:1)	56	75
3	Peat:SFC*** (1:2)	52	62
4	Peat:SFC (1:1)	54	69
5	Peat:SFC (2:1)	71	90
6	Vermiculite:Polystyrene (1:1)	69	84
7	Vermiculite:Perlite (1:1)	71	77
8	Vermiculite:SFC (1:2)	50	71
9	Vermiculite:SFC (1:1)	65	84
10	Vermiculite:SFC (2:1)	52	67
11	SFC:Polystyrene (1:2)	65	75
12	SFC:Polystyrene (2:1)	48	65
13	SFC:Perlite (1:2)	52	77
14	Roockwool	56	62

* - F-value found between treatments was 1.65 (at $\alpha = 0.05$, critical F = 1.76).

** - Polystyrene = shredded polystyrene (flakes).

*** - SFC = Sandy Flint Clay.

Table B.2. Rooting of Eucalyptus cuttings as affected by bottom heating of the medium, mist frequency and genotype

Cutting source		Cuttings per temperature and mist frequency combination.	Rooting (%)				
Clone	Kind of stockplant		No bottom heat (25°C)		With bottom heat (30°C)		Mean
			Mist frequency		Mist frequency		
			Low	High	Low	High	
ILA-I	Own roots	72	4	14	11	16	11
ILA-I	Grafted	48	4	6	6	4	5
ILA-II	"	24	26	34	42	71	43
ILA-III	"	24	23	38	34	42	34
ILA-IV	"	24	0	0	0	8	2
Mean			11	18	19	28	
			14		23		

Table B.3. Average rooting percentage by clones for the two heat treatments

Treatment	Clone					Means
	1	2	3	4	5	
Bottom Heat	88	96	100	33	79	79
Control	0	33	42	0	62	27

Table B.4. Height and dry weight of shoot, number and dry weight of roots, and shoot/root ratio as affected by bottom heating time (weeks).

Time (weeks)	Height (mm)	Number Primary Roots	Root Dry Weight (mg)	Shoot Dry Weight (mg)	Shoot/ Root
0	112	6.2	200.79	1065.00	5.303
6	114	5.4	169.21	940.13	5.558
9	123	5.8	165.16	922.03	5.582
12	117	6.6	139.74	919.24	6.576

Table B.5. Percentage of cuttings rooted under different mist and fungicide treatments

Mist Amount (seconds/ 2 minutes)	Percent Cuttings Rooted			
	Fungicide Rate			Mist Means (%)
	None (%)	Once/Week (%)	Twice/Week (%)	
4	20	22	30	24
8	33	38	42	38
12	43	37	50	43
Fungicide Means	32	32	41	35

Table B.6. Percentage of cuttings rooted under different mist and fertilizer treatments.

Mist-x-Fertilizer Experiment				
Mist Amount (seconds/ minute)	Percent Cuttings Rooted			Mist Means
	Quantity Fertilizer per Liter of Medium			
	0g/l	2. 5g/l	5. 0g/l	
	(%)	(%)	(%)	(%)
5	47	45	30	41
10	50	40	50	47
15	33	38	37	36
Fertilizer Means	41	47	36	41

Table B.7. Rooting of Eucalyptus cuttings taken from the mature crown and from epicormic shoots which developed at different heights of the trunk

Cutting source	Number of cuttings taken	Rooting (%)
Basal epicormic shoots	21	29
Trunk (1-4 m) epicormic shoots	25	4
Mature crown	20	0

Table B.8. Rooting (%) of cuttings taken from different stem heights above ground

Height above ground (m)	Cuttings that rooted (%) ^{1/}
0.0 - 0.5	10
0.5 - 1.0	37
1.0 - 2.0	29
2.0 - 3.0	18
3.0 - 5.0	12
5.0 - 7.0	8
7.0 - 9.0	3
9.0 - 11.0	3
^{1/} Out of 100 cuttings per height	

Table B.9. Rooting of Eucalyptus cuttings of two clones taken from upper (U) or basal and middle (L) stem position

Clone	Stem position	Number of cuttings taken	Days under mist	Rooting (%)
BH	U	19	30	21
BH	L	21	30	57
ILA-IV	U	27	45	4
ILA-IV	L	68	45	22

Table B.10. Rooted (%) and flushed (%) cuttings as related to section's distance from the shoot base

Segment	Rooted (%)	Flushed (%)	Rooted and Flushed (%)
3	69ab	69a	66a
4	78a	66a	59ab
5	81a	69a	66a
6	59b	62a	50b

Within columns, means sharing a common letter do not differ significantly (p = 0.05)

Table B.11. Effect of cutting base diameter on rooting of Eucalyptus ILA-II clone

Class	Diameter (mm)		Rooting (%)
	Range	Average	
Small	1.8-2.4	2.1	28
Medium	2.5-3.2	2.7	54
Large	3.8-4.2	4.0	48

Table B.12. Effect of cutting base diameter on rooting and flushing of three Eucalyptus clones

Class	Number of cuttings	Diameter range (mm)	Rooting (%)	Flushing (%)
Small	1105	2.0-3.9	5	44
Medium	2505	4.0-6.9	8	67
Large	759	> 7.0	10	82

Table B.13. Effect of cutting source and age of stockplants on rooting of ILA-1 Eucalyptus cuttings

Stockplant		Rooting (%)
Kind	Age [*] (Months)	
Grafted	4	27 a ^{**}
"	12	15 b
Own roots	7	9 b
"	11	16 b

* Age from grafting or potting of the rooted cutting.

** Mean separation according to Duncan's Multiple Range Test at P=0.05.

Table B.14. Rooting percentage of cuttings from two Eucalyptus clones as affected by stockplant and basal bleaching treatment

Source of cuttings	Basal bleaching	ILA-I		ILA-II	
		Days under mist			
		30	40	30	40
Coppice from mature tree	-	7	9a [*]	13	36b
Grafted stockplants	-	7	11a	22	60c
" "	+	11	13a	44	82e
Stockplants on own roots	-	17	38b	40	67e
" "	+	33	36b	44	71c,d

*Mean separation according to Duncan's Multiple Range Test at P = 0.05.

Table B.15. Effect of basal bleaching of Eucalyptus ILA-I cutting on rooting after 30 days under mist in two seasons

Start of mist	Rooting (%)		Significance (t-test)
	Control	Bleached	
Feb. 2 (winter)	0 ^b	15 ^a	+
June 21 (summer)	14 ^b	27 ^a	+

Table B.16. Responses of E. camaldulensis cuttings with 4 foliar nodes to trimming or removal of leaves

Treatment	Rooting and Flushing Quantity		
	Cuttings that rooted (%)	Cuttings that flushed (%)	Cuttings that rooted & flushed (%)
4 whole leaves	66b*	52b	48b
4 leaves cut in half	78a	81a	72b
4 leaves removed	0c	34c	0c

*Within columns, means sharing a common letter do not differ significantly (p = 0.05).

Table B.17. Relative rooting and flushing speeds of E. camaldulensis cuttings with 4 foliar nodes with leaves trimmed or untrimmed

Treatment	Response Speed*		
	Rooting	Flushing	Rooting & Flushing
4 whole leaves	0.64	0.48	0.48
4 leaves cut in half	0.62	0.65	0.57

*The proportion of number of cuttings that had rooted and/or flushed by the 34th day of the experiment to the number rooted and/or flushed by the 67th day.

B.18. Responses of E. camaldulensis cuttings with 4 foliar nodes to trimming or removal of leaves

Leaf treatment	Relative leaf area	Cuttings that rooted (%)	Cuttings that flushed (%)	Cuttings that rooted & flushed (%)
4 whole leaves	1.00	53	72	50
2 whole leaves	0.50	66	81	66
4 leaves cut in half	0.50	69	94	69
1 whole leaf	0.25	44	94	44
4 leaves cut to $\frac{1}{4}$ length	0.25	75	91	75
1 leaf cut in half	0.12	25	88	25

Table B.19. Relative rooting and flushing speeds of E. camaldulensis cuttings with 4 foliar nodes following leaf trimming and removals

Leaf treatment	Response Speed*			
	Relative leaf area	Rooting	Flushing	Rooting & Flushing
4 whole leaves	1.00	0.41	0.43	0.31
2 whole leaves	0.50	0.48	0.60	0.23
4 half leaves	0.50	0.59	0.83	0.55
1 whole leaf	0.25	0.57	0.97	0.50
4 quarter leaves	0.25	0.54	0.90	0.50
1 half leaf	0.12	0.38	1.00	0.38

*The proportion of number of cuttings that had rooted and/or flushed by the 37th day of the experiment to the number rooted and/or flushed by the 75th day.

Table B.20. Rooting and flushing response of E. camaldulensis cuttings with 4 and 2 foliar nodes

Cutting type*	Rooted (%)	Flushed (%)	Rooted and Flushed (%)
4-leaves (4 nodes)	66	91	66
2-upper leaves (2 nodes)	28	56	28
2-lower leaves (2 nodes)	25	47	25

*Leaves were trimmed to one half length

Table B.21. Cumulative rooting and flushing of cuttings with two or four half-leaves taken from young Eucalyptus coppices

Leaves cutting	Number of cuttings taken	Weeks under mist							
		Rooting (%)				Flushing (%)			
		3	4	5	6	3	4	5	6
Two	193	29	48	57	62	66a	69a	70a	73a
Four	154	33	46	53	64	42b	47b	51b	66b
Significance ^z		NS	NS	NS	NS	**	**	*	*

^zWithin columns, means with different letters are significantly different according to the t-test at P=0.05 (*) or P=0.01 (**). NS = nonsignificant.

Table B.22. Rooting (%) and flushing (%) of cuttings as related to senescence and dehiscence of leaves

Leaves remaining on cutting	Cuttings that rooted (%)	Cuttings that flushed (%)
0	12	12
1	41	29
2	68	59
3	91	86
4	80	78
<u>Leaf color</u>		
green	90	79
Changing to red	61	63
Red	22	22

Table B.23. Number of cuttings used and rooting percentage of three Eucalyptus clones in an unheated medium on different dates.

Clonal source	Date of rooting trial initiation					
	Sept. 1982		June 1983		Aug. 1983	
	Number of days under mist					
	40		30		45	
	Cuttings (no.)	Rooting (%)	Cuttings (no.)	Rooting (%)	Cuttings (no.)	Rooting (%)
ILA-I	1238	6.7	374	9.6	770	5.2
ILA-II	1854	10.0	434	10.8	392	8.0
ILA-III	1277	3.7	-	-	392	10.7

Table B.24. Rooting of six randomly selected "local" clones of Eucalyptus
camadulensis

Clonal source	Number of cuttings	Rooting (%)
1	56	45
2	29	4
3	62	40
4	27	52
5	68	93
6 (ILA-11)	18	17

Table B.25. Rooting of four-leaved cuttings after 4 weeks under mist, of
eight Eucalyptus clones from various seed sources

Provenance seed source	Clone no.	1st experiment		2nd experiment	
		Cuttings (no.)	Rooting (%)	Cuttings (no.)	Rooting (%)
"Local"	1	20	2	23	0
"	2	24	2	42	5
Lake Alvacutya, Victoria (6845)	3	15	44	36	22
"	4	38	57	31	23
Katherine, N. Territories (6869)	5	13	92	22	86
"	6	20	69	24	79
Menger's Hill, S. Australia (6970)	7	14	80	27	78
"	8	10	74	24	83

Table B. 26. Rooting (%) and flushing (%) of cuttings as related to different clones

Clone	Rooted (%)	Flushed (%)	Rooted & Flushed (%)
1.1	81	62	62
1.2	81	81	69
2.1	100	100	100
3.1	56	62	50
4.1	88	50	50
5.1	38	44	31
6.1	62	69	62
7.1	69	62	56

Table B.27. Cumulative rooting percentage after different times under mist of cuttings with two or four half-leaves taken from eight Eucalyptus clones of various seed sources

Provenance seed source	Clone No.	Weeks under mist			
		3	4	5	6
"Local"	1	2	2	4	4
"	2	2	2	5	21
Lake Albacutya, Victoria (6845)	3	22	44	58	69
"	4	20	57	73	83
Katherine, N. Territories (6869)	5	78	92	92	94
"	6	57	69	74	86
Menger's Hill, S. Australia (6970)	7	67	80	87	87
"	8	57	74	91	96

Fig. B.1. The effect of etiolation of the cutting base (ETIOLATED) of two Eucalyptus clones on cumulative rooting percentage, as compared with non-etiolated (GREEN) cuttings of the same and other clones. Experiment started December 1982.

Fig. B.2. The effect of etiolation of the cutting base (ETIOLATED) of four Eucalyptus clones on cumulative rooting percentage, as compared with non-etiolated (GREEN) cuttings of the same clones. Experiment started April 1983.

Fig. B.3. Number of leaves retained under mist on rooted and non-rooted cuttings of different *Eucalyptus* clones, with and without base etiolation. Experiment started December 1982.

Fig. B.4. Number of leaves retained under mist on rooted and non-rooted cuttings of different Eucalyptus clones, with and without base etiolation. Experiment started April 1983.

Fig. B.5. Petiole retention (%) on nodes of four *Eucalyptus* clones during incubation at 28°C.

C. In vitro CULTURE - O. Reuveni, Israel

Clonal propagation of several eucalypt species by in vitro techniques has been reported and reviewed (Durand-Creswell et al., 1982; Hartney, 1982). Although this technique is at present more expensive than propagating by rooting of cuttings, it has the advantage of enabling us to multiply difficult-to-root clones, which might be important in breeding programs, and to establish stock plants of difficult-to-root plants from which cuttings can be taken for conventional rooting. The techniques developed for eucalypts are similar to those developed for other plants (George and Sherrington, 1984). Of the various initial explants and methods used, the simple method of using stem nodals was chosen. With this method stem nodals are surface-sterilized and then placed on a sterile medium. After a certain period, axillary shoots usually developed and then were subcultured for further shoot multiplication, or for root formation and potting. During the study we faced two main problems: (i) Explants taken from mature trees failed to establish and grow in culture; a phenomenon known from the literature with other eucalypts (Durand-Creswell et al., 1982). (ii) At the stage of hardening and potting many plants, although rooted, were lost.

Objectives: 1) To study the effect of the physiological status of the plant material source, on the establishment and multiplication rate in culture. 2) To optimize the media used at different stages of multiplication and rooting. 3) To compare the multiplication and rooting capability of four clones. 4) To harden and pot multiplied plants.

Methods: Nodal stem segments (3-5 mm diameter) carrying an axillary bud were used as initial explants. Surface sterilization was done in ethanol (70%) for 2 min, followed by sodium hypochlorite (3% NaOCl) for 3 min; then the segments were rinsed twice with sterile water and finally dissected to fragments in a sterile solution containing citric acid (2,000 mg/l) and ascorbic acid (200 mg/l). They were planted on a shoot multiplication medium and subcultured every 6 weeks. The developed axillary shoots were cut into 5-mm-long pieces and inoculated again on a multiplication medium, or on a rooting medium. The composition of the media used for multiplication or rooting is that given in Table C.1, if not otherwise mentioned.

Media were sterilized by autoclaving of 105 kPa for 15 min. Cultures were maintained in test tubes (25 x 150 mm) at $28 \pm 1^{\circ}\text{C}$ under 40 W Gro-Lux fluorescent illumination ($80 \mu\text{ES}^{-1} \text{ m}^{-2}$), with a 16-h light and 8-h dark photoperiod. For rooting and hardening of plantlets before potting, a special procedure was developed which is described in Figure C.1.

Results: The results are presented according to the different steps of operating the in vitro propagation technique and not necessarily in the same order, in which they were done. At each step, results of the final decisive experiments are presented excluding many preliminary experiments (e.g. the development of the plug system for rooting and hardening of explants).

1) Effect of explant source on establishment and growth in culture

Explants were derived from four clones used in the experiments on rooting cuttings. Thirty explants were inoculated from each of the following sources: mature trees, cut-back stockplants grown in the field, and cut-back stockplants grown in a greenhouse. For one clone a fourth source was field-grown coppice shoots (Table C.2). The rate of external contamination was the highest in explants derived from mature trees, and only a small percentage of explants which survived started to produce a new growth. The rate of contamination was very low in the two kinds of explant sources derived from cut-back stockplants and the percentage of uncontaminated plants which started new growth was quite high after 40 days. Explants derived from coppice shoots were inferior to the cut-back stockplants in this respect but were better than those of mature plants (Table C.2).

Fifty percent of the explants of the ILA IV clone from greenhouse-grown stockplants rooted after 40 days in culture. Roots were formed on the original explant.

The uncontaminated and growing explants were subcultured and when the axillary or the main shoot was large enough, the explants were cut into new segments. The rate of multiplication at transfers was calculated as the ratio between the new cultures obtained and the initial number of cultures. This ratio increased with time in culture until it reached a more or less steady state which was typical for each clone (Table C.3), without differing between explant source.

2) Effect of plant growth regulators on growth and rooting

The effect of plant growth regulators on the growth and rooting of the four clones was studied in a series of experiments. Explants were derived from cultures in shoot multiplication medium. In the first test the effect of the two auxins NAA and IBA, in the presence and absence of the cytokinin BA, was compared in 10 cultures of each combination of the four clones (Table C.4). The main findings were that BA suppresses rooting even if auxin is added; the auxin IBA is more effective than NAA in inducing rooting; both cytokinin and auxin are needed to achieve maximum growth; and as found in other cases, the rate of growth and response was not the same in the four clones compared (Table C.4).

In another experiment, conducted as above, the effect of NAA and IBA applied at different concentrations, without BA, was studied. It was confirmed again that without BA, rooting of explants occurs at a high rate and in some clones even without adding an auxin. Of the two auxins, IBA was more effective than NAA in promoting mass production of both shoots and roots (Table C.5). Of the different concentrations tried, 0.25 mg/l seems adequate in most cases, as higher concentrations induced callus production and some suppression in shoot growth.

In four other experiments, explants of the ILA IV clone were used to study more carefully the interactions between different concentrations of the cytokinins BA and kinetin with the auxins NAA and IBA. In reference to rooting, it was confirmed again that BA suppresses rooting even if IBA is added, while kinetin does not suppress it at the same rate, especially when IBA is added. Again, IBA was found to be much more efficient than NAA in inducing rooting (Table C.6). Maximum shoot production was achieved with a combination of both cytokinin and auxin in the range of 0.25-0.50 mg/l of each (Table C.7). At higher concentrations, shoot production was lower and, in most cases, leaves were smaller. Of the two cytokinins, BA was more effective than kinetin in inducing shoot production. At the higher concentrations of the auxins, and especially of IBA, callus was formed in addition to shoots and roots (Table C.8).

3) Rooting, hardening and potting

In the experiments conducted with plant growth regulators, it was easy to induce root formation in all four clones as explants (and others not reported)

(Tables C.4 and C.5). Rooting of the ILA IV clone was not difficult, whereas as a cutting under mist it was difficult to root (Table C.6).

The next step was hardening (and potting) of the rooted plantlets. At this stage, many failures occurred when small plantlets with a few leaves and a well developed root system were potted. Keeping the plantlets for a while at a high relative humidity did not solve the problem. Plants died as a result of leaf desiccation, shortly after removal from the high humidity environment. After many preliminary experiments aimed at developing a functional root system and hardened shoots, the procedure described in Figure C.1 evolved. The technique was developed with the aim of solving three main problems: (i) to obtain root development in a well-aerated medium; (ii) to get new leaves able to develop under relatively higher light intensity and a certain temperature fluctuation (the conditions prevalent in the greenhouse); and (iii) to perform hardening gradually, without disturbing the root system and the shoot.

Using a plant with the plug potted was found to be successful, and was used for potting hundreds of plants. The rate of success measured as potted growing plants was found to be 60-80% of the initial number of explants used.

Discussion: The results obtained with the in vitro multiplication demonstrated that it is possible to use this technique for vegetative propagation of Eucalyptus camaldulensis, as has been shown by others (Durand-Creswell et al., 1982; Hartney, 1983). In order to succeed, there are three main stages which are crucial: establishment in culture; multiplication; and hardening and potting.

As with many other woody plants (Bonga, 1982), it is difficult or even impossible to establish in culture mature tissue of eucalypts, or it takes a very long time (McComb and Bennett, 1982). To overcome this problem, it is necessary to rejuvenate the stockplants from which the initial explants are taken. This stage was found to be crucial in our study, as well as in other studies (Durand-Creswell, et al., 1982). Initial explants taken from such rejuvenated stockplants have two advantages: the rate of external contamination is much lower (Table C.2), and they start to develop much faster, as compared with explants derived from mature trees. In our case (except for some better rooting after 20 days in the greenhouse), no clear difference was found between explants derived from cut-back stockplants grown on their own roots in the field or in the greenhouse. In cases

where it is very difficult to establish stockplants on their own roots, grafted plants might serve as a good source of explants, as they are rejuvenated (Durand-Creswell et al., 1982).

At the multiplication stage, when a great amount of shoot production is desired, a proper ratio of cytokinins and auxins has to be used. It was found that both are needed to induce shoot proliferation. The question is whether the maximum or the optimal shoot production is aimed. By optimal, we refer to shoot formation without callus formation. The avoidance of callus formation is in order to prevent plant regeneration from this tissue which might induce a higher somaclonal variation (Larkin and Scowcroft, 1981), undesirable in *vegetative* propagation. Therefore, the optimal kind and concentration of the plant growth regulators has to be considered. Of the two cytokinins tried, BA was more efficient than kinetin in inducing shoot formation. Of the two auxins, NAA was more efficient than IBA in avoiding callus formation (Table C.8). The optimal concentration of both NAA and BA was in the range of 0.25-0.50 mg/l, which is in agreement with the findings of other researchers (Hartney, 1983).

For the last stage of rooting, hardening and potting, a special procedure was developed which enables us to perform this stage gradually, ending with a hardened plant with a functional root system. Although no evidence is presented, it is believed that the success with this procedure is a result of obtaining a functional root system, as opposed to the root system obtained when explant roots are formed in agar. The growth of the plantlets at a higher light intensity and at temperature fluctuations might lead to a different kind of wax on the leaves (Giese, 1975; Grout and Aston, 1978; Sutter, 1984), which prevents free water loss from the plants once they are transferred from in vitro to in situ conditions. Further development of this technique and a study of other factors (such as pH of the medium and scaling of the test tubes with different films) might improve the results obtained so far and simplify the technique. Another advantage lies in the fact that the last stage is carried out in a greenhouse, which saves room in the more expensive incubation rooms, and thus makes the in vitro propagation of eucalypts cheaper than hitherto. In general, this technique enables the propagation of difficult-to-root clones - which are important in breeding programs - to produce stockplants from which cuttings can be taken for rooting under mist.

Table C.1 Composition of the media used for multiplication and rooting

Component	Shoot Multiplication	Rooting
	Medium	Medium
Murashige and Skoog (1962) Major elements	$\frac{1}{2}$ strength	$\frac{1}{2}$ strength
" " " " Minor elements	full strength	full strength
Sucrose	3.0%	3.0%
Agar	0.8%	-
Benzylaminopurine (BA)	0.2 mg/l	-
Naphthaleneacetic acid (NAA)	0.2 mg/l	-
Indolebutyric acid (IBA)	-	0.5 mg/l
Kinetin	-	0.1 mg/l
Activated charcoal	-	0.5%
pH before autoclaving	5.6	5.0

Table C.2 Effect of explant source on the cumulative rate (%) of contamination and in the death and start of new growth in the uncontaminated cultures 20 and 40 days after initial incubation.

Culture situation		Clone	E x p l a n t s o u r c e							
			Mature trees	Pruned stockplants grown in the:						Coppice shoots
				Field		Greenhouse				
20	40	20	40	20	40	20	40			
Days after incubation										
Contamination	ILA I	70	80	3	7	0	0	3	3	
	ILA II	83	83	3	3	0	3	-	-	
	ILA III	57	60	0	0	0	0	-	-	
	ILA IV	90	97	0	3	7	7	-	-	
	Average	75	80	2	3	2	3	3	3	
Death	ILA I	0	3	7	57	0	0	0	37	
	ILA II	0	7	0	37	0	0	-	-	
	ILA III	0	7	0	30	0	0	-	-	
	ILA IV	0	0	0	3	0	0	-	-	
	Average	0	5	2	32	0	0	0	37	
New growth	ILA I	0	0	3	55	23	37	0	6	
	ILA II	0	0	31	77	40	41	-	-	
	ILA III	8	11	30	67	43	47	-	-	
	ILA IV	0	0	0	21	36	39	-	-	
	Average	2	3	16	55	36	41	0	6	

Table C.3 Culture multiplication ratio of four clones in the first five sub-cultures of initial growing explants

Subculture order	C l o n e			
	ILA I	ILA II	ILA III	ILA IV
1	1.2	1.7	1.5	1.6
2	1.5	1.6	1.3	2.0
3	2.8	3.7	3.7	2.9
4	5.3	8.0	5.8	3.6
5	4.0	6.0	4.5	8.2

Table C.4 Percent of cultures in which roots were formed, and the average fresh weight (10^{-1} g) of shoot per culture of four clones grown on media with different concentrations of BA, NAA and IBA

Plant growth regulator (mg/l)			C l o n e							
			ILA I	ILA II	ILA III	ILA IV	ILA I	ILA II	ILA III	ILA IV
BA	NAA	IBA	Cultures with roots (%)				Shoot fresh weight (10^{-1} g)			
0	0	0	60	90	20	30	4.0	4.6	2.0	2.1
0.25	0	0	0	0	0	10	6.3	11.2	4.7	6.7
0.25	0.25	0	10	20	0	20	5.4	9.6	4.3	5.7
0.25	0.50	0	0	0	10	10	7.8	12.0	5.5	7.1
0.25	1.00	0	30	10	10	20	7.3	7.4	4.6	5.1
0.25	0	0.25	40	40	20	10	6.4	9.2	4.1	5.0
0.25	0	0.50	10	30	30	10	7.6	9.6	5.0	5.9
0.25	0	1.00	0	60	0	0	8.0	8.5	4.2	5.8

Table C.5 Percent of cultures in which roots were formed, and the average fresh weight (10^{-1} g) of shoots and roots per culture of four clones grown on media with different concentrations of NAA and IBA.

Plant growth regulator		C l o n e											
NAA	IBA (mg/l)	Cultures with roots (%)				Shoot fresh weight (10^{-1} g)				Root fresh weight (10^{-1} g)			
		ILA I	ILA II	ILA III	ILA IV	ILA I	ILA II	ILA III	ILA IV	ILA I	ILA II	ILA III	ILA IV
0	0	40	100	0	60	1.1	1.7	0.4	1.7	<	<	<	0.1
0.25	0	90	100	90	100	0.9	1.4	0.9	2.2	<	0.1	0.1	0.3
0.50	0	100	100	100	100	1.2	2.3	0.6	1.5	0.2	0.4	0.3	0.4
1.00	0	100	100	100	100	1.7	2.3	0.8	2.1	0.2	0.7	0.8	0.5
0	0.25	100	100	100	100	1.8	3.2	1.1	3.0	0.6	1.5	0.6	0.9
0	0.50	100	100	100	100	2.4	3.3	1.2	2.2	1.1	1.9	1.2	1.3
0	1.00	100	100	100	100	3.1	4.4	0.2	2.1	1.5	3.2	2.1	2.8

Table C.6 Percent of ILA IV clone cultures in which roots were formed in four experiments of adding different concentrations (mg/l) of cytokinins and auxins to the media

Exp. 1

NAA \ BA	0	0.25	0.50	1.00
0	40	0	0	0
0.25	50	0	0	0
0.50	80	0	0	0
1.00	50	0	0	0

Exp. 2

IBA \ BA	0	0.25	0.50	1.00
0	50	0	0	0
0.25	100	0	0	0
0.50	100	0	0	0
1.00	100	0	0	0

Exp. 3

NAA \ KIN	0	0.25	0.50	1.00
0	40	30	30	10
0.25	80	70	20	0
0.50	70	70	50	10
1.00	80	80	60	30

Exp. 4

IBA \ KIN	0	0.25	0.50	1.00
0	50	20	50	10
0.25	100	90	50	20
0.50	100	90	60	20
1.00	100	90	80	30

Table C.7 Average shoot fresh weight (10^{-1} g) of ILA IV clone cultures in four experiments with different concentrations (mg/l) of cytokinins and auxins added to the media

Exp. 1

NAA \ BA	0	0.25	0.50	1.00
0	1.0	3.0	3.5	3.6
0.25	1.3	3.4	3.6	3.5
0.50	1.6	2.5	3.7	3.8
1.00	1.3	2.4	2.9	2.7

Exp. 2

IBA \ BA	0	0.25	0.50	1.00
0	1.7	3.6	4.5	5.0
0.25	2.5	5.9	9.1	3.9
0.50	1.5	4.0	2.9	2.4
1.00	1.2	2.1	1.9	1.8

Exp. 3

NAA \ KIN	0	0.25	0.50	1.00
0	1.0	1.9	2.2	2.7
0.25	1.5	1.9	2.5	2.9
0.50	1.5	2.8	3.0	3.9
1.00	2.0	2.2	2.5	2.6

Exp. 4

IBA \ KIN	0	0.25	0.50	1.00
0	1.3	1.4	2.8	1.6
0.25	2.8	1.6	2.9	2.3
0.50	2.9	2.0	2.2	2.3
1.00	3.6	2.6	2.5	2.9

Table C.8 Average fresh weight (10^{-1} g) of callus produced in ILA IV clone cultures in four experiments with different concentrations (mg/l) of cytokinins and auxins added to the media

Exp. 1

NAA \ BA	0	0.25	0.50	1.00
0	0	0	0	0
0.25	0	0	0	0
0.50	0	0	0	0.1
1.00	0.1	0.2	0.2	0.2

Exp. 2

IBA \ BA	0	0.25	0.50	1.00
0	0	0	0	0
0.25	0	0	0	3.6
0.50	1.6	2.2	3.1	4.3
1.00	3.3	3.2	4.8	4.3

Exp. 3

NAA \ KIN	0	0.25	0.50	1.00
0	0	0	0	0
0.25	0	0	0	0
0.50	0	0	0	0.2
1.00	0.1	0.4	0.2	0.2

Exp. 4

IBA \ KIN	0	0.25	0.50	1.00
0	0	0	0	0
0.25	0	1.3	0.3	0.4
0.50	0.5	1.3	1.1	0.6
1.00	1.2	1.7	1.4	1.0

Fig. C.1 Description of operation and schematic drawing at different stages of eucalypt plantlets' growth; rooting, hardening and potting.

Stage	Description of operation	Schematic drawing
I	Autoclaving of polyurethane plugs (P) (20 x 40 mm) in a test tube (25 x 150 mm) containing a liquid medium (M).
II	Inoculation of explants (E) and incubation for 7-10 days at 28°C, with 18 h light.
III	Transfer of test tubes to a controlled temperature (18-32°C) greenhouse under reduced light intensity until shoot and roots are formed. Cups are fastened to the test tubes by a parafilm band (B).
IV	Original solution is rinsed, plugs are washed a few times with tap water. Half-strength non-sterile Hoagland solution (H) is added. The test tubes are kept for 7-10 days in a humid chamber in the greenhouse.
V	Further hardening continues in the test tubes, which are transferred to greenhouse atmosphere for another 7-10 days. At the end of this period, they are ready for potting (RP).
VI	Potting of plants is done with the plug. Proper irrigation and high relative humidity around the plants at the first stages are essential for good take.

D. Container Studies - T. Geary and M. Cunningham, U.S.A.

Rooted cuttings must be grown to a size and hardness that insures high survival over a wide range of environmental conditions--from dry to saturated soils, from cold to hot. Container-grown plants are needed so that growth can be controlled in order to have plants of the proper kind ready when they are needed.

A precedent has been established for this--seedling eucalypts are almost universally grown in containers.

A few basic requirements for growing eucalypt seedlings in containers are well known although not always followed. The container design should prevent roots from spiraling and allow roots to air prune. Root spiraling if started in the container, continues in the field and often results in self strangulation; air pruning produces a fibrous root system.

There is a bewildering array of containers used. The kind selected by each nursery has been based mainly on empirical research or the practical expedient of using what was available. Studies on the comparison of container types typically confound spacing, volume, and shape. Qualitative studies that control the variables are rare for any containerized tree species. Diameter of the container was more important than the depth of the container for five tree species (Endean and Carlson, 1975). Diameter of the container, however, did not influence the growth of picea glauca (Carlson and Endean, 1976).

Spacing of the containers was not included as a variable in these studies. Despite the contradiction with one species, the evidence suggests that the wider the diameter of the container, the better, while depth of the container is not important. Despite this, the tendency is to produce long narrow containers, because practicing foresters feel that long root plugs result in better field survival of seedlings, particularly when the topsoil is dry. Also, narrow diameter containers permit closer spacing of the seedlings in the nursery, which reduces nursery costs, especially when the seedlings are grown in houses or under shade.

The effect of spacing has not been quantified on the quality of eucalypt species. It does seem, though, from experience in south Florida that E. camaldulensis can be grown at a closer spacing than other eucalypt species because of its narrower crown.

In order to minimize nursery cost as much as possible, the period of growing a cutting to planting size should be as short as possible, the smallest rooting volume possible should be used, and the closest spacing possible should be used. One of the most expensive items in the nursery is the cost of the potting mix and the transporting of plants. The less mix used and the lighter the plant, the better.

Methods, Results, and Discussion: Three container studies were conducted. The first 2 experiments used seed and seedlings, to make progress in container systems, while we were developing cutting methods. In all experiments, containers were constructed to PVC pipe of various diameters and lengths to represent container configurations that are commonly used to grow Eucalyptus. These were tested over a range of spacings used in Eucalyptus nurseries. In the first study, spacing treatments were used as whole plots, with one of each of the container size combinations present in each spacing block. Total height was measured every week for 15 weeks. The results for container size are given in Table D.1.

The analysis of variance showed that both tube diameter and tube length were significant at the 0.05 level. Seedling height increased as tube diameter increased but was equal for all tube lengths except the longest, where seedling height decreased. Container spacing effects were not significant. Average heights were relatively the same for all densities except the highest one, where seedlings were a little taller (Table D.2.).

Linear regressions were run, using seedling height as the dependent variable and either tube volume, tube surface area, or tube area to tube volume ratio as independent variables. None of these were good predictors of seedling height with correlation coefficients of 0.085, 0.165 and 0.0003 for tube volume, surface area and volume/area, respectively.

For the density of 200 seedlings/m², a fourth tube diameter was included. The mean seedling heights for container sizes, using only the widest spacing, are given in Table B.3.

The analysis of variance showed only tube diameter effects as significant. The 64mm tube produced a considerably taller seedling than the other diameter containers.

The second experiment used the same size containers but only the three highest densities (300, 400 and 500 seedlings/m²). For each container size by spacing treatment, a nine-seedling square plot was used. This was to eliminate the possibility of competition effects of seedlings in different container sizes growing together. Using this type of design, the 38mm diameter tubes could not be used for the highest density because of the thickness of the PVC container. Seedlings were grown for 18 weeks and then total height, root-collar diameter, and root-and-shoot dry weights were measured.

The treatment averages for each variable are shown in Table D.4. The statistical analysis had not been completed on these data but it appears that the larger the tube diameter and length, the taller the seedling produced; or, more simply put, the larger the container, the taller the seedling produced. However, neither container size nor spacing appears to influence root-collar diameter. Root dry weights also increased with increasing tube size. This also seems obvious, since a larger tube volume can hold a larger mass of roots. Shoot dry weight responded in a similar manner. Shoot dry weight also appears to be influenced by seedling density in an inverse way. This may be due to the tendency of seedlings at wider spacings to retain more leaves than seedlings at closer spacings, and leaves were included in shoot dry weights. Shoot-root ratios using dry weights increased as diameters of tubes increased but decreased as tube length increased. As with seedling height, the response of shoot-root ratio was variable for densities.

The third container experiment used cuttings as the planting stock material. Standard cuttings were prepared, two from each of five clones, making a total of ten cuttings for each treatment combination. Again, three container diameters and four container lengths were tested. The ten containers were suspended on wire as row plots under the mist nozzles. The cuttings were kept under mist for ten weeks. The mist was then turned off and the cuttings were grown for an additional eight weeks.

Treatment averages for tube diameters and length are shown in Table D.5. The results for the effects of tube diameter are similar to those found with seedlings--the larger the diameter, the larger the cutting. However, for tube length the data show that the larger the container the better--up to a point. The 15 cm tubes produced more shoot and root biomass than the 20 cm tubes, but cuttings grown in the 20 cm-long tubes were taller on average.

Table D.1. Seedling height (mm) as related to container length and diameter

Container Diameter (mm)	Container Length (cm)				Means
	5	10	15	20	
----- Seedling Height (mm) -----					
19	530.25	568.69	558.69	515.62	543.31
25	585.31	570.94	578.38	555.88	572.63
38	629.69	613.75	640.81	630.75	628.75
Means	581.75	584.46	592.63	567.42	

Table D.2. Seedling height as related to spacing

Spacing (Seedlings/m ²)	Average Height (mm)
200	571.73
300	576.81
400	570.63
500	607.08

Table D.3. Seedling height (mm) as related to container length and diameter

Container Diameter (mm)	Seedling Height (mm)	Container Length (mm)	Seedling Height (mm)
19	514.94	5	554.38
25	536.5	10	584.75
38	578.0	15	555.0
64	670.19	20	605.5

Table D.4. Treatment averages for second container experiment

Tube Diameter	Final Height	Root-Collar Diameter	Root Dry Weight	Shoot Dry Weight	Shoot/Root
(mm)	(mm)	(mm)	(mg)	(mg)	
19	359	3.5	409	1076	2631
25	374	3.7	448	1252	2793
38	374	3.8	469	1336	2849
<u>Tube Length</u>					
(cm)					
5	341	3.5	306	1024	3344
10	361	3.7	404	1227	3040
15	375	3.7	478	1271	2660
20	396	3.7	566	1306	2309
<u>Tube Spacing</u>					
Seedling/m ²					
300	372	3.7	446	1226	2747
400	360	3.6	430	1218	2833
500	376	3.6	441	1163	2639

Table D.5. Influence of container size on the growth of rooted cuttings

Tube Diameter	Height	Root Dry Weight	Shoot Dry Weight	Shoot/ Root
(mm)	(mm)	(mg)	(mg)	
19	89.3	246.9	867.1	3.87
25	105.1	254.3	1014.5	4.23
38	138.2	280.6	1078.0	3.88
Tube Length (cm)				
5	102.7	191.4	912.9	5.02
10	108.4	218.4	968.4	3.84
15	104.5	318.3	1076.4	3.42
20	122.4	277.0	988.2	3.60

E. STRATEGY FOR MASS PRODUCTION OF SELECTED CLONES

To succeed in the vegetative propagation of a selected ortet of eucalypt, there is need to go through intermediate stages of preparing the plant material before it is multiplied by one or another method. The steps, methods, possibilities and options suggested are presented in Fig. E.1. They are based on results obtained in this study and those reported in the literature. Each stage was discussed in detail in the previous chapters and here a general summary only of the procedure to be followed is given. The decision which route to follow depends on many factors. For instance, if there is need to keep the original tree, coppice cannot be produced by felling, and girdling or grafting on young seedlings is to be performed. If cuttings produced in the first stage fail to root under mist, in vitro propagation is recommended to produce stockplants grown under a regime involving their cutting-back all the time. The strategy illustrated in the attached flow chart should permit to reach the required objective - vegetative propagation of selected trees.

Fig. E.1. Flow chart of options and operations for vegetative propagation.

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Description of cooperation

The division of research work between the Israeli and American scientists involved the Israelis with grafting, coppicing and in vitro culture studies, while the American scientists dealt with girdling and effects of container configuration and spacing on nursery growth of seedlings. Both Israeli and American scientists studied rooting of difficult-to-root cuttings. Exchange of letters and presentation of common reports made us acquainted with each other's progress in the research. Visits by the Israelis to Florida and by the American scientist to Israel, contributed largely to exchange of ideas and increased knowledge of research progress in both countries. The cooperation of the scientists of the two countries resulted in achieving the objective - development of a technique for mass production of rooted cuttings of Eucalyptus camaldulensis.

Benefit to forestry

The technique developed in this project to mass-produce rooted cuttings of superior trees of Eucalyptus camaldulensis enables Israeli and American foresters to increase the productivity of Israel's and Florida's eucalypt plantation. Breeding for timber yield (quantity and quality), drought avoidance, salt and lime resistance, is now a practical proposition for application in the two countries as well as in many of the developing countries where E. camaldulensis can be grown. These countries can use the technology to establish high-yielding fuelwood plantations. Results of our research can also assist the U.S. Forest Service in its lead role within the framework of AID in planning, developing and managing a bioresource energy program for the less developed countries of the world.