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Grafting is not feasible for propagating jarrah (*Eucalyptus marginata*) selected for resistance to Phytophthora Dieback caused by *Phytophthora cinnamomi*

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ABSTRACT

Lines of jarrah, possessing a high level of genetically-controlled resistance to *Phytophthora cinnamomi*, have been selected for use in rehabilitation plantings of diseased forest sites. Some Dieback Resistant Jarrah (DRJ) lines cannot easily be propagated by the standard tissue culture procedure. Grafting was assessed as an alternative propagation method to enable these DRJ lines to be included in seed production orchards. Two trials were carried out at separate locations, each using a range of different combinations of DRJ rootstock and scions. Only six grafts finally survived from a total of over 900 across the trials. It was concluded that the cleft graft method, as applied here to one-year-old rootstock, is not a successful cloning method for jarrah.

Keywords: jarrah forest, rehabilitation, plant propagation, seed orchards, selection, genetics

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INTRODUCTION

Phytophthora Dieback, caused by the soil borne water-mould *Phytophthora cinnamomi*, is the most serious disease of Western Australia's jarrah forests. In affected sites, jarrah dies but does not regenerate since seedlings do not survive, and once-productive jarrah stands are gradually replaced by open woodland dominated by a more resistant tree species. Broad-scale eradication of *P. cinnamomi* is not a viable option, so to re-establish jarrah in these diseased sites it will be necessary to use planting stock that has a sufficiently high level of *P. cinnamomi*-resistance for long term survival and growth in the presence of the pathogen.

Certain lines of jarrah possess a high level of resistance to *P. cinnamomi* which is under strong genetic control (ie, the resistance shows a high level of heritability) (Stukely & Crane 1994). This means that it is feasible to select these Dieback-Resistant Jarrah (DRJ) for use in rehabilitation plantings of diseased forest sites, degraded bushland remnants on farms, open-cut mine sites, and cleared land including river catchments.

Tissue-culture techniques have been developed for the propagation of DRJ, and validation trials of clonal DRJ selections have been carried out in the field (Stukely *et al*., 2007). However, the use of tissue-culturing is too expensive for the routine production of the large numbers of plants required for broad-scale field planting; furthermore, some DRJ lines cannot easily be propagated by standard tissue culture procedures.

Tissue-cultured DRJ have been used to establish open-pollinated seed production orchards. When they reach reproductive age (after about five years), these seed orchards will begin to supply seed in sufficient quantity for use in planting programs. High genetic diversity can be maintained by using many unrelated DRJ lines in the seed orchards.

Grafting is a possible alternative method for propagating jarrah to capture and multiply additional dieback-resistant selections for use in DRJ seed orchards. It is the most common method used for establishing clonal eucalypt seed orchards (Eldridge *et al.* 1994: 221–225). The advantage of using a graft compared to the successfully developed tissue culture system is that grafting can maintain the age of the plant by taking scion from the highest point in the tree, whilst tissue culture success relies upon working with juvenile plants or on rejuvenation. Maintaining the maturity of the plant progresses the plant towards the flowering stage, and hence to earlier seed production.

Grafting has been used successfully by the Forest Products Commission (FPC) for the propagation of several eucalypt species, eg *Eucalyptus globulus* (Barbour &

Butcher 1995), *Eucalyptus saligna, Eucalyptus viminalis, Eucalyptus occidentalis, Eucalyptus kochii, Eucalyptus horistes, Corymbia maculata* and *Corymbia ficifolia*. Preliminary trials with jarrah had met with limited success (one in 50 grafts was successful), but this suggested that it might be possible to improve the grafting success rate to an acceptable level. The aim of this project was to improve grafting performance in jarrah so that grafting can be used for the propagation of DRJ for establishing production seed orchards.

METHODS AND MATERIALS

Two major grafting trials were carried out, using a range of different DRJ rootstocks and scions.

One trial was conducted at DEC Science Division's Kensington Research Centre in 2001–2002 (382 grafts), and the second at FPC Seed Technologies Section's Research Nursery at Wanneroo in 2003 (522 grafts).

Trial 1

Dieback resistant jarrah families from a range of provenances were selected. These families also covered a range of levels of root production for their clones in tissue culture, from very low (family codes 162, 626) to consistently high (family codes 405, 700). The grafting program was planned so that, where possible, each rootstock was grafted with family-related as well as non-related scion and where non-related, the scion covered a range of rootstock families (Table 1). Eighteen unrelated rootstock and eleven scion families were used.

To produce rootstock, half-sib seed-lots collected from the previously-selected individual mother trees of proven DRJ "family" lines in the forest and in planted trials were sown in January 2001, and seedlings were grown in the shade-house at Kensington. After re-potting in May they were transferred to the Kensington No. 2 glasshouse and grown on for grafting.

The scion material was collected from 4- to 5-year-old seedlings of proven DRJ lines in the jarrah open-pollinated seedling seed orchard (OPSSO) in Manjimup and couriered overnight to Perth. To minimise the duration of storing the scions, their collection was divided up so that between 2 and 4 trees were collected at a time.

The scion was harvested, tagged and wrapped in damp newspaper and then placed into an esky with frozen ice bricks for transportation to Perth.

On arrival in Perth the scion was refrigerated until required. The scion was pruned of unusable material and placed into a sterilised bucket with Benlate (1tsp per 5L of water). All grafts were carried out using the cleft graft technique. The scion was shaped so that the diameter of the scion matched that of the pruned rootstock. The sources of the scion and rootstock were recorded by a two-label system on each graft. Steri-prune was used to seal the scion wound at the top of the graft and the union was sealed with grafting tape.

In this experiment both the genetic compatibility between the scion and the rootstock and the physical attributes of the grafting technique were recorded so that

Table 1

The grafting compatibility plan for Trial 1, showing the relationship of *Eucalyptus marginata* rootstock and scion, and numbers of grafts completed. Cells highlighted in grey indicate where the scion was related to the rootstock. 'Sib' indicates the half-sib relationship between five rootstocks and three differently numbered scion lines.

		Rootstock																	
	Code	722	742	744	724	729	019	091	162	405	624	626	671	672	692	694	700	734	745
	Sib	001	001	001	005	012													
Scion Code	001	5	5	5	5	0	5	0	5	0	5	0	3	0	5	0	5	0	5
	005	0	5	5	0	0	5	0	5	0	5	0	0	0	5	0	5	0	5
	012	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0	5	0
	019	0	5	5	0	0	5	0	5	0	5	5	0	0	0	0	0	0	0
	091	0	5	5	0	5	0	5	0	5	0	0	0	0	0	0	0	0	0
	162	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	671	0	0	5	0	2	0	5	0	2	5	5	5	5	5	5	5	0	5
	672	5	0	4	2	0	5	5	0	0	0	0	0	5	0	0	0	0	0
	692	2	0	4	0	5	0	0	0	0	2	0	0	0	10	5	0	0	0
	694	0	5	5	0	0	0	10	0	5	0	5	5	5	0	5	5	5	0
	700	0	0	5	0	5	0	4	3	0	0	4	0	0	0	0	5	0	0
	Total	17	25	48	7	22	20	34	18	17	22	24	13	20	25	20	25	10	15

common links on the successful grafts might provide valuable information as to how to improve the technique.

A total of 62.5 hours was required to make 382 grafts and record their details for the physical observation part of the experiment.

The physical attributes recorded on the rootstock were:

- stem height to the graft union (cm)
- number of leaves on the rootstock
- number of axillary branches from the rootstock
- whether the stem was round or square at the union
- a rating of health from 1 (good) to 5 (poor)

The physical attributes recorded on the scion were:

- stem diameter (mm)
- number of nodes
- number of leaves on the scion
- the amount of leaf cropped (%)
- a rating of health from 1 (good) to 5 (poor)

The grafting was carried out in mid-February 2002. The glasshouse used for grafting had shade cloth on the roof and the overhead irrigation system was set to water twice a day. Irrigation was reduced to once daily after grafting. The shade cloth over the glasshouse was removed when signs of necrosis at the graft union were observed, to reduce humidity. However, with the rapid change in temperature, this caused visible stress in the grafts. Similar symptoms had been observed with an *E. globulus* grafting program in this facility, which produced poor grafting results.

Trial 2

Rootstock and scion were prepared in a similar way as for Trial 1. Half-sib seedlots for rootstocks were sown in April 2002, and seedlings were grown in the shade-house at Kensington. After re-potting in August they were transferred to the Kensington No. 1 glasshouse and grown on for grafting. In November, the rootstocks were transferred from Kensington to Wanneroo, where the grafting was to be conducted in the shade house. The latter facility was regarded as a better grafting environment than that at Kensington.

The grafting program spanned the period from 10th to 21st March 2003 and was undertaken by the most experienced grafter on FPC staff. A total of 522 grafts were made, using 21 unrelated rootstock families and 16 scion families from the Manjimup OPSSO. The scion was again spread across the rootstock so that compatibility between scion and rootstock could be investigated. The two most promising families from Trial 1 (families 700 and 694) were included.

RESULTS

Trial 1

Of 382 grafts completed only nine produced shoots, with three grafts finally surviving (Tables 2 and 3).

The following observations and trends were noted for the shooting grafts:

- Three families of rootstock (families 700, 626 and 091) each had two grafts shooting. Of these, the two from family 091 and one from 626 survived. This indicates that these two families may favour the grafting technique although no statistical analysis can be applied to the data. Other rootstock families whose grafts produced shoots were 162, 694 and 744.
- Of the successful grafts, each involved a scion grafted onto an unrelated rootstock. However, of all the scion that produced shoots, family 700 had three grafts that produced shoots with one surviving, family 694 had two grafts that produced shoots and the following families each had only one graft that produced shoots: 012, 019, 671 and 672. One of these shooting grafts with scion from family 700 was on related rootstock. Family 700 and family 694 appear to be families that produce scion that is compatible with grafting.
- Graft union height above the soil ranged from two to 32 cm (average 17.4 cm). Of the successful grafts,

Table 2

Rootstock and scion attributes for the nine *Eucalyptus marginata* grafts (ramets) that produced shoots in Trial 1.

Graft Identity			Rootstock				Scion					
Rootstock code	Scion code	Ram. #	Graft Height (cm)	# Leaves	# Axillary Branch	Health (1 good - 5 poor)	Stem dia. (mm)	# Nodes	# Leaves	% Leaf crop	Health (1 good - 5 poor)	Survival
91	672	1	16	6	3	1	3	1	1	90	1	Alive
91	700	4	13	5	2	1	3	1	2	30	1	Alive
162	700	2	10	1	0	1	3	1	1	30	1	Died back
626	12	1	26	4	3	1	4	1	2	30	1	Died back
626	671	1	17	1	2	1	3	1	1	90	1	Alive
694	694	2	15	5	2	1	3	1	2	30	1	Died back
700	694	2	21	1	2	1	4	1	2	90	1	Died back
700	700	1	20	6	0	1	3	1	2	90	1	Died back
745	19	4	22	1	4	1	4	1	2	70	1	Died back

the union height above soil ranged from 13 to 17 cm (average 15.3 cm). Of all the grafts that produced shoots, the range of grafting height was between 10 and 26 cm (average 17.8 cm).

- The number of leaves on the rootstock ranged from 0 to 11 (average 3). The successful grafts had 1, 5 or 6 leaves, with an average of 4 leaves remaining on the rootstock. Of all the grafts that produced shoots, the rootstock had from 1 to 6 leaves (average 3.3).
- The rootstock had between 0 and 22 axillary shoots coming from the stem (average 2). The three successful grafts had 2, 2 or 3 axillary shoots (average 2.3). Of all the grafts that produced shoots, the number of axillary shoots was between 0 and 4 (average 2).
- All the grafts that produced shoots had healthy rootstock on grafting.
- All three successful grafts had a scion diameter of 3 mm, from a range of 2 – 8 mm tested. Of the nine grafts that produced shoots, the range of scion diameter was 3 to 4 mm (average 3.3 mm).
- All three successful grafts and the nine that produced a shoot had a one-node scion, from a range of either one or two nodes.
- The three successful grafts had either 1 or 2 scion leaves, from a range of 0 to 4 leaves. Of the nine grafts that produced shoots, the number of scion leaves was 1 or 2 (average 1.7).
- Two of the successful grafts had 90% of their leaves cropped and the other only 30%, from a range of 0 to 90% cropping. Of the nine grafts that produced shoots, the average cropping was 61.1%.
- Every scion was healthy at grafting with no axillary branches on the scion.
- Two of the three scions that produced shoots and survived were described at grafting as dry and woody.

Trial 2

Only three grafts finally survived of the 522 grafts completed. After grafting, it soon became apparent that the success rate would again be very low, so no detailed observations were recorded during the course of this trial.

The three surviving grafts showed no pattern to explain their survival in preference to other grafts, and no statistical analysis of the data was possible.

Table 3

Grafting compatibility plan for Trial 1, showing number of grafts completed for each *Eucalyptus marginata* rootstock/ scion combination, with success overlay. For the nine grafts that produced shoots during the trial period (highlighted grey), 1A indicates the number of surviving grafts and 1D the number of grafts that died after producing shoots. 'Sib' indicates the half-sib relationship between five rootstocks and three differently numbered scion lines.

	Rootstock																		
	Code	722	742	744	724	729	19	91	162	405	624	626	671	672	692	694	700	734	745
	Sib	1	1	1	5	12													
Scion Code	1	5	5	5	5	0	5	0	5	0	5	0	3	0	5	0	5	0	5
	5	0	5	5	0	0	5	0	5	0	5	0	0	0	5	0	5	0	5
	12	5	0	5	0	5	0	5	0	5	0	5 1D	0	5	0	5	0	5	0
	19	0	5	5 1D	0	0	5	0	5	0	5	5	0	0	0	0	0	0	0
	91	0	5	5	0	5	0	5	0	5	0	0	0	0	0	0	0	0	0
	162	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	671	0	0	5	0	2	0	5	0	2	5	5 1A	5	5	5	5	5	0	5
	672	5	0	4	2	0	5	5 1A	0	0	0	0	0	5	0	0	0	0	0
	692	2	0	4	0	5	0	5	0	0	2	0	0	0	10	5	0	0	0
	694	0	5	5	0	0	0	5	0	5	0	5	5	5	0	5 1D	5 1D	5	0
	700	0	0	5	0	5	0	4 1A	3 1D	0	0	4	0	0	0	0	5 1D	0	0
	Total	17	25	48	7	22	20	34	18	17	22	24	13	20	25	20	25	10	15

DISCUSSION AND CONCLUSION

The aim of this project was to improve the grafting success of jarrah (*Eucalyptus marginata*) so that this propagation technique could be used for the capture and multiplication of dieback resistant selections for use in establishing open-pollinated seed production orchards.

Two extensive trials were conducted with jarrah, the second being carried out in a different facility (Wanneroo) to avoid disease problems that were evident in the first trial at Kensington. The grafting procedures, facilities and conditions employed here have been used successfully over many years by the Forest Products Commission for the propagation of several other eucalypt species.

Very few grafts initially produced shoots, and in each trial, only three of these grafts finally survived. Each of these successful grafts involved a different scion grafted onto an unrelated rootstock, so no consistent pattern of scion/rootstock combinations for grafting success was apparent.

We conclude that the cleft graft method, as applied here to one-year-old rootstock, is not a successful cloning method for jarrah.

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