PART 1

TECHNIQUES OF VEGETATIVE PROPAGATION OF CONIFERS

AIR-LAYERING OF GRAFTS TO OVERCOME INCOMPATIBILITY PROBLEMS IN PROPAGATING OLD PINE TREES

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ABSTRACT

A technique of air-layering and subsequently deep-planting 20-month-old grafts has been developed as a practical method of propagating 20- to 35-year-old plus trees of eight pine species. The method has been used successfully to preserve incompatible clones and to produce ramets of these for seed orchards. Various modifications of technique were tried to improve the rooting rate of scions but yearly variation in environmental conditions appeared to be more important than any of the treatments. Success rates of more than 90% should be attainable with a controlled environment. It is relatively easy to induce roots to develop from rootstock tissue at the graft union and further experimental work is needed to assess the value of this.

INTRODUCTION

In most pine breeding programmes production of both commercial and control-pollinated seed is from clonally propagated orchards. Progress in meeting seed requirements and in developing breeding populations is therefore largely dependent upon successful techniques for vegetative propagation. Initially, grafting is a very satisfactory method of propagating pine species, but it is becoming progressively more evident that delayed incompatibility between scion and rootstock can result in serious loss of productive ramets in a seed orchard. On this account it is not uncommon for clones to be unusable in seed orchards; in extreme cases it may be impossible to propagate an ortet permanently by grafting (Barnes, 1969; Sweet and Thulin, 1973). The alternative of producing clones by cuttings is not often successful because the plus trees are generally physiologically old and rooting becomes more difficult to induce (Libby and Conkle, 1966; Thulin and Faulds, 1968). The third possible technique for vegetative propagation is air-layering. Making layers in the tops of plus trees is unlikely to be successful because of the practical difficulties of access and possibly also because of the morphological position and nutrient status of the layerable material in forest trees (*see* Sweet, 1964).

Layers are much easier to make and maintain, and are more likely to be successful (*see* Anon., 1964) in grafted material of plus trees.

Despite widespread scion-rootstock incompatibility failures in the pine breeding programme in Rhodesia, all clones used so far have been graftable initially with a very high degree of success and none of the incompatibility failures has occurred less than about 24 months after grafting. It has been possible, therefore, to layer grafted material of any clone on very young grafts. This has led to the successful development of a practical method for propagating incompatible clones both for breeding and seed production purposes (Barnes, 1969). This paper reports on, and discusses, the present status of the work.

MATERIALS AND METHODS

Throughout the development of this layering technique the grafts used for experimental work have been largely those left over from grafting programmes undertaken to produce ramets of 20- to 35-year-old ortets for routine seed orchard formation. Both compatible and incompatible clones were used and scions originated from ortets and seed orchard ramets. This has been far from ideal experimental material and it has meant that the work has been in the form of field trials designed to show, from gross effects, the areas in which future formal experimental work might be profitable. Grafting of all the pine species concerned was by the tip cleft method, normally carried out between August and October when a 12- to 18-month-old 50 cm-high rootstock raised in a 22 cm (length) × 25 cm (flat width), 250 gauge (0.625 mm) black polythene tube was used. The best month for layering was May but the scion was not woody enough for successful layering until the second May, about 20 months after grafting. During a period of 9 years the technique has been tried on a varying number of grafts of the nine different pine species shown in Table 1. Both compatible and incompatible clones were used.

In the standard method used for layering the scion on a graft, a 2.5 cm-section was ringbarked at the base of the scion in the callus tissue across the graft union. This section was located as low as possible on the scion without allowing any rootstock tissue to remain in the upper ring of exposed phloem and cambium. A proprietary rooting hormone powder (Seradix B No. 3, containing β-indole-butyric acid (IBA)) was rubbed onto the upper exposed tissues which were then packed round with sphagnum moss contained in a clear 150 gauge polythene tube, 20 cm (length) × 12 cm (flat width), tied above and below the ringbarked section with plastic binding tape. The binding was waxed to prevent water from running down the stem and entering the bag, and the moisture level of the rooting medium was maintained by periodic replenishment with a hypodermic syringe. The layered grafts were lined out in the open in full sunlight in the John Meikle Forest Research Station nursery at 1608 m a.s.l., where the mean annual rainfall is 1839 mm, the mean maximum temperature 21.4°C, the mean minimum temperature 11.4°C, the mean relative humidity 77.7% and the mean daily evaporation 3.1 mm.

During the last 9 years various modifications of the standard technique were tried in an attempt to improve success. These modifications (listed in Table 1) included layering at different distances above the union, omitting the application of rooting hormone, fertilising the rootstock with double superphosphate, pruning the rootstock

TABLE 1—Summary of air-layering results, 1964 to 1972

SPECIES	YEAR (Layered in May/June)	1964	1965	1966	1967				1968				1969				1970											1971	1972				1964-1972 **
	DEVIATIONS FROM STANDARD TREATMENT																																
	Layering point 2-5 cm above graft union	+	+	+	+	+	+	+	+	+						+		+					+	+	+					+			
	Layering point 15 cm above graft union																														+		
	No IBA				+		+																										
	Rootstock fertilized with 60g 38% P_2O_5						+	+																									
	Rootstock pruned (all branches)									+		+																					
	Rind slit for 2 cm above layering point														+																		
	8 cm wide ringbarked section												+																				
	Small section of rootstock tissue left										+	+							+								+	+					+	
	Salicylic acid (0,01g/1)																			+				+		+							
	Salicylic acid (1,00g/1)																				+				+		+						
	Stockholm tar painted on ringbarked section																					+	+										
P.elliottii	Total number of grafts layered		8	12	5	5	5	5	21	5	5	5	40	40			13	24	19			33	23					116	5	7	7	5	311
	Rooted (percent)		13	9	0	0	0	20	24	20	100	100	88	93			23	13	63			15	17					16	20	14	0	60	35
	Number of clones represented		4	4	5	5	5	5	8	5	5	5	17	15			9	18	10			18	13					10	1	5	5	1	
P.kesiya	Total number of grafts layered													19			8			3	4			3	3								40
	Rooted (percent)													79			88			67	100			100	100								85
	Number of clones represented													8			5			2	4			2	2								
P.montezumae	Total number of grafts layered													10																			10
	Rooted (percent)													60																			60
	Number of clones represented													3																			
P.oocarpa	Total number of grafts layered																											19					19
	Rooted (percent)																											63					63
	Number of clones represented																											5					
P.palustris	Total number of grafts layered													3																			3
	Rooted (percent)													100																			100
	Number of clones represented													3																			
P.patula	Total number of grafts layered		50	9	10	10	11	10	49	3	4	3		23	24		5	7	4	6	5			8	8	2	2	113	3	6	6	4	360
	Rooted (percent)		60	33	50	70	46	60	86	33	75	33		57	79		20	29	75	50	60			38	25	50	100	27	0	33	17	75	49
	Number of clones represented		13	6	9	10	10	9	8	3	4	3		14	17		5	6	4	5	5			6	6	2	1	22	2	6	5	3	
P.pseudostrobus	Total number of grafts layered													20																			20
	Rooted (percent)													80																			80
	Number of clones represented													7																			
P.radiata	Total number of grafts layered	11	89	30	10	10	10	10	29	30	28	28		88		85												386					788
	Rooted (percent)	64	69	33	50	50	10	30	59	47	68	72		48		55												6					30
	Number of clones represented	2	26	18	10	10	9	9	29	29	28	28		30		31												31					
P.taeda	Total number of grafts layered		30	50	11	10	10	12	12	13	13	14		9	9		10	16	10	10	9			16	17	9	9	46	65	17	17	64	372
	Rooted (percent)		20	8	9	20	10	17	17	15	38	50		56	67		0	6	40	10	11			19	18	44	22	15	32	18	6	53	19
	Number of clones represented		8	19	9	8	9	12	12	12	13	13		7	9		7	7	6	5	6			6	7	6	5	15	9	11	12	9	

** Excluding layers where rootstock tissue was left and treatments which were clearly prejudicial to success, viz. application of stockholm tar and layering at 15 cm above the graft union.

(removing all branches at layering), making two vertical slits 2 cm-long immediately above the layered point, ringbarking an 8 cm-wide section, leaving a small triangular portion of rootstock tissue above the ringbarked section, applying salicylic acid by soaking a piece of cottonwool in the solution and placing this in contact with the upper exposed tissue for 30 minutes before applying the rooting powder, and painting Stockholm tar on the exposed wood of the ringbarked section. The modified treatments tried in any one year were, where possible, distributed equally between the available clones, and alternatively to the lined-out grafts.

No experiments have been made with the layers after rooting, but they have been used for preserving certain clones and for establishing ramets in routine seed orchards. Initially the layer was severed from the rootstock, potted, and planted out in the field after a further year in the nursery; later, layers were left unsevered and deep-planted with the whole of the rootstock below ground and the soil level approximately 12 cm above the point of layering.

RESULTS AND DISCUSSION

After layering in May, roots appeared between the following September and March. The results of the trials carried out between 1964 and 1972 are shown in Table 1. The percentage rooted included all layers which produced any roots at all regardless of subsequent survival. Except where rootstock tissue is recorded as having been left above the ringbarked section, rooting was taken as having originated from scion tissue. However, it is possible, but unlikely, that sometimes, where the ringbarking was carried out at the union, some strands of rootstock cambium were left on the exposed wood surface and these may have given rise to roots which were not identified as different from those of scion origin. Certain clones did root more easily than others but as more or less the same wide cross section of clones was represented in each year's work and clones were allocated equally to different treatments, results have been taken as being relatively free from clonal influences. The results were not amenable to statistical analysis although in the case of the first three treatments on *P. taeda* in 1972 a chi-squared test indicated that the differences between the percentage rooted at the three layering points were significant at the 0.05 level.

Where it was possible to deep-plant layers immediately after roots appeared, this proved a much more reliable method for establishment in the field than that of first severing and potting the layer. Where the deep-planting of layered grafts has been used for establishing ramets of certain clones in a seed orchard, the layers have proved to be slightly slower growing than grafts, a little more susceptible to windthrow and, in a subjective assessment, a little less branchy. Healthy ramets of *P. patula, P. elliottii, P. taeda* and *P. radiata* clones from 25- to 30-year-old ortets now exist in seed orchards or tree banks and some of these have reached heights of up to 9 m ir years from planting in the field.

The last column in Table 1 shows the overall rate of success in rooting each species during the period from 1964 to 1972. Of the four main pine species the highest proportion of rooted layers was obtained with *P. patula* where 49% of the 360 layers made were successful. *P. patula* was followed by *P. elliottii* with 35%, *P. radiata* with 30% and then *P. taeda* with 19%. The chief point of interest in these results is the wide fluctuation of percentage success for all four species from year to year. For instance, 1969 was a particularly good year for all four species when rates of 57, 93, 48, and 56 % respectively were achieved. In 1971 rooting was generally very poor. When results from these two years, and others, were compared on the basis of the standard technique without modification, the year effect was apparently more important than any of the treatments in obtaining roots from the scion. Since the same staff were associated with the layering exercise every year and since the environment in which the grafts were growing was not under control, it seems likely that the weather rather than any variation in technique was the contributory factor particularly as temperature, humidity and rainfall do vary greatly at the John Meikle Forest Research Station from year to year. In 1971 a large number of scions of layered grafts died before rooting could occur, and this was attributed to the particularly hot, dry, windy conditions which prevailed in the latter part of the year. During the course of the work, failure to root has frequently been associated with waterlogging of the layering medium in the polythene tube. Weather conditions have affected the rate of deterioration of the polythene tube and binding. This factor and the yearly variation in rainfall affect susceptibility to waterlogging. If rooting is delayed the upper binding on the polythene tube has tended to cause a constriction of the scion stem at that point, and this could cause an accumulation of root-promoting hormones at a point where rooting cannot take place. If weather conditions affect the rate at which roots develope, they might thus be an indirect cause of failure to root on this account as well. Clearly, therefore, it would be advantageous to keep the layered grafts under controlled environmental conditions.

There were indications that rooting success decreased with increasing height of the layer above the graft union. Whether this was due to the ability to root being greatest near the rootstock roots or whether it was because the scion material became less woody, and therefore less suitable for layering with increasing height is not known. Callus tissue already exists in a graft union and this may be conducive to rooting in layers at the union. Further, if there is an incipient condition of incompatibility in the graft, the typical phloem blockage associated with this condition may lead to an accumulation of root-promoting hormones in the area.

When the rooting hormone application was omitted, there were slight decreases in overall rooting success for the four main pine species. Although there were too few grafts used for these differences to be statistically significant, the effect was consistent and it might have proved to be greater had a more efficient means of applying and retaining the rooting hormone been used. For example, Cameron (1968) found that auxins are leached from air-layers into the rooting medium and a modified technique to minimise this effect would be worth trying.

The possible importance of the nutritional status of pine cutting or layering material is frequently referred to in the literature (e.g., Jesinger, 1967; Cameron, 1968b). There was little obvious benefit from the application of double superphosphate to the rootstocks in 1967 but more work should be done on this aspect since potted grafts are ideal subjects in which to control, and therefore investigate, the effects of nutritional status on rooting.

Pruning the rootstock of all branches at the time the layer was made appeared to be marginally prejudicial to rooting and certainly led to a greater number of early deaths among the layered grafts. This indicated that the nutritional reserves of the rootstock were barely sufficient to support the graft through the rooting period without supplementation from the foliage.

In 1969 the use of vertical slits above the layer gave marginally better results with both *P. patula* and *P. taeda.* Some roots were produced from callus tissue which originated from the slits in *P. patula,* and there seemed to be less inclination for the callus tissue to bridge the ringbarked section in slit layers so the practice is probably worth adopting.

Many failures to produce roots were attributed to bridging of the ringbarked section by callus tissue. This tendency was most marked in *P. elliottii* where even careful scraping of all cambial strands from the exposed wood of the ringbarked section was not an adequate safeguard against bridging. Trial of the 8 cm-wide ringbarked section, and painting the exposed wood with Stockholm tar, were treatments designed to minimise bridging. In 1969 when the former treatment was tried with *P. elliottii,* success was particularly high but there appeared to be little advantage of the 8 cm-wide section over the 2 to 3 cm-wide section. Failures in the narrow-ringbarked grafts were mostly attributed to bridging; in the wide-ringbarked grafts failures were often due to the death of a graft before rooting could occur. The use of Stockholm tar was decidedly prejudicial to success as it caused the death of many grafts well before roots could have been produced.

The salicylic acid treatment was used because of its rôle as an inhibitor of the formation of indolacetic oxydase, an enzyme which breaks down indole-butyric acid (G. D. Scott, pers. comm.). Very few grafts were available for testing this treatment but there appeared to be some improvement in rooting with both *P. patula* and *P. taeda* and it would be worth trying the treatment on a more formal experimental basis.

In 1968 when the layering point was first tried at the union, very small pieces of rootstock tissue were inadvertently left above the ringbarked section. This was easily done because portions of the rootstock which originally form the tips of the cleft still project up on either side of the scion tissue 20 months after grafting. Many of the layered grafts produced roots from these pieces of rootstock tissue. The roots were generally much more numerous, more fibrous, and thinner than those produced from the scion. In the original assessment (Barnes, 1969) it was thought possible that the presence of roots on the rootstock tissue had a repressive effect on the rooting of the scion, a reasonable assumption since hormones produced in roots may inhibit rooting (*see* Brix and Barker, 1971). However, Brix and Barker (1971) also found that cuttings from mature Douglas fir trees can be induced to root by grafting them at the stem base with cuttings from seedlings, and the indications in their work were that roots of the young cuttings provide a stimulation to root in the mature material.

With improved techniques and environmental control and careful selection of rootstock material it should not be difficult to achieve 90-100 % success in inducing rootstock tissue in most pine species to root in the region of the graft union, and therefore the long-term practical value of this technique should be investigated further. Even if the scion is not induced to root immediately, this technique makes it possible to deep-plant the graft and place the scion in a position where it could root later. Physiologically, the graft may possibly not be as susceptible to incompatibility failure when the graft union is effectively in the root collar zone. Lastly, a better balanced root system is produced from rootstock tissue and, provided incompatibility failure does not follow, this may make layers less susceptible to windthrow than those dependent entirely on the less well balanced root system of scion origin.

The above discussion has dealt with the four main pine species only. Table 1 also shows results with small numbers of grafts of five other minor pine species. A high degree of success has been achieved with *P. montezumae, P. palustris, P. pseudostrobus, P. oocarpa* and particularly *P. kesiya* where, over 2 years, 85% of the 40 layered grafts produced roots. The scion material for all these species except the *P. oocarpa* was from 20- to 30-year-old ortets. The *P. oocarpa* ortets were only 7 years old.

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