Propagation of Jarrah Forest Plants for Mine Restoration: Alcoa's Marrinup Nursery©

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INTRODUCTION

Plant propagation has a useful role to play in disturbed land restoration. Alcoa of Australia (Alcoa) operates a nursery and tissue culture laboratory to produce plants for restoration following mining. This paper provides an overview of a 16-year program to develop ex situ propagation and large-scale production methods for plants absent from mine restoration. In Western Australia Alcoa operates two bauxite mines and Marrinup Nursery in the Darling Range south of Perth, and has three alumina refineries on the coastal plain. The principal vegetation of the Darling Range is Jarrah Forest. This forest has at least 784 plant species (Bell and Heddle, 1989) and is part of one of the world's top 25 biodiversity hotspots (Myers et al., 2000). Alcoa aims to establish a self-sustaining jarrah forest ecosystem on its bauxite mine-sites (see Koch 2007a and 2007b for details on the general mining and restoration processes).

With a large area to restore each year (over 550 ha) and such a large number of plant species in the pre-mining forest, any propagation and restoration work is complex. Southwest Australia has a dry Mediterranean-type climate (Beard, 1990), and this further challenges plant propagation for mine restoration. The nursery's entire annual production has to be held onsite throughout the year, then planted in the first 2 months of the short winter wet season. Many of the Jarrah Forest plant species had not been propagated at a commercial scale prior to Marrinup Nursery's production, so considerable propagation research and development has been undertaken since 1990. Propagation supplied 1% of the restored mine pit biodiversity in 1992, and we had less species in our restored forest than occurred in the unmined forest. The opening of a tissue culture laboratory in 1992 allowed research and development to focus on a wider range of species. A concurrent effort occurred in cuttings and seedling propagation.

Mined lands restored in 2000 had a similar number of species as unmined forest (Gardner, 2001) yet still lacked several plants; Alcoa calls these "recalcitrant" species. While most of the plant species in the restored forest were successfully reestablished from the direct return topsoil seedbank, or from the Nursery-supplied broadcast seedmix, over 20% of species were still establishing poorly or were absent. A focus of Marrinup Nursery's research has been on studying the interactions between each species wild biology, propagation, and revegetation survival. Alcoa's mining department is both the propagator and the primary customer for the plants, so the nursery's products need to have proven field performance before large-scale operational production can be justified. The propagation methods chosen have to deliver the highest field survival but at the lowest cost per plant leaving the nursery.

Each year since 2000 Marrinup Nursery has supplied up to 20% of the species found in restored Jarrah Forest by propagating and planting "recalcitrant" species.

Figure 1. Small legumes seed orchard (top), *Chorizema dicksonii* orchard plant (bottom).

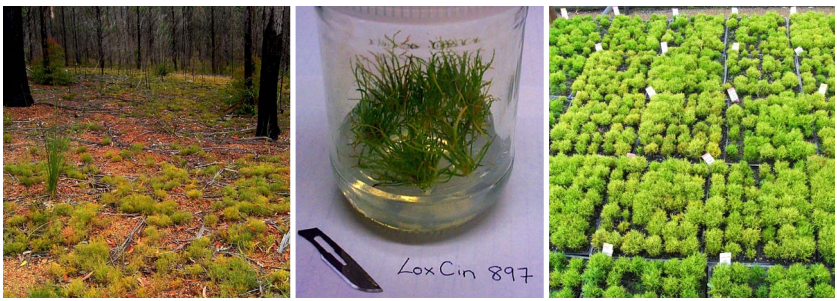

Figure 2. Dryland rush, *Loxocarya cinerea*: post-fire re-sprouting (left); tissue culture (centre); nursery crop acclimatization (right).

Four examples are presented demonstrating the different propagation research and plant production approaches we have used to enhance Alcoa's bauxite mine restoration, as an overview of how propagation can be applied to add value to disturbed land restoration.

CASE STUDY 1: RE-SPROUTING SHRUBS

Re-sprouting shrubs are an important element in the forest's ability to cope with frequent forest fires — they rapidly resprout and spread vegetatively after fires. Two shrub species of interest to Alcoa have low seed production so seldom recruit from the topsoil seedbank. This makes them ideal for cuttings propagation, but we are currently reliant on forest fires to provide source material. *Leschenaultia biloba* and *Dampiera linearis* cuttings have a high strike rate and vigorous root production at Marrinup Nursery. We do benefit though from our low summer humidity for propagation success; during rare humid conditions we can get fungal rots.

CASE STUDY 2: SMALL LEGUMES

We must establish nitrogen-fixing plants in the restored mined areas to assist with sustainable nutrient cycling in the restored ecosystem. Many of the small legumes prevalent in unmined forest have difficult-to-collect seed. The plants are often far apart in the wild, plus the seed ripens and is released in 1 or 2 days, often explosively. We have set up seed-orchards-in-pots to make seed collecting at the correct time easier. As a bonus we also get higher seed production. For several small legume species all seed in the broadcast seed mix comes from these orchards.

CASE STUDY 3: FOREST DISEASE

A team from the Department of Environment and Conservation, Murdoch University, Edith Cowan University, and Alcoa identified Jarrah (*Eucalyptus marginata*) individuals with enhanced resistance to *Phytophthora cinnamomi*. These disease-resistant individuals were tissue cultured but the plants proved to be too expensive for broad-scale planting. Enough plants were produced at Marrinup Nursery for establishing large seed orchards. Seed has been collected for the last 2 years and low-cost seedlings have been produced. These seedlings were given to community conservation groups for field testing. We will collect and share the results from these tests as the plants mature.

CASE STUDY 4: GEOPHYTES

What was the problem? Plants with tubers, rhizomes, corms, or bulbs (geophytes) are often absent from disturbed land restoration. The prolonged annual dry season during summer in the southwest of Australia has favoured the evolution of a large number of geophytes in the Jarrah Forest flora**.** Geophytes are significant for ecosystem resilience (Pate and Dixon, 1982). They rapidly re-sprout from their underground storage organs following fire or drought, which particularly benefits grazing marsupials and minimizes soil erosion. Many of the plant species absent from Alcoa's bauxite mine restoration in 1992 were geophytes.

Large-scale propagation and restoration methods have been developed for 24 species of Jarrah Forest geophytes including *Clematis pubescens*, *Pteridium aquilinum* (syn. *Esculentum*) (Austral bracken fern), two species of tuberous *Drosera*

and eight species of dryland rushes and sedges. This latter group will be discussed further as it provided a good demonstration of the key findings.

Dryland Rushes and Sedges. Why did Marrinup Nursery need a large tissue culture laboratory? The first reason was for producing the dryland rush and sedge species of the Jarrah Forest. Most of these species were highly recalcitrant, having very low production of viable seed, so we couldn't establish them from the returned topsoil seed-bank nor from broadcast seed. This left ex situ vegetative propagation and planting as the only viable restoration methods. Cuttings propagation was not possible with these rhizatomous monocots. Division of mature plants did work but growth rates were very slow, producing enough plants each year was unreliable, dividing the plants was very tiring, and the plants were too expensive. Tissue culture was the last realistic option.

Identifying the optimal time for wild seed collection was critical to improving tissue culture initiation success. Embryos were extracted from the few seed collected and placed on filter-paper bridges with half-strength Murashige and Skoog (MS) liquid culture medium using a modification of Meney's method (Meney and Dixon, 1995). When these embryos germinated they were used to establish shoot cultures.

The rushes and sedges were healthy and reliable in tissue culture, but the growth rates were below the commercial minimum of ×3 multiplication each month. These are naturally slow growing species in the forest. This made them expensive to produce compared to other tissue cultured species.

I thought we could produce all plants by in vivo division and remove the species from tissue culture. I'd previously done this at Marrinup Nursery with wetland rushes. The result wasn't as good though, due to the slower growth rates of the dryland rushes and sedges. I developed a comprise method for production where we produce waves of tissue cultured plants, divide them in vivo once or twice, and then plant them all during the wet season. We then start again, with new waves of tissue cultures.

The tissue cultures rooted well on average, but were variable batch to batch. This made production scheduling more difficult. Accommodating and reducing this variation has been an important development area.

It has been critical to acclimatize plants to the field planting conditions. If you produce your plants well away from the restoration sites then they need to be transferred to a nearby irrigated holding site several weeks prior to planting, to adjust to the planting site environment. Unlike the frost-free coastal plain, due to its 260-m altitude Marrinup Nursery experiences hard frosts, the same as the mine restoration planting sites, so our plants are toughened for field conditions.

MAIN FINDINGS

1) Plant propagation has a useful role to play in mine restoration.
2) Production propagation methods were developed for all the Jarrah Forest plant species investigated, but different propagation methods were needed for each species, even within genera. We have to use a very wide range of propagation methods as a response to the complex biology and life histories of the Jarrah Forest plant species.
3) Wild plant biology studies are crucial before commencing propagation. Collecting seed or spore at the optimum time greatly assisted the germination studies.

4) Re-sprouter spreading shrubs typically propagate well by cuttings. Wild shoots collected within 6 months after forest fires produced the highest percent rooting.
5) To get small legumes seed (for broadcast restoration seed-mixes) requires on-site orchards and close attention to collection time.
6) Dieback-resistant Jarrah tissue cultured plants were expensive. Off-site seed orchards provide seed for low-cost seedlings.
7) To improve field survival it is important to acclimatize nursery-produced plants for several weeks prior to planting in the same environment as the planting sites.
8) Tissue culture can be a valuable first stepping stone when developing production propagation methods for species new to commercial horticulture.
9) Tissue culture production can increase the growth rate of species that are naturally slow growing in the forest.
10) To lower the cost of spreading species tissue cultures, we produce waves of tissue cultures throughout the year, then divide each batch several times in vivo prior to field planting. If left in vivo too long plant growth gradually reverts to the slower wild growth rate.
11) Long-term plant spread and seed production in the field are good restoration sustainability measures. For post-fire re-sprouter species high spread rates of surviving plants in the field can "make-up" for any early losses.
12) In restoration projects the "cost-per-surviving-plant" 2 years after planting is more relevant than the nursery plant price.
13) Larger plants had higher restoration survival and spread; they cope better with the first dry season.
14) The adoption of research and development methods into operational plant production is a key research success measure.
15) By working closely with the mine restoration research team over 10 species have been removed from propagation either by developing seed germination treatments allowing use of broadcast seed, or by increased natural recruitment once we started using direct return fresh topsoil on all mine restoration.
16) Integrated study of each plant species adaptive biology, propagation, and revegetation offers considerable potential for increasing the number of species that can be successfully propagated and established in disturbed land restoration.

SUMMARY

- Propagation now supplies 20% of the species richness of the restored mine area. We routinely have more species in our restored forest than occur in any one patch of unmined forest.
- Seventy-nine recalcitrant (difficult) Jarrah Forest plant species (26 families) have been propagated at Marrinup Nursery since 1992.

- We have propagated 1.94 million recalcitrant plants to date and planted them in 6,537 ha of Jarrah Forest Restoration.
- Thirteen external Marrinup Nursery-based research papers have been published to date and seven internal publications as well as two TAFE diplomas and seven university degrees. Marrinup Nursery's internal publications are available for download on Alcoa's Mine Environmental Department webpage: <http://www.alcoa.com/australia/en/info_page/mining_research.asp>.
- Alcoa runs regular weekly mine restoration and mining tours and Marrinup Nursery tours by arrangement.

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