The germination, cultivation and initial translocation success of four rare Western Australian plant taxa.

Anne Cochrane, Leonie Monks* and Sophie Juszkiewicz#*

*Department of Conservation and Land Management,
CALMSCIENCE Division, Locked Bag 104 Bentley Delivery Centre WA 6983
Botanic Gardens and Parks Authority, West Perth WA 6005

Abstract

The survivorship of plants from four rare Western Australian taxa (*Lambertia orbifolia, L. echinata* subsp. *echinata, Daviesia bursarioides* and *Grevillea calliantha*) was monitored over 33 months from initial germination of seed under controlled laboratory conditions, through nursery cultivation to planting in the field. These plants were used in a series of translocation programs designed to increase the survival of critically endangered taxa in the wild. This paper presents some preliminary data collected by the Western Australian Department of Conservation and Land Management in collaboration with Perth's Botanic Gardens and Parks Authority.

Introduction

The South West Botanical Province of Western Australia has one of the most genetically diverse floras in the world, with over 8,000 taxa. Loss of habitat, weed invasion, salinity and dieback disease are major threatening processes to native plant survival in this region (Coates and Atkins 1997). More than 300 taxa are considered under threat from one or several of these processes, with over 100 of these taxa listed as critically endangered. Many require recovery actions to prevent possible extinction including the long term storage of genetic material and the translocation of that material into new or existing populations.

Lambertia orbifolia, L. echinata subsp. *echinata,* (Proteaceae), *Daviesia bursarioides* (Fabaceae) and *Grevillea calliantha* (Proteaceae) are taxa endemic to Western Australia. All occur in the South West Botanical Province of the State, and are gazetted rare flora (Government Gazette 1999). *Lambertia orbifolia* is known from two groups of populations, one located just north of Albany and the other located in the Scott River Plain area, east of Augusta. *L. echinata* subsp. *echinata* is known from less than 20 plants located east of Esperance. *Grevillea calliantha* is restricted to six populations in an 8km^2 radius on the sandplains north of Perth. *Daviesia bursarioides* is restricted to an area of 7 km^2 west of Three Springs where there are only five populations.

In all cases, these taxa are experiencing threatening processes, such as dieback disease, weed invasion, rising salinity, inappropriate fire regimes and clearing for agriculture. These threats reduce chances of plant survival in the wild.

In recent years, the Western Australian Department of Conservation and Land Management (CALM) has developed a comprehensive *ex situ* conservation program

to address this loss of genetic diversity and potential extinction of populations in the wild. A long term seed storage facility, the Threatened Flora Seed Centre (TFSC), was established in 1992 for the *ex situ* storage of seed material from rare and threatened taxa. Sampling is designed to capture a diverse sample of the genetic variation within each taxon, prior to testing and long term storage under low moisture and low temperature conditions (Cochrane and Coates 1994). Seed viability testing is an integral part of the monitoring program established for all seed that is held in the TFSC, and ensures that seed remains viable over the long term. The storage of sufficient genetic resources for each taxon will enable its successful reintroduction and establishment in the wild following a significant reduction in plant numbers or extinction from the natural populations assuming all other mitigating factors can be controlled.

In 1997 CALM commenced the first of a series of multi-species experimental translocation trials. A translocation is the deliberate transfer of plant propagation material from one site to another (Guidelines for the Translocation of Threatened Plants in Australia 1997). A translocation is a conservation measure that attempts to safeguard the species by halting the decline in the number of individuals and conserve the genetic diversity of the species. The aim of a translocation project is to establish self-sustaining populations with a broad genetic base to ensure the potential for adaptation. The process of translocation requires careful planning, the establishment of specific guidelines and long term commitment to monitoring.

CALM's experimental translocation project has involved the cooperation of Perth's Botanic Gardens and Parks Authority (BGPA) and community groups (recovery teams). A total of ten critically endangered taxa are being targeted for translocation over the lifespan of the project. Seven were selected for translocation in 1998. Presented here is preliminary data from four of these taxa. This is the first time that a large scale systematic approach to plant translocation has been undertaken in Western Australia. The ultimate aim of this project is to develop and implement protocols for the establishment of viable populations of a range of taxa. The seed resources of CALM's genebank, the TFSC, are being utilised to provide a broad genetic base for the re-establishment of these new populations. The resulting seedlings are raised by BGPA, under strict hygiene conditions, until they are of sufficient size to plant out at the translocation sites. Monitoring of vegetative and reproductive condition of the transplants is undertaken every 2-3 months for a period of one year and with monitoring less frequent in the following two years.

This paper details some of the problems encountered in the three phases of plant cultivation.

Materials and Methods

Seed Germination

Seed from all four taxa was collected from wild stock between 1993 and 1997. Controlled laboratory germination trials were conducted in 90mm glass Petrie dishes on a 0.75% agar solution in temperature and light controlled incubation cabinets, using a 12 hour photoperiod. Cabinets were set at a constant 15^0C. The addition of a 2% solution of Previcure fungicide to the agar solution aimed to inhibit fungal growth. In most cases, seed was surface sterilised with a 10% solution of 40g/L of sodium

hypochlorite for 5 minutes prior to incubation. Seed of *Daviesia bursarioides* was pre-treated with boiling water prior to incubation to crack the hard seed coat to allow moisture imbibition. Seed of *Grevillea calliantha* was nicked and soaked in an aqueous smoke solution for 24 hours prior to incubation on an agar mix with the addition of the naturally occurring plant growth hormone Gibberellic Acid (as GA_3). The two *Lambertia* taxa required no pre-treatment to elicit germination. Petrie dishes were checked twice weekly and germination was determined by radicle emergence. After germination, all seedlings were transferred to the accredited BGPA nursery.

Nursery Cultivation

Seedling establishment in the nursery incorporated two stages. The first stage involved hardening of the germinants, pricking them out and then establishment of the seedlings. This was the most critical stage and usually took about 3 to 4 months depending on the individual development of seedlings. During this time standard horticultural practices were applied with an integrated disease approach that involved excellent hygiene, good ventilation and fungicide treatment. Gradual acclimatisation of the seedlings took place in the propagation glasshouse with a 75% shade screen and high humidity maintained by intermittent light misting or fogging. The hardening-off time of plants took between 3 to 10 days depending on the vigour of the plants. Seedlings were pricked out at the cotyledon stage into 50mm pots containing a propagation mix (peat/perlite/sand) with no fertilisers incorporated. The young seedlings required constant attention at this stage and were not allowed to dry out. Once established they were transferred into 75mm pots and moved into the growing part of the glasshouse with a harsher environment (more light and reduced humidity). At around 8 to 9 months, seedlings are potted into a free draining, pasteurised, standard, composted jarrah potting mix containing low phosphorous slow release fertilisers with a pH level of 5.8. Under these conditions the resulting well-established seedlings exhibited good root development and subsequently required less maintenance.

The second stage of plant development in the open environment allowed seedlings to adjust for future planting out on the translocation sites and took from 2 to 3 months. General plant health was monitored on a regular basis during this stage, especially for possible fungal contamination.

Translocation

After five to seven months in the nursery, plants were transferred to the field either at the existing site of the wild population (population enhancement or restocking - *L. echinata* subsp. *echinata* and *D. bursarioides*) or at new secure sites (introduction - *G. calliantha* and *L. orbifolia*). All seedlings were planted into grids to allow for replication and then tagged with an individual number and surrounded by a circular wire cage to prevent herbivore predation. Seedlings were planted under a range of treatments that were designed to test the techniques needed to successfully establish them. Establishment techniques used a range of standard horticultural practices including irrigation, mulching and covering with shade cloth.

Results

The success of seed germination, seedling survival during nursery cultivation and survivorship in the field post translocation is presented in Table 1.

Seed Germination

Seed germination ranged from 58% in *Daviesia bursarioides* to 99% in *Lambertia orbifolia*. None of these taxa are considered difficult to germinate compared to many other native West Australian plants that exhibit complex dormancy mechanisms (pers. comm. A. Cochrane). Germination time for the two *Lambertia* taxa ranged from 20 to 60 days; 10 to 40 days for *G. calliantha* and 30 to 70 days for *D. bursarioides*.

Nursery Cultivation

Little difficulty was experienced with cultivation of *Grevillea calliantha* and *Lambertia orbifolia.* Germinants received in the summer months grew rapidly reaching full potential for field planting within 4 months. Most plants of these two species reached between 20cm and 30cm in height during that time and both species developed excellent root system with proteoid 'clusters' present. Both species were relatively pest free. However, plants of *L. orbifolia* were affected by an aerial-borne canker in the winter months. These fungal problems were easily overcome using cultural and environmental procedures such as pruning and removing dead branches and fungicide control. Treated and pruned plants responded well and remained healthy.

Daviesia bursarioides was considered easy to establish and the survival rate was high. The species was disease free and there were no cultural problems. Due to initial slow growth and vigour of the plants, at least 6 months was considered the optimum time for nursery cultivation prior to planting in the field.

L. echinata subsp. *echinata* proved to be unreliable in cultivation. Poor plant health was possibly attributed to nutritional imbalance in the potting media and leaf burning was visible in many plants. With changes in general nursery cultural management the problem were overcome and general plant health improved. Due to the early difficulties, at least 6 months was required before plants were ready for the field.

Translocation

Percentage survival of translocated seedlings from time of planting (seedling survival at nine months) is shown in Table 1. Survival data has been grouped across treatments and replicates for the purposes of this paper. Very few plant deaths were recorded prior to the hot dry conditions of the first summer.

Discussion

These data highlight some of the problems that may be encountered in a translocation program that utilises wild seed sources and standard nursery practices for the growth and transferral of genetic material to new and existing sites. The survival of plants described in this paper show considerable variation and ranges from 13% to 71% of germinated seed, 33 months after initial seed germination. Losses have occurred throughout all growing phases.

Losses to *D. bursarioides* and *L. echinata* subsp. *echinata* occurred predominantly during the translocation phase. Heavy losses of the former were sustained due to late planting, and root establishment prior to the hot summer months may not have been optimal for survival. Problems with bird damage (possibly cockatoos) to the irrigation

system meant that watering was not sustained for the duration of the summer period. Dieback disease appeared to have a considerable effect on survivorship of *L. echinata* subsp. *echinata* plants in the field. Flooding in the area during the warm weather of summer 1998 created ideal conditions for the spread of dieback throughout the site, despite the application of the fungicide Phosphite to the whole site and the individual seedlings.

Survivorship of both *G. calliantha* and *L. orbifolia* from seed germination through nursery cultivation and translocation of seedlings has been reasonably high (53% and 71% respectively). Both these taxa have been successfully grown in cultivation for a number of years and potential problems with propagation overcome during that time.

These data have plotted the fate of seed and seedling survival over 33 months. The importance of maximising seed germination and seedling survival throughout the nursery and planting phases is vital to the maintenance of the integrity of the genepool of each taxon, and ultimately, to the success of the reintroduction. The goal of a reintroduction is to establish self-sustaining populations that retain the genetic resources necessary to undergo adaptive evolutionary changes (Guerrant and Pavlik 1996). Losing those resources throughout the three phases of growth can diminish the survival chances of the new populations. Closely examining the fitness, growth rates and survivorship of offspring for indications of inbreeding depression in future generations may be necessary where substantial loss of material has occurred. The ultimate success of the reintroductions will only be evident after many years of monitoring. Augmentation of those populations hardest hit by death will be necessary in coming years.

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