Conservation and Restoration Research Proposal
Darwinia masonii and *Lepidosperma gibsonii*

An integrated research program into *ex situ* and in situ conservation, restoration and translocation *of Darwinia masonii* and *Lepidosperma gibsonii* 2007-2010

Version 5, 26 August 2008

DURATION: 2007 – 2010. The research program may be extended subject to achieving requirements as detailed in Ministerial Statement 753.

Botanic Gardens and Parks Authority (Kings Park and Botanic Garden) ("BGPA")

In Association with Project Proponents and Sponsors:

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1 INTRODUCTION

1.1 Project Background

Mount Gibson and the adjoining ridges lie 350 km north east of Perth in Western Australia. The range is largely composed of banded ironstone, with significant deposits of both hematite and magnetite. The range has been investigated for many years with the view to extracting iron ore.

The project was assessed as a Public Environmental Review (PER) under Part IV of the Western Australian *Environmental Protection Act* 1986. In addition the proposal is considered to be a controlled action under the Commonwealth *Environmental Protection and Biodiversity Conservation Act* 1999

The PER was released for public review from 18 April to 30 May 2006. The Environmental Protection Authority (EPA) released its Report and Recommendations on the Mt Gibson Iron Ore Mine and Infrastructure Project (Bulletin 1242) on 27 November 2006.

The EPA recommended that the project be given approval subject to a number of conditions, many of which had previously been foreshadowed by the proponents following the results of initial investigations.

The Mt Gibson Iron Ore Mine and infrastructure Project was approved by the Western Australian Minister for the Environment on 24 October 2007 (Ministerial Statement 753). The project received approval to undertake a controlled action under the *Environmental Protection and Biodiversity Conservation Act 1999* on the 18 December 2007.

During the assessment process, a species of Declared Rare Flora (*Darwinia masonii*) was known to be endemic to the Mt Gibson Range, and the then project proponent, Mount Gibson Mining Limited contracted ATA Environmental to survey the plants, and BGPA to investigate critical biological factors relating to the rarity and reproductive potential of the species.

In early 2006, a second species endemic to the range was discovered, which was referred to in the EPA Bulletin 1242 as *Lepidosperma* sp. Mt Gibson, but has recently (December 2007) been described as *Lepidosperma gibsonii* R.L. Barrett, and will be referred to as *L. gibsonii* in the current document (Barrett, 2007). ATA Environmental (now Coffey Environments) and BGPA were again contracted to conduct similar preliminary research for *Lepidosperma gibsonii* as previously done for *Darwinia masonii*.

In August 2006, Mount Gibson Mining Limited sold Asia Iron Holdings Limited and Extension Hill Pty Ltd including the mining tenements and overall project to Sinom Investments but retained the rights to mine hematite ores verses the magnetite ores that were to be mined by the new independent company.

Since this time and following State Ministerial approval (24 October 2007) and Commonwealth Approval (18 December 2007), Mount Gibson Mining Limited (MGM) and Extension Hill Pty Ltd (EHPL) have become joint proponents ("the proponents") in the Mount Gibson Iron Ore Mine and Infrastructure Project defined by Ministerial

Statement 753 (WA *Environmental Protection Act 1986*) and the Commonwealth approval under the *Environment Protection and Biodiversity Conservation Act 1999*.

The proponents now propose to develop the iron ore mine at the Extension Hill deposit in the northern part of the Mt Gibson Ranges consisting of both hematite and magnetite mining infrastructure (MGM and EHPL respectively). The magnetite which will be produced in a purpose built concentrate plant on site will be transported to Geraldton as a slurry in a buried pipeline within a services corridor while the hematite will be hauled by road to a rail terminal at Perenjori whereby it will then be railed to the port at Geraldton.

1.2 Scope

This research proposal has been developed in response to the two project proponents' commitment to fund a 3+ year research program on *Darwinia masonii* and on *Lepidosperma gibsonii*, leading to the preparation and implementation of Recovery Plans for both species.

This research proposal specifically addresses the objectives of Conditions 6.1 and 7.1 of Ministerial Statement 753 to facilitate the continued *in-situ* survival and improvement in the conservation status of *Darwinia masonii* and *Lepidosperma gibsonii* over time through targeted research which assists the development of a recovery plan for each species.

The Research Plan is also relevant to and addresses in part Conditions 6.2.2, 6.2.3, 7.2.3, 7.2.3, 8.1, 8.1.7, and 8.1.8.

The research proposal builds on the initial research plan prepared for *Darwinia masonii* (BGPA 2004-2006), workshops held with the WA Department of Environment and Conservation (DEC) and Environmental Protection Authority (EPA) Service Unit on 3 February 2005 and 15 May 2006, Versions 1, 2, 3 and 4 of the *Conservation and Restoration Research Proposal Darwinia masonii and Lepidosperma gibsonii 2007-2010* (BGPA, February 2007, April 2008, June 2008 and August 2008 respectively), and DEC comments on Versions 1, 2, 3 and 4 of the research proposal.

This research proposal has been structured to:

- (i) describe the rationale underpinning the research (Section 2);
- (ii) provide background on *Darwinia masonii* and *Lepidosperma gibsonii* (Section 3);
- (iii) outline the research work plan (Section 4)
- (iv) provide a timeline for the research (section 5); and
- (v) cross reference the requirements of the Ministerial statement to the research proposal (Section 6).

1.3 Related Documents

Interim Recovery Plans (IRP's) have been prepared for *Darwinia masonii* and *Lepidosperma gibsonii* which detail the recovery actions and the monitoring to be undertaken for each species as required by Conditions 6.1.1, 6.2, 7.1.1 and 7.2 of Ministerial Statement 753.

An Environmental Management Plan has been prepared for the Mt Gibson Iron Ore Mine and Infrastructure Project that details management measures to minimize the direct and indirect impacts of mining on significant flora including *Darwinia masonii* and *Lepidosperma gibsonii* as required by Condition 8 of Ministerial Statement 753.

Other Research

Whilst the Research Plans apply specifically to the two individual Declared Rare Flora (DRF) species, *Darwinia masonii* and *Lepidosperma gibsonii,* other funded research initiatives and monitoring activities being undertaken as part of the broader site environmental management program will also provide complementary information on associated species and floristic communities that will assist in the restoration management of these two species and other priority flora within a more complex floristic and landscape setting.

2 RATIONALE UNDERPINNING RESEARCH

The proposal sets out a detailed research plan to provide strategies for *in situ* (and secondarily *ex-situ*) conservation of *Darwinia masonii* and *Lepidosperma gibsonii*.

Conservation of rare species can be divided into the following categories:

- *In-situ* conservation
- Augmentation of existing populations, including:
  - Rehabilitation of disturbed areas;
  - Increasing population size; and
  - Increasing population density.
- Translocations to create new populations in undisturbed areas
- Restoration on re-created surfaces
- *Ex-situ* conservation, including:
  - Propagation of living material;
  - Seed storage; and
  - Cryostored germplasm.

In-situ conservation efforts form the bulk of the research in this proposal, and is aimed at understanding natural variability, understanding responses to natural and anthropogenically-induced changes, and developing and implementing strategies to successfully alleviate detrimental effects to ultimately ensure long-term sustainability of the rare species by:

(1) Surveying population requirements, processes and dynamics to give baseline information against which population health can be monitored, and the necessary requirements should rehabilitation, translocation, or restoration be deemed desirable;
(2) Researching propagation methodology to generate plant material should rehabilitation, translocation, or restoration be deemed desirable; and
(3) Researching the effectiveness of restoration, translocation and rehabilitation of the two target species.

The baseline population research (1 above) is the most intensive area of research due to the complexity and range of critical factors involved in: (A) ensuring long-term survival of existing populations; (B) assessing the necessary requirements for augmenting / re-establishing populations; and (C) the use of the two key species in rehabilitation works.

Critical research topics include:

(a) Collecting basic information on population demography and recruitment in that current population dynamics provide baseline information on ‘healthy’ populations, and allow prediction of future population changes.
(b) Collecting detailed information on habitat requirements, especially water relations, topological requirements, and vegetation associations. Such requirements allow an assessment as to whether the current population is fully occupying its niche, or whether it is a result of past stochastic processes (eg. fire history). Knowledge of habitat requirements are critical for any restorative works or rehabilitation.
(c) Investigation of the ecological factors limiting the distribution of the species, establishment and survival of the study species (including mycorrhizal and pollinator associations and predation). Any beneficial

associations (eg. mycorrhizas, pollinators) need to be considered as limiting factors, and included in restoration works or rehabilitation, while detrimental impacts may limit distribution or affect population dynamics.

(d) Assessing threatening processes (eg. predation, drought).

(e) Generating important information on breeding and reproductive biology including mating systems, dispersal and fecundity (necessary to understand population regenerative capacity and limitations following disruptions, eg. fire events).

(f) Detailed research on seed biology, a critical factor in population persistence, recruitment, recovery, and also important in generation of plant material for restoration (if deemed necessary).

(g) Comparative studies with other closely related species where necessary to understand key adaptations to the Mt Gibson environment.

Ex-situ conservation is a small component of the project, aimed at inclusion of the species in long term seed banks and cryostorage banks as appropriate for localised endemic species.

Translocation, restoration and revegetation are a key focus of the research. Such activities are however likely to have complex interactions with existing populations, and need to be carefully evaluated for their local impacts before proceeding. The main possibilities are:

(1) *Use of the two DRF species in rehabilitation works*, thus partially offsetting the plants removed due to mining activities. Any rehabilitation activities need to be carefully planned to avoid loss of genetic variation in neighbouring populations (in particular to avoid genetic swamping by introducing large numbers of one or a few genotypes). However, the possibility of increasing population numbers of DRF species without disturbing any existing vegetation (outside the approved disturbance footprint) merits extensive investigation and potential rehabilitation effort.

(2) *Translocation or augmentation of existing populations*. Native populations may decline due to numerous processes, including fire, drought, population size below a critical limit, predation, or due to mining or other land use activities (e.g. pastoral grazing). Translocation activities have the potential to improve the overall health of the species, however could also have negative effects such as lowering the local genetic diversity, increasing density-dependent predation, damaging an existing seedbank, or decreasing regrowth of other species (competition effects). Any restoration activities will need to be carefully planned, taking into account the outcomes of the proposed research into biology, ecology and topology, to ensure that existing populations (including the seed bank) are not negatively impacted in the process. The same translocation techniques could however be used to rehabilitate disturbed surfaces such as old drill pads and tracks, with less potential impacts than planting into undisturbed areas.

(3) *Creating a new population of DRF* in undisturbed habitat outside of the species' current distribution. Both DRF taxa have never been collected outside the general Mt Gibson Range area (attribution of herbarium collections of *D. masonii* to Mt Singleton are likely to be incorrect), and are likely to have evolved *in-situ* to the local geology, and may never have had wide distributions once adaptations to their specific range of substrate types became fixed.

Other nearby hills potentially have their own restricted community and species types, as per those recently described for Mt Gibson, and at least in the case of *Lepidosperma gibsonii* which appears that it may have closely related species already occurring in similar niches nearby. For these reasons, new populations in new localities of the two DRF taxa should be created only with caution and after much more information regarding adaptations and requirements have been determined. If feasible, the creation of a new population on the disturbed waste rock dump is the preferred option.

3 RESEARCH BACKGROUND

3.1 *Darwinia masonii*

Darwinia masonii is endemic to banded ironstones and possible granitic areas of the Mt Gibson Ranges. The species forms part of a complex of c. 58 species in the endemic Western Australian genus *Darwinia*. The genus is highly unusual in having a high proportion of species that are considered rare and endangered as a result of *intrinsic rarity* – i.e. a species naturally limited as a result of limiting natural ecological factors such as edaphic preference or breeding biology constraints. *Darwinia masonii* represents a highly specialised case of a nationally significant, intrinsically rare species.

Darwinia masonii is Declared Rare Flora (DRF) under Western Australian government legislation (WA Wildlife Conservation Act 1950) and is therefore afforded the highest level of statutory protection on the basis that:

- The species is under threat due to its poor representation in the conservation estate;
- The species is represented by critically low numbers, threatening factors continue to impact upon populations and/or plants exhibit biological attributes that are likely to significantly limit the species (i.e. inherently low seed production; predators); and
- The species has been systematically searched for in probable habitats for at least five years by technically competent persons.

As a result of these criteria *Darwinia masonii* is deemed to represent a species of high scientific significance and conservation value.

As Declared Rare Flora, any loss of plants or ecological intrusion on *Darwinia masonii* will have an impact upon the overall conservation status of the species. Unlike more common taxa, DRF require highly specialised consideration in any proposal that will impact adversely on the conservation security of the species.

The rare status for *Darwinia masonii* also reflects the paucity of knowledge regarding the biology, reproductive success, propagation, off-site conservation (seed banking, cryogenics), genetic diversity (genetic importance values for individual plants, populations and locations) and recovery status of the species.

A recent comprehensive survey of *D. masonii* by (ATA Environmental, 2004: report 2004/227) located 16 032 adult plants in nine discrete populations along 6 km of the Mt Gibson range system. Despite extensive searching for other populations of the species in other areas of likely habitat (ATA Environmental, 2004), *D. masonii* appears to be restricted to the Mt Gibson Ranges, and impacts within this range system must be carefully evaluated for long-term survival of *D. masonii*.

This proposal sets out a research and development program aimed at deriving critical scientific data for the long-term conservation security of the species.

3.2 Summary of Previous Research on *Darwinia masonii*

The preliminary study of *Darwinia masonii* was undertaken to provide benchmark indicators of the research needed to develop an effective integrated conservation and recovery plan for *D. masonii* (BGPA 2004). The preliminary study (Stage One) works occurred between August 2004 and January 2005 and investigated primarily the extent of genetic partitioning within *D. masonii*, and to develop principles for compiling a synopsis of research required in other areas such as propagation and seed biology.

The preliminary study of the genetic diversity of the species used the powerful genetic fingerprinting technique Amplified Fragment Length Polymorphism (AFLP) to assess genetic diversity within *D. masonii* and degree of population subdivision. Four of the populations were intensively sampled (c. 20 plants per population) for genetic examination. Genetic diversity was generally low, with nearly all (94%) of variation occurring within rather than between populations, and no significant population structuring could be detected between populations (BGPA 2004)

Since completion of Stage One works, DNA sequencing research has shown that *D. masonii* belongs to a complex of four species, (the other three being *D. acerosa*, *D. purpurea* and *D.* sp. *Chiddarcooping*). All four species are found in the drier parts of the Wheatbelt north of Perth, north and east into the semi-arid “interzone” as far as Kirkalocka Station and Chiddarcooping Nature Reserve. The four species do not overlap, suggesting an allopatric speciation model. The four species occur on different substrates; *D. masonii* on Banded ironstone Formation (BIF) and possibly granite breakaways, *D. sp. Chiddarcooping* around granite inselbergs, *D. purpurea* on sandplain, and *D. acerosa* on sandy to loam soils (sometimes near Granite outcrops).

A preliminary translocation trial of *D. masonii* plants grown from cuttings at Mt Gibson currently has 89% survival (50% reaching reproductive maturity) 18 months after planting, providing plants are watered through summer. Growth rates for these plants were exceptional, and much faster than observed in existing stands, and faster than unwatered plants, suggesting that growth of *D. masonii* is substantially water limited, and that *D. masonii* may be water-harvesting in the fine cracks between the BIF substrate. Translocated plants that were not watered showed only 20% survival (in a very dry season), and a greatly reduced growth rate more consistent with wild plants.

3.3 *Lepidosperma gibsonii*

Species of the dry-land sedge genus *Lepidosperma* (Cyperaceae) are an ecologically important and common element of most vegetation communities of south-western Australia. The taxonomy and identification of *Lepidosperma* species in Western Australia has long been problematic, and the WA Herbarium has identified *Lepidosperma* as the largest taxonomically problematic genus remaining in the state flora. At the start of 2006 there were over 3000 specimens in the WA Herbarium of *Lepidosperma* that were unidentified to species level.

In January 2006, Russell Barrett (BGPA) carried out a preliminary sort of the unsorted specimens, after many years of field evaluation and examination of types in foreign herbaria. As a result of that preliminary study, it is clear that there are many more taxa of *Lepidosperma* than has previously been realised (from 24 described species and 11 phrase names to 57 taxa now recognised as clearly delimited, and more than 100 putative taxa await detailed study before they can be even informally distinguished). Furthermore many of these species are apparently highly restricted in distribution and may require a degree of protection, including several that appear to be unique to localised mineral deposits.

Previous concepts of the genus *Lepidosperma* composed of a moderate number of mostly widespread species therefore need to be radically updated to a genus of numerous species, many of which are highly adapted to specific ecological niches.

Lepidosperma gibsonii
An ecologically restricted species of *Lepidosperma,* newly recognised in January 2006 was until recently known informally as '*Lepidosperma* Mt Gibson', and referred to as such by the PER, EPA Bulletin 1242 and Ministerial Statement 753. Until early 2006 the species was not represented by any specimen in the WA Herbarium., The species was formally described as *L. gibsonii* by Russell Barrett, who is revising the genus *Lepidosperma*, in December 2007 (Barrett, 2007).

An intensive survey for the species recorded over 17,000 plants on the Mt Gibson Ranges (ATA Environmental, 2006 Report No 2006/090). Recent intensive surveys on low granitic and regolith breakaways east and west of the Mt Gibson Range have discovered further populations up to 4 km from the range, giving a total extent of distribution of 7 x 5 km, and a total number of 45,013 plants of *L. gibsonii* (Coffey Environments unpublished 2008). Recent surveys in June 2008 have identified additional plant populations within this same overall distribution area. Extensive searching of likely habitat within 10,000km^2 external to this local area did not locate additional populations (ATA Environmental, 2006, unpublished BGPA surveys in 2007). Based on current knowledge, *Lepidosperma gibsonii* is apparently restricted to the gullies and slopes of the Mt Gibson Ranges, and immediately surrounding breakaways 350 km north-north-east of Perth on the edge of the Yalgoo Botanical Region.

3.4 Summary of Previous *Lepidosperma* Research

Lepidosperma is a large genus with representative taxa on many mine sites involving land clearance of native vegetation in the south west of Western Australia. The genus is of significance in restoration due to:

- Widespread occurrence; high density populations; intense clonal behaviour for most species and high level of fire-resistance (re-sprouting) (Meney and Dixon, 1998);
- Species with wide edaphic and ecological tolerances (coastal to desert taxa – such as the keystone coastal restoration species *Lepidosperma gladiatum*;
- Sand-binding rooting patterns increase soil stability capabilities (Pate and Dixon, 1996);
- Resistance of all taxa to *Phytophthora* (dieback) and most other common root-rots (Meney *et al.* 1999); and
- Ability to produce specialised rooting structures for uptake of nutrients (dauciform roots) (Shane *et al.* 2005).

The necessity for evaluating mining impacts and post-mining recovery of *Lepidosperma* has led to a broad and complex array of research being undertaken. Long term research has been conducted by Iluka Resources during sand-mining operations at Eneabba and Capel (Meney and Dixon, 1995a) and Alcoa World Alumina across all their bauxite extraction mines in the Darling Range near Perth (Meney and Dixon, 1995a,b).

Studies of post-mining recovery of *Lepidosperma* have found the following:

- Broadcast seeding fails to recruit seedlings due to seed dormancy (complicated by seed quality and viability) issues.
- Seed dormancy in *Lepidosperma* falls within the Cyperaceae dormancy state and represents one of four Australian families with deep intractable seed dormancy (Roche *et al.* 1997a and b). Recent research by BGPA researchers suggests that timing of seed collection of at least some species of *Lepidosperma* is critical, and that a complex after-ripening process in conjunction with germination stimulants such as gibberellic acid or smoke may be necessary to promote germination (S. Turner unpublished data).
- Topsoil reinstatement of species depends upon precision in stripping, storage and replacement (Meney and Dixon, 1988).
- Topsoil reinstatement may only enable reinstatement of some species and potentially at low abundance.
- Vegetative propagation is only possible through rhizome division and has been demonstrated to be labour intensive and costly (including transfer to site).
- Micropropagation and tissue culture techniques have been successfully employed to mass propagate some target species (S. Turner unpublished data) and have been successfully used for broad acre restoration of dryland Cyperaceae at the Alcoa mining operations in the Darling Range south of Perth. Somatic embryogenesis research underway for increasing propagule frequency.
- Genetic diversity in some taxa is broader than for most invertebrate/vertebrate pollinated native plants (Krauss *pers. comm.*).

A preliminary study focussed on genetic variation of *Lepidosperma gibsonii* is currently underway at BGPA. Preliminary findings are only available for sequence data, which suggest that *Lepidosperma gibsonii* is closely related to a group of species around *L. costale*, which all share a narrow inflorescence and narrow, terete to angular or winged culms.

Following a meeting on 15 May 2006 with representatives from Mt Gibson Iron Ltd, Botanic Gardens and Parks Authority, Conservation and Land Management and Department of Environment, it was agreed that genetic work should be carried out immediately (during the approvals process) to address three critical issues relating to the species *Lepidosperma gibsonii*. The three critical research topics were defined as:

(1) Clarification of the relationships and status of *Lepidosperma gibsonii*;
(2) An assessment of clonality in *Lepidosperma gibsonii*; and
(3) Examination of genetic variation and population structure in *Lepidosperma gibsonii* within the Mt Gibson Ranges.

4 DETAILED RESEARCH WORK PLAN

The Research Plan addresses seven broad areas of research on *Darwinia masonii* and *Lepidosperma gibsonii*, which are listed below. This following section describes the aims, proposed methods and anticipated outcomes for research in each of the seven areas. An indicative timeline for the research is provided in Section 5.

1 - Conservation Genetics	Population structure, comparison with other species, phylogeny, clonality (for breeding biology - see 3).
2 - Population Demography	Fire, demography, tools for monitoring plant demographic changes, (but not seed demography or reproductive parameters, see 3 – Breeding Biology)
3 – Breeding/Recruitment Biology	Seed research, seed production, soil seed bank, phenology, genetic analysis of breeding systems and dispersal.
4 - Population Viability Analysis	Modelling of population persistence using parameters obtained from Population Demography and Breeding Biology
5 - Environmental Interactions and Plant Health	Biotic and abiotic associations, habitat requirement assessment, ecophysiology, impacts of associations on plant health, and tools to monitor plant health
6 - Restoration and Translocation	Reconstruction of habitat, propagation, translocations, and tools to monitor rehabilitation, dust, and water table lowering.
7 – *Ex-situ* Conservation	Propagation for long-term seed storage, and cryostorage.

4.1 Conservation Genetics

The characterisation of patterns of genetic variation and the processes leading to the observed pattern is critical for the future conservation and management of DRF species. Conservation genetics research involves applying powerful molecular tools to the assessment of genetic variation within and between populations and to the investigation of processes operating to create these patterns - such as: realized mating systems, dispersal of pollen and seed and inbreeding depression. Tools for population genetic analysis include AFLP DNA fingerprinting (Krauss 2000) and simple-sequence repeats (SSR, or microsatellites).

Aims:

- Identify hierarchical genetic structure between populations for both *D. masonii* and *L. gibsonii*;
- Estimate between-population gene flow in both species; and
- Identify the closest phylogenetic relatives of DRF taxa.

Methods

A preliminary assessment of genetic variation has been undertaken for both *D. masonii* (BGPA 2005) and *L. gibsonii* (BGPA 2006). Further work will focus on three areas to benchmark the genetic study and recommendation to benchmark data with other species of *Darwinia* and *Lepidosperma*.

4.1a Phylogenetic context

- DNA sequencing of other *Lepidosperma* and *Darwinia* species to determine species relationships and place *D. masonii* in a phylogenetic perspective. Partial phylogenies have already been constructed for each species, using nuclear ribosomal DNA regions ETS and ITS (both *Darwinia* and *Lepidosperma*), and chloroplast matK and atpB-rbcL spacer regions (*Darwinia*), or chloroplast trnL intron and trnL-trnF spacer regions (*Lepidosperma*).

These studies aim to place the genetic diversity and other characteristics of *D. masonii* and *L. gibsonii* within a firm phylogenetic framework in order to benchmark this study relative to other species. The phylogenetic study in particular will provide considerable insight into the evolutionary history of the DRF and their closest relatives in south-western Australia. This in turn helps evaluate the evolution of breeding systems and ecological adaptations (or lack thereof) to particular environments, and thus understand the key characteristics that lead to the restricted distribution of the study species (see also 4.5 – Environmental Interactions and Plant Health, reciprocal translocation study). Importantly, this study may provide indications as to why, in recent geological history, the species has become restricted to the Mt Gibson Ranges and not nearby matching habitats (e.g. Mt Singleton).

4.1b Landscape scale genetic structure

- Genetic survey of remaining populations (interstitial populations between the sampled population nodes of *D. masonii*, and recently discovered outlier *L. gibsonii* populations on breakaways immediately adjacent to the Mt Gibson Ranges). Between 20-30 samples from each discrete population (200+ samples from the each species) will be genotyped using microsatellite markers, and analysed using standard population-genetic methods (AMOVA, FST, STRUCTURE etc).
- Comparative survey of other species (using similar methods to above, but with fewer populations) to benchmark the observed variation in DRF species compared to closely related species.

Knowledge of genetic structure and diversity is critical to identify the consequences for genetic diversity on removal of any plants during mining activities, and to determine the number and location of genotypes to be incorporated in future *ex-situ* and *in-situ* conservation efforts to adequately represent the total genetic variation. The more complete genetic characterisation of these taxa is also required for analyses of mating system and pollen and seed dispersal (see 3 – Breeding Biology below for other genetic measurements).

4.1c Monitoring genetic threats

- Identifying if populations are experiencing, or are under threat of, genetic decline and whether genetic decline threatens the long-term viability of populations.

Changes in plant mating patterns can indicate the possibility of future genetic decline as, increased inbreeding (for instance) may lead to a future genetic decline. Research on predominant out-breeders suggests that a decline in genetic variation increases the risk of extinction of a population. More generally, research is required on how populations such as *Lepidosperma gibsonii* have survived for presumably a long time as small and isolated populations when theory predicts their extinction. Plants such as *Isotoma petraea* have undergone chromosomal rearrangements to mask deleterious alleles and maintain high levels of heterozygosity. Another strategy is to invest in longevity rather than reproduction, such as displayed by the Western Australian granite endemic *Eucalyptus caesia and E. phylacis*, where individuals are possibly thousands of years old and very rarely successfully reproduce. This research objective will involve the genetic characterisation of offspring related to the genetic characterisation of their parents. An increase in the average genetic similarity among offspring compared to their parents may indicate genetic decline. The assessment of inbreeding depression, through growth experiments of offspring and the comparison of vigour with genotype, will identify whether genetic decline is associated with an expression of inbreeding depression. These genetic variables will be linked to the characterisation of life history traits and demography (see below) and will provide vital information for composing the genetic makeup of translocated populations.

Outcomes:

- Estimates of population allele frequencies for analysis of mating systems and dispersal in section 3 – Breeding biology
- Identification of closely related species for section 5-Environmental Interactions and Plant Health (comparative studies with closely related species)
- Identification of genetic provenances for section 6-Restoration and Translocation (for maintenance of genetic diversity in translocation and restoration by ensuring a diverse mix of genotypes is utilized and avoiding genetic swamping by a few genotpypes)
- Ensuring that gene flow between populations is maintained
- Ensuring that pollination processes are maintained
- Evidence for historical population size fluctuations.
- Addresses EPA criteria, Conditions 6-1 and 7-1, sub conditions 1- and 2.
- Estimates of genetic diversity, population structure, gene flow, and species relationships are necessary for construction of the *Darwinia masonii* recovery Plan.

4.2 Population Demography

Population monitoring of *D. masonii* and *L. gibsonii* will occur on site for two purposes:

1. to identify impacts of the mining operations (ie dust, hydrological, biotic) on population sizes and function; and
2. to quantify natural demographic processes.

Demographic understanding provides a fundamental basis for investigation of the ecology of any species, as well as its requirements for restoration and conservation.

Some saving in monitoring efforts may be made by incorporating the two programs where possible, but only after each is fully designed to meet its particular requirements.

The aims, methods and outcomes of the demographic assessment monitoring are described here: monitoring for impacts is described in the Environmental Management System (EMS), specifically 10.15 – Environmental Monitoring. Note that the principles, parameters, and reporting in relation to dust monitoring on vegetation and monitoring in the vegetation management procedure, shall be agreed with the DEC through the EMS.

Aims:

- Assess patterns of mortality, growth, fecundity and recruitment in *D. masonii* and *L. gibsonii*
- Identify the pace and variability of population changes in undisturbed populations of *D. masonii* and *L. gibsonii*
- Establish a fire history for the Mt Gibson Ranges
- Identify the response of populations to fire (survival and regeneration rates)

Methods:

Demographic data will be collected for each species from tagged plants in permanent plots distributed across the range of both species. At least ten long term monitoring plots (5 × 5 m or 10 × 10 m depending on density) will be established to cover >2% of the total population of each species, and to incorporate a range of fire-histories (e.g. burnt in a recent wildfire, long unburnt, etc), topographic positions, vegetation associations and distance from the mine (including the populations closest to the pit edge).

The following will be completed for each plot: record of GPS location, site factors (slope, aspect, vegetation canopy height and openness, cover of rock, gravel, bare ground, litter, biological soil crusts) and vegetation structure and composition; Map (x, y) and tag all plants, measure *L. gibsonii* base diameter and longest leaf length, *D. masonii* canopy height, canopy and stem diameter and base diameter; Assess plant health (qualitative scale), herbivory (% eaten); Count number of old and new inflorescences and; Collect 20 newly produced infructescences from plants at each site in each year to assess seed production per infructescence, seed viability and seed predation and its variability in relation to demographic and environmental factors. The plots will be re-assessed annually, with size (growth), mortality, change in condition, flowering/fruiting and any new recruits (also tagged) recorded. Growth, flowering and fruiting phenologies will be recorded in separate studies (sections 4.3a, 4.5d – below).

A regional fire history will be developed from satellite imagery and air photos which will enable populations in different locations to be dated and a size-age curve fitted so that age of long-unburnt stands and large individuals can be estimated. Survival, growth and fecundity data can also be stratified by stand age as well as plant size.

Fire intensity, season and interval may all impact on survival of both species. An experimental fire in a small area of the mine footprint (not scheduled to be disturbed for >18 months) containing individuals of both species is planned. Flame heights, surface temperatures (at n= 60 points within the fire using thermocouples attached to protected dataloggers), scorch intensity and fire patchiness will be assessed at the time of the fire. The experimental fire will enable assessment of mortality rates as a result of fire in relation to adult size and health, of seedling recruitment density and vigour (relative to recruitment and vigour in the absence of fire), seedling distributions in relation to the distribution of pre-fire adults and environmental features (i.e. potential microsites/safe sites for recruitment including pre-fire ant colony nests, rock outcrops and crevices etc.), and of the growth and survival of seedlings through their first three years (following the approach above).

A series of small cage exclosures will be constructed shortly after fire to exclude herbivores from a proportion of seedlings, so that survival and growth of seedlings protected from, and exposed to, herbivory can be compared. A recent wildfire in the southern part of the range will allow the continuation of this sequence with seedling growth and survival data for plants 4-7 years old. Survival data will include investigation of post fire effects including grazing. The impact of fire (and other parameters including grazing) is further addressed in Section 4.4 Population Viability Analysis (PVA).

Smoke (aerosol and karrikinolide) application experiments will be undertaken to audit the size, germinability characteristics and the spatial extent of seed banks relative to parent plants. Smoke provides a more decisive tool for auditing soil seed banks often enabling a more precise measure at the niche scale of maximum germination capability of soil stored seed (Roche *et al.* 1998).

Outcomes:

- demographic life table data for input to PVA;
- population structure data for analysis in fire regime modelling in PVA;
- vital rates data for modelling of climate variation in PVA;
- contribution to population growth baseline data for impact monitoring;
- provision of baseline data for comparison with restoration and translocation efforts;
- improved understanding of species distribution i.e. fire regime data contributes to analysis of species distributions in (section 5 below) and interpretation of floristic communities;
- Compliance against Ministerial Statement 753 conditions 6-1 and 7-1 sub conditions 1 and 5.

4.3 Breeding Biology

Breeding biology is used here as a broad term incorporating phenology (timing) and rates (quantities) of pollination, seed development, dispersal and germination, from one generation to the next. The research outlined in this section is aimed at determining the necessary requirements for successful recruitment of the next generation, and the limiting processes during reproduction. Such parameters are necessary to estimate potential population growth rates and extinction probabilities, and determine the most vulnerable stages of the DRF species.

Aims:

- Determine timing and rates of flower and seed production
- Determine seed survival rates *in-situ*
- Determine seed germination requirements
- Develop methodologies to alleviate seed dormancy
- Estimate inbreeding/outcrossing rates among individuals
- Estimate dispersal distances for pollen and seed

Methods:

4.3a Phenology

Recent research at BGPA suggests that seed maturation is a critical factor for obtaining viable seed in *Lepidosperma*. Many species are known to hold non-viable seed in the inflorescence long after the viable seed has fallen; dropped seed may have viability rates of 80-90% while the persistent seed may have less than 10% viability. Timing of seed collection is likely to be critical for most *Lepidosperma* species. The sequence of reproductive events leading to seed maturation, as well as timing of seed dispersal provide key information for planning and modelling experiments.

In addition to the assessment of phenological state associated with the quarterly measurement of physiological behaviour (section 4.5d – below), more frequent monitoring will occur through the part of the year including flower initiation, anthesis and seed ripening to capture the timing of these events more precisely, and to relate them more clearly to recorded climate events.

4.3b Production of viable seed [cf. Demography]

The reproductive potential of a species is determined by many factors such as rates of flower production, percentage of successful pollinations, selfing rate, seed maturation rate, seed viability, seed predation, as well as factors relating to long-term storage and germination (see soil seed banks below). Hence the following investigations will be undertaken:

- Annual variation in flowering and seed production and predation rates assessed in the demographic study; and
- Assessment of seed viability by application of a range of testing procedures including cut-tests; embryo excision; X-ray imagery and seed weight calibration.

4.3c Seed germination requirements (and methods)

Research undertaken by Kings Park scientists has, over the past 15 years indicated that south west dryland Cyperaceae possess some of the deepest and most intractable dormancy in the Australian flora (eg Meney & Dixon 1988). In some cases, deep dormancy is complicated by low seed quality thought to result from suppressed seed production (may be linked to genetic issues), seed predation both on the plant and in soil and limits on seed maturity due to environmental factors (soil nutrients and moisture deficits).

The dormancy syndromes in Cyperaceae are related to a form of physiological dormancy where the embryo requires certain environmental stimuli to release dormancy and empower the embryo to grow through the seed tissues. Studies by Roche *et al*. (1997) have shown that bushfire smoke (under controlled experimental conditions can elicit high germination from the soil seed bank but only for seed that has been through an ageing (after ripening) phenomenon within the natural soil matrix. What the phenomenon is and how to replicate this for restoration of *Lepidosperma* forms the basis for this important area of the research program.

The study will use the latest breakthrough research in two areas:

1. the alteration in warm and cool stratification regimes on the release of dormancy; and

2. the action of the recently discovered chemical in smoke that promotes germination (Flematti *et al.* 2004a and b).

The research will, for the first time, relate germination outcomes to vitality and growth of excised embryos taken from seeds and cultured on *in-vitro* media. Plants generated in this way will also form part of the micropropagation program.

Seed germination of *Lepidosperma* is typically very low. There are some indications that a complex after-ripening process followed by a stimulant such as smoke may be required to break dormancy, but considerable experimentation remains to be done to characterise such processes for even one species of *Lepidosperma*.

These studies would be replicated for *D. masonii*, which is also has a soil stored seed-bank. Such research is critical in order to germinate seed for restoration, and to assess the ability of the species to recover from adverse impacts. Hence this work will focus on:

- Assessing dormancy release requirements in seed including need for after-ripening (dry and moist states), temperature optima for dormancy release (seasonal temperature rhythms) and emergence from soil; and
- Testing of germination rates for replicate samples of seed treated with three temperature treatments, three chemical (control, smoke water, karrikinolide and 100ppm GA) treatments, and two different (±) light treatments.

4.3d Seed bank demography

Seed of *Lepidosperma* and *Darwinia* species are known to germinate following smoke treatment. Audits of the soil seed bank will be undertaken using the smoke stimulated germination technique to understand the level of smoke requirement for germination, season of application and temporal and spatial extent of the soil seed bank.

This component will provide the necessary data on size and nature of soil seed banks to underpin long term population management strategies. This research objective extends the preliminary study of the soil-stored seed bank to include a more detailed assessment of natural seed banks, and includes an assessment of the utility of soil-stored seed banks for the establishment of translocated populations and the optimal handling and treatment of the soil for maximum germination of seed. The details of the program are:

- Collect (n>50) surface soil samples from within one target population of each species, identifying distance from nearest plant (recording its size and health) and away from population edges. Apply smoke and heat treatments to samples, place in seedling trays and germinate in a glasshouse, record seedling emergence.
- Bury samples of collected and X-ray viability (non-destructively) tested seeds (n=25) in sewn bags in the field, remove and re-test viability at 6 month intervals
- Map seedling emergence in the field following experimental fire and smoke application (over successive years), in relation to pre-existing plants and microtopography.

4.3e Breeding and mating systems

An understanding of the realized patterns of mating is critical for the management of genetic variation. A population's mating system influences its future genetic makeup of a population and is in turn influenced by the current genetic makeup of the population

(Krauss 1999; Krauss *et al.* 2002). For example, if *Lepidosperma gibsonii* is self incompatible, then the loss of variation at self-incompatibility loci (which may occur with a severe reduction in population size) would effectively render the population sterile. At this stage there is no knowledge of the mating system of *Lepidosperma gibsonii* or related species. Hence this work will focus on:

- Field and glasshouse pollinator exclusion studies with seed set estimates;
- AFLP DNA fingerprinting to be used to provide an accurate assessment of outcrossing rates (proportion of seeds that are the product of cross pollination versus those that are the products of self pollination).
- AFLP analysis to also allow the possibility of assigning paternity to individual seeds. Paternity assignment will additionally be employed to provide data on the distance that pollen moves in a population as well as its longevity and the behaviour and effectiveness of pollinators.

4.3f Dispersal

DNA fingerprinting enables the unique genetic characterisation (or genetic barcoding) of individuals. Genetic markers will be used to to assign parentage to offspring (Krauss 2000a, 2000b). Genetic barcoding of a seed, where the maternal parent is known, will be used to attempt to identify the identity of pollen donors. This in turn will enable the characterisation of pollen flow within the population. The characterisation of realized pollen flow will be used to identify the “paternity pool” of *L. gibsonii*, and *D. masonii* which in turn influences the minimum viable population size. Information on the realized dispersal capability of seeds is critical for an understanding of how populations may increase their range naturally. These data on realized dispersal of pollen and seed will be considered with more direct observations of pollen movement (e.g. following pollinators) and seed dispersal (see below) to build up a detailed picture of the dynamics of gene flow within natural populations, which can then be applied to the creation of translocated populations.

Outcomes:

- Timing of and variation in flowering and seed ripening and production documented
- Dormancy requirements understood and germinability rates assessed
- Soil seed bank density, longevity and dynamics described in detail
- Mating systems for *D. masonii* and *L. gibsonii* determined, including: outcrossing rates, self-compatibility, and paternity mapping
- Realized pollen and seed dispersal distances determined
- Paternity pool identified
- Model of gene flow in and between populations.

4.4 Population Viability Analysis (PVA)

Aims:

- To assess population security, growth rates and extinction likelihoods *for D. masonii* and *L. gibsonii* incorporating variation in climate and fire regimes;
- To assess impacts of the loss or augmentation of populations on the species’ overall survival and extinction likelihoods; and
- To assess impacts of varying fire regime on the species’ overall survival and extinction likelihoods (based also on use of smoke auditing to derive seasonal data implications for germination and recovery of seedlings).

Methods:
Demographic models (matrix projection analysis and population viability analysis - PVA) will be constructed from annual life table data derived from the demographic survey, reproductive biology and restoration/translocation studies, covering a range of climate and site conditions. Life tables will be constructed from the demographic survey results to express vital rates (growth, survival, fecundity and recruitment) per individual per size class for two full years of observation (longer if possible). These models will enable the calculation of population growth rates, mean generation times, mean age to maturity, etc., as well as extinction probabilities under different scenarios (e.g. Morris and Doak 2002). Sensitivity of these population parameters to variation in vital rates can also be assessed in these models, allowing estimation of the relative effects of variation in seedling survival (for instance) versus seed predation on population. The effect of fire, varying fire regime, inter-year climate variability, and potential impacts associated with mining (loss of populations and restoration of populations) and other management activities on population growth rates and extinction probabilities will be analysed by manipulating the parameters within these models.

Outcomes:
- Models of future population growth rates (including population decline) and extinction probabilities for each species;
- Estimation of population growth rates and extinction probabilities for each species under scenarios of different management and wildfire conditions;
- Estimation of the relative significance of restoration, rehabilitation or specific management actions (such as herbivore control) for population growth rates and extinction likelihoods; and
- Addresses Ministerial Statement 753 conditions: 6/7-1-1; 6/7-1-2; 6/7-2-2; 6/7-3-2; 6/7-3-4; and 6/7-3-5.

4.5 Environmental Interactions and Plant Health

Aims:
- To determine the factors limiting the distributions of both species
- To identify target locations for translocation of populations
- To identify locations of potential as-yet unknown populations
- To identify environmental factors essential for successful restoration
- To identify the adaptations of both species that limits their distribution and enables persistence in the environment
- To measure the effects of environmental variation on plant growth and population processes
- To identify key biotic interactions that contribute to the persistence of the species in their environment
- To identify key biotic interactions that assist in the operation of population processes
- To develop key indicators of plant health for monitoring detrimental impacts

Methods:
4.5a Abiotic associations
The occurrence and density of *D. masonii* and *L. gibsonii* populations (detailed mapping by ATA/Coffey 2004, 2006, 2008) will be analysed in relation to geology, soils (including depth, rockiness, clay content, chemical composition), slope, aspect, heat load, fire-

history and floristic communities (the Mt Gibson Range has been mapped for some of these). GIS and multivariate methods (e.g. PCNM; principal coordinates of neighbour matrices – Borcard *et al*. 2004) will be employed to interpret and extrapolate spatial patterns and to analyse for significant correlations between distributions and environmental variables.

4.5b Translocation study of environmental boundaries

A translocation study will be used to test for the relative influence of environmental parameters (aspect, slope, rockiness, geology, soil depth) on plant growth and establishment. In this study, replicate groups of at least 10 propagated individuals will planted in areas selected from the distribution models described above. The models will be used to identify the most significant parameters relevant to the species' distributions and the threshold or range of boundary conditions of these parameters associated with the limits to the distributions of each species. Translocation sites will be selected from the distribution models to cross the range of these boundaries, and will include control plantings within the current distribution. The growth, survival and reproduction (if maturity is reached) of translocated individuals will be assessed, together with physiological measures of plant health and vigour. These last will include: stomatal conductance, carbon dioxide assimilation, water transpiration, water use efficiency, pre-dawn and daytime xylem pressure potential (indicating soil moisture availability and the ability of plants to utilise it) and leaf fluorometry – indicating levels of plant stress.

4.5c Drought study

A drought study assessing these same variables will additionally be undertaken using plants grown in controlled conditions and a standard substrate in pots. Seedlings of each species will be transplanted into washed river sand in tall upright pots. These will be flushed once with a low strength nutrient solution and watered daily (to field capacity), after a period of establishment the size and physiology (as above) of seedlings will be assessed.

At this time, one quarter of the plants (5 replicates of each species) will also be harvested and plant biomass and architecture (basal diameter, root depth and length, total leaf area, leaf SLA, stem, leaf and root fresh and dry weights) of these assessed.

The remaining plants will be split into three treatments, one control sample watered as before, and two drought treatments, one of which will receive no further water application, the other supplied weekly with 10% of the calculated water use at first harvest. Subsequent harvests, measuring all plants and the harvested plants as before, will be performed after 2 months and 6 months from the onset of treatment. After the 3rd harvest, plants will be watered again to field capacity, and a final measurement and harvest will be made one month later.

4.5d Plant response to environmental variation

The pattern of variation in plant growth, vigour and physiological activity in relation to temporal and environmental variation will be assessed for tagged plants in the field. For each species, ten plants will be tagged within each of five populations, one of which was burnt in 2003 and consists only of seedlings (i.e. >50 tagged individuals per species).

Each plant will be monitored of plant phenology, physiology parameters (water status, gas exchange, stress) and small scale growth patterns. For each plant the following will be recorded: plant size and growth as for the demographic survey, local site factors, plant health and physiology (as above), leaf growth (*L. gibsonii*) and leaf, inflorescence and infructescence production. Development, growth and survival of new branchlets (*D. masonii*) / rhizomes (*L. gibsonii*) will also be measured for one tagged branch / rhizome on each of these plants. Plants will be assessed over the course of a day at least four times per year in different seasons. Further assessment of the physiological behaviour of plants will take place following significant climatic events (e.g. major summer rains).

Results will indicate patterns of plant physiological behaviour in relation to annual, seasonal and diurnal variation in climate and soil moisture conditions and will help to determine patterns of water use in relation to variation in abiotic site factors. This will be of value in restoration studies as it identifies likely response curves of restored populations depending on the hydrological properties of restored soils. Additional monitoring for timing of reproductive phenology events in the demography survey plots is described in section 4.3a above.

Weather data is currently obtained from the Bureau of Meteorology recording station on Ninghan Station, located approximately 22 km from Extension Hill. The proponents will install an automatic weather station on site to record daily temperature, rainfall and wind data. The data will be made available to the research program to enable correlation of observed physiological, demographic and phenological patterns with climate events, and to allow rapid response for post-rain measurements in case of unusual events.

The results of the investigation into plant response to environmental variation will provide the baseline for assessing changes in plant response due to any potential perturbations from the mine operation, such as high fugitive dust loads. This information will identify any effects from the mining operation, and may also be used in planning translocation and rehabilitation works where these potential perturbating influences may be active. To facilitate this investigation, monitoring sites will be located across a range of areas where potential secondary influences may occur.

Dust load will be measured through the installation of dust deposition monitors at increasing distances from mining activities, along the major seasonal wind directions based on wind rose data. This data will be made available to the research program for correlation against plant health data, and correlated against the proponents' internal vegetation monitoring program to ensure any dust-related impact is identified and addressed in a timely manner.

4.5e Plant health

Plant health will be assessed visually for each tagged plant in the demographic survey based on a qualitative scale. Survival, reproduction and growth data will also be available for each of these individuals from this same study. Detailed plant physiological behaviour will be assessed four times per year for 50 plants of each species (for which the same growth, survival, reproduction and qualitative health data will also be recorded). For these plants, a more detailed colour chart will also be used to describe mean foliage colour. The possibility of constructing a general plant condition score from visual estimation will be investigated by correlating the plant growth (and reproduction etc) with health indices and of these with physiological measurements and colour scores.

4.5f Below ground adaptations

Root depth, architecture and fissure penetration will be assessed in the field. Vertical profiles will be excavated through soil and regolith beneath populations of *D. masonii* and *L. gibsonii* down to bedrock and to within the bedrock using mining technology (dug within the approved mine footprint and prior to the commencement of full mining operations). Samples will be taken of soil, regolith and weathered rock down the profile, as well as within rock fissures. Soil water will be extracted and soil chemistry assessed, soil root length densities, root tip mycorrhizal infections and mycorrhizal length densities will be assessed from each sample (following Bornyasz *et al*. 2005). Samples of plant material will be taken for chemical and isotope composition analyses. Oxygen and hydrogen isotope ratios will be assessed for soil-extracted water and for plant materials; these can be used to identify the depth and materials from which plants are extracting water in the soil (e.g. Chimner & Cooper 2004). Roots will be traced within fissures to depth and, in large samples of fissured rock into fine cracks (following Zwieniecki & Newton 1995), root diameters, root anatomy and presence of fungal associations will be examined. If fragments of root material are found at greater depth, genetic analysis will be undertaken to identify whether the material is from either of the target species. These studies will identify the below-ground adaptations of the two species to their substrates, and the features of the substrates that are most relevant to the persistence and the restoration of populations.

In addition, detailed greenhouse studies of *adaptations / responses to shallow soils and to low and variable water availability will be undertaken*. These studies allow more controlled analysis of the adaptations investigated above. Recently rare *Hakea* (Proteaceae) species restricted to shallow-soil in drought prone environments differed from common congeners in their root system morphology: species invested more in deep roots and showed considerably more lateral spread of their root system at depth.

It is hypothesized that the specific root morphology of shallow-soil endemics confers an advantage by increasing the chance of detecting fissures in the underlying rock, but may not be competitive on deeper soils, and thus may provide an explanation for the rarity of many shallow-soil endemics. As the DRF in this proposal inhabit a similar shallow-soil habitat we will test these hypotheses in a series of innovative glasshouse experiments.

For both the DRF species we will ascertain their root system morphology and compare it with at least three congeners from deeper soils. Plants will be grown on individual long (1.80m) and shallow (0.2m) containers with a transparent bottom sensu Poot and Lambers, 2008. Root system morphology will be assessed by determining root length and mass in different parts of the pot (a minimum of 2 vertical and 14 horizontal compartments will be harvested separately; Poot and Lambers, 2008).

To test the effectiveness of the root morphology/root foraging of these species for locating cracks, custom-made containers will be built containing numerous small holes that lead into a water-filled transparent bottom. The latter will allow a visual determination of the effectiveness of two-dimensional root foraging for cracks.

To demonstrate the hypothesized advantage of the ‘shallow-soil’ root system morphology in a more realistic situation the *Darwinia* and *Lepidosperma* species will also be grown in competition. At least 5 seedlings of each of the species in both the *Darwinia* as well as the *Lepidosperma* ‘series’ will be placed close together in one end of a long container. At the other end the container will have a few holes leading to a bottom compartment with a transparent bottom. When roots of the first individuals have grown into the bottom compartment, drought will be simulated in the top compartment by withholding watering. Leaf gas exchange measurements over time (LICOR 2000) and careful root system excavations at the final harvest will reveal which of the seedlings reached the bottom compartment. The results of this experiment will be compared with one in which seedlings of both ‘series’ will be grown together in deep containers.

To test whether the BIF species have an inherently higher drought tolerance then their common congeners, seedlings of all species (n=8 per species) will be grown in individual custom-made containers that will be placed on a rack under an angle of 45 degrees with the bottom side being transparent to allow root growth observations. Moisture probes will be placed at several depths to allow continuous measurement of soil moisture status. Several times during the development of the young seedlings, watering will be withheld and the seedling responses to these droughts will be monitored by measuring root and shoot extension, and leaf gas exchange. When the seedlings have attained a larger size, these measurements can be accompanied by destructive sampling of leaves to determine leaf water content and leaf water and osmotic potentials. Importantly this design will allow plant responses to be directly associated with soil moisture status and will reveal the soil water content at which root extension is halted.

Field monitoring, comparing the physiological response of plants across varying season and soil water capacity in restored and natural environments s detailed in Sections 4.5b and 4.5d of this research plan.

The above experiments will deliver invaluable insights in the mechanisms that allow the DRF to survive in their inhospitable environments, which is essential for successful future restoration. In addition they may provide further insights as to why so many shallow-soil endemics worldwide are rare.

4.5g Biotic interactions

Pollinator identity, activity and movements will be assessed through a systematic observational study of flowering plant populations. Seed dispersal will be studied through a cafeteria style experiment with samples of collected seeds placed in containers arranged in a grid at ground level. The grid will be systematically surveyed to observe for seed removal at intervals over the course of a day (or daily over a week if removal rates are low) during the peak period of ripe seed production. The identity of dispersers will be determined, if observed, and studies targeted to their behaviour to assess numbers, timing, distance of seed removal events and the fate (e.g. burial depth, predation) of removed seed. Plant herbivory rates will be assessed for all tagged plants in the demographic survey, and the impact of herbivory on plants can be assessed in relation to rates of plant size, growth, fecundity and survival. If mycorrhizal infections are observed in the study of plant roots (4e, above), pot studies on the role of these associations in plant water and nutrient uptake will be performed.

Outcomes:

- Knowledge of factors limiting the distributions of both species particularly role of water harvesting as a key driver of species survival on shallow BIF and granitic soils.
- Understanding of the adaptations of both species to the environment
- Understanding of the habitat characteristics necessary for successful restoration and translocation
- Understanding of biotic interactions of the species and the need to monitor or manage the restoration of disperser and pollinator populations

- An index for the simple classification of plant condition suitable for monitoring of population health
- Baseline physiological data for monitoring success of restored and translocated populations
- Input to translocation and restoration program, identifying locations for potential translocations and site requirements for successful restoration
- Addresses Ministerial Statement 753 conditions 6/7-1-2; 6/7-1-3; 6/7-1-5; 6/7-2-1; 6/7-3-1; 6/7-3-2; 6/7-3-3; 6/7-3-4; 6/7-3-4; 6/7-3-6; and 8-1-8

4.6 Restoration and Translocation

Translocation and restoration are related concepts that apply to locations with different degrees of disturbance. At one extreme, the introduction of plants to a site with an entirely constructed surface and profiles (e.g. waste rock dump) is restoration, at the other, introducing plants into an intact environment is translocation.

In between, sites may have a range of degrees of disturbance and may have either title and 'rehabilitation' may also be used in some circumstances. Here we restrict the terms 'restoration' and 'translocation' to the use described above and use 'rehabilitation' for intermediate degraded sites. The purpose of these three actions varies. All three may be performed to create new, secure populations or significant species as investment against the loss of natural populations, while restoration and rehabilitation may additionally provide stability to surfaces (e.g. resistance to erosion, leaching or dust production), or create or improve provision of other ecosystem services or ecosystem continuity or to ameliorate impacts on biodiversity.

In terms of benefits to species of conservation concern, translocations are most appropriate when populations are few and threatened or diminished in number, and where secure locations with appropriate habitat are available. Translocating into undisturbed and fully functioning populations is itself a disturbance with potential negative consequences for long-term conservation (eg. density-dependent predation, disruption of seed bank), and each site must be evaluated for such risks. Restoration of populations of species of conservation significance is appropriate when continuity or connections with remnant populations are required, or when no other suitable habitat exists. This is most likely when species have narrow habitat requirements. Rehabilitation may be appropriate if degraded sites are available in appropriate locations with suitable habitat, and if the degrading or threatening processes are removed.

Both *Darwinia masonii* and *Lepidosperma gibsonii*, occur in populations experiencing varying degrees of disturbance (by anthropogenic influences), from almost completely undisturbed to moderately disturbed – due to earlier geological survey tracks and grazing. The largest disturbance associated with mining will be the complete removal of populations on Extension Hill North and Extension Hill. The specific aims of restoration and translocation research below are therefore to:

(1) develop methods for translocating plants, including propagation, substrate and ecological requirements, and planting methodologies and success rates;
(2) identify when existing populations are in need of augmenting (translocation);

(3) determine whether translocation to new populations either on or off the range or breakaways is beneficial and feasible, and if so identify sites for translocation; and
(4) develop methodologies for reconstructing surfaces for restoration on the waste rock dump.

In-situ conservation of remaining populations is therefore a critical goal, however existing populations are large, potentially with a well-developed seed bank, and with complex interactions, so translocations in such populations should not be undertaken without careful consideration. Similarly, the creation of new populations on nearby hills where the species do not currently occur has the potential to cause detrimental impacts to other species and communities, and must be carefully considered.

Restoration of new populations on the waste rock dump to "replace" populations lost from Extension Hill is very unlikely to have negative consequences (beyond those caused by mining activities), and so is the preferred location for the development of new populations, however there are substantial challenges to such restoration, and it is currently difficult to predict long-term success.

The strategy for the research is therefore to:
1) ensure existing populations do not deteriorate;
2) develop methods to ameliorate any deterioration should it occur;
3) restore populations to the waste rock dump; and only
4) create new populations of DRF outside of its current area of occurrence if research demonstrates it to be:
   a. necessary for long term survival of the species;
   b. not likely to be detrimental to existing communities; and
   c. practically feasible.

Aims:
- To identify suitable locations and methods for successful translocation of populations
- To identify environmental factors essential for successful restoration
- To identify site treatments for successful restoration of populations of both species
- To identify methods for establishment of populations of both species in rehabilitated environments

Methods:
4.6a Storage of propagation material for translocation
Propagation by cuttings (*D. masonii*), and shoot divisions (*L. gibsonii*) has been demonstrated at BGPA to be effective methods of propagation of the DRF species. These methods produce clonal material which may affect the genetic structure of populations if planted out *en masse*. Impacts to genetic structure of populations from clonal material may be minimised by propagating a large number of genotypes (>100). Material from 200 plants for long-term propagation for translocation and restoration works will be taken after approval of the EMP (but prior to ground-breaking activities), from the area of Extension Hill and Extension Hill North that are to be cleared for mining activities, in order to avoid impacts on any other population of DRF.

4.6b Translocation methods

Potential translocation sites will be identified from the environment associations study (4a above), and methods for enhancing the success of populations in rehabilitated areas (e.g. unused tracks, drill pads) will be trialled. These will include ripping, mulching and (initial) irrigation treatments, as well as application of gravels and coarser debris and artificial wind baffling.

Analysis of the environmental associations of the habitat of both species will be employed to identify a preliminary set of substrate properties (soil rockiness, texture, infiltration and water holding capacity and underlying features – depth, fissure densities) for restoration treatments. Surface rock (overburden) will be extracted in large volume units – of several m^3 – to be used in restoration trials, while other substrate components (e.g. gravels, soils, soil seedbanks) will also be extracted and stored.

These materials, together with materials from ore processing (waste rock and fines), and the clay soils of the waste-rock dump area, will be the materials used both in the final waste-rock dump restoration and in the restoration trials. Analysis of these materials (settling properties, stability, dispersion, infiltration, water holding capacity) alone, and in mixture, will be performed to facilitate the design of profiles with key properties within the range required by the target species. Profiles of different depths (over rock) and soil/waste mixtures will be constructed in a devoted restoration trial area and planted with seedlings, or spread with treated soil-seedbanks. Other treatments will include different applications of surface soil depths, gravels, mulched (trittered) organic material and/or coarse woody debris ± baffling. Surface ripping treatments may also be trialled.

Finally, positive (facilitative) and negative (competitive) interactions with other plant species will be investigated in restoration trials with DRF planted in replicated pairs, close to and away from other significant restoration species. Development of soil surface functionality (infiltration, erosion stability, nutrient cycling) will be assessed using landscape function analyses. Treatment success will be assessed through measurement of plant growth and survival and plant physiology (photosystem stress, Carbon assimilation and water use) – all of which are proposed to be assessed four times each year.

Outcomes

- Methods developed for translocation of *D. masonii* and *L. gibsonii* populations
- Methods developed for restoration of *D. masonii* and *L. gibsonii* populations
- Addresses Ministerial Statement 753 conditions: 6/7-1-2; 6/7-1-4; 6/7-2-2; 6/7-2-3; 6/7-3-3; 6/7-3-4; 6/7-3-5; 6/7-3-6; 8-1-6; 8-1-7; 8-1-8; and 14-3-5.

4.7 *Ex-Situ* Conservation

Conservation of the two DRF species on the Mt Gibson Ranges must be focussed on *in-situ* long term viability of existing populations, however *ex-situ* storage of germplasm (seeds, somatic tissue collections, DNA banking) is an important backup strategy in the conservation of any DRF species, and is proposed to be implemented for *D. masonii* and *L. gibsonii* regardless of any impact from mining.

Aim:
To ensure the existence of long-term backup germ-lines for the DRF species in the event of any catastrophic event

Methods:
Genetic analysis will first identify the optimal number and composition of individual genotypes to store off site, so as to adequately represent the genetic diversity of the species. Research and implementation of *ex-situ* storage will then be conducted through the following strategies:

4.7a Seed storage
Recent studies (Merritt *et al.* 2000; Merritt 2001) have found that storage temperature and seed water content may significantly influence seed viability over short to medium term storage time frames in Western Australian native species (unlike established procedures for seed storage). Therefore, to maintain a viable seed bank, optimal conditions for seed storage need to be determined. Seed with a range of water contents (air dried, dried over silica gel) will be stored at a range of temperatures -18^{o}C; 5^{o}C; and 20^{o}C) to evaluate germination potential and provide measures for establishing the longevity potential of seed banking for conservation. Such investigations are dependent on the ability to overcome seed dormancy, which in the case of *Lepidosperma* is likely to be challenging, and storage of germplasm (eg. obtained from embryo rescue) may be a more viable option.

Several collaborative programs with the BGPA have recently been set up with various institutions, including the Millennium Seed Bank (MSB) Project, and Royal Botanic Gardens, Kew. Once optimal germination and storage requirements have been determined, seed will be sent to to Wakehurst Place (UK) as part of the MSB project. In addition, seed will also be stored at the West Australian Seed Technology Centre (Kings Park) and at the West Australian Threatened Flora Seed Centre (DEC) at –18°C

4.7b Germplasm storage
Long-term storage of germplasm through cryopreservation (i.e. storage in liquid nitrogen at -196^{o}C) will be initiated from both seeds and shoot apices (derived from genetically important in vitro grown mother plants). BGPA is a leading research institution in the use and development of cryogenic methods for the long-term storage of genetically important germplasm. The technique has the advantages of ultra-long storage intervals (>100 years) of genetically stable germplasm and is space and cost effective. At the temperature of liquid nitrogen (-196^{o}C) there is a total cessation of all water movement, chemical reactions and metabolic processes, and under such conditions material can be maintained for potentially hundreds of years without the loss of viability (Turner 2001).

Seed from many native species covering a diverse range of plant families can be stored in liquid nitrogen with minimal pre-conditioning (Touchell and Dixon 1995). With appropriate adjustments in seed water content prior to liquid nitrogen immersion, seed viability can be maintained at pre-storage levels (Merritt *et al.* 2000, Merritt 2001). In addition, several studies by Turner *et al.* (2000, 2001a, 2001b) have found that shoot apices from some rare and threatened species derived from mother plants maintained as tissue cultures, could be successfully stored in liquid nitrogen.

For germplasm storage, it is necessary to develop methods for propagating tissues *in-vitro*. The research on *D. masonii* and *L. gibsonii* will:

- Evaluate requirements for initiating cultures from meristematic tissue and seed embryo rescue especially tissue culture media optimisation.
- Evaluate a selection of standard tissue culture media to determine their affect on standard growth parameters such as: number of leaves; number of shoots; general appearance (health); and vigour.
- Evaluate several lower temperatures (10^{o}C and 12^{o}C) commonly used to maintain cultures for longer durations. Such temperatures reduce growth and therefore allow cultures to be maintained for extended periods of time (up to 12 months) without the need for sub-culturing.

Once germplasm techniques have been optimised it will be necessary to optimise several key factors necessary for successful seed and germplasm storage, including:

- Optimal seed water content prior to liquid nitrogen immersion.
- Germination outcomes during freeze-thaw cycles.
- Germination and growth rates (with and without liquid nitrogen treatment) and impacts on successful transfer to soil.
- For storage of shoot apices, which due to their very high water contents are more difficult to store in liquid nitrogen, methods to suppress freezing (and ice crystal formation) need to be evaluated such as vitrification and encapsulation.

Following completion of propagation and germplasm studies, selected germplasm will be distributed both nationally and internationally as part of a risk management strategy for *ex-situ* storage of DRF. Collaborative programs with the BGPA and Royal Botanic Gardens, Kew facilitate reciprocal storage of germplasm between these institutions. A sample of each liquid nitrogen stored accession will also be sent to the Royal Botanic gardens Kew, to be maintained in their liquid nitrogen germplasm repository.

Outcomes:

Long-term backup germplasm for *D. masonii* and *L. gibsonii*, stored both locally and internationally.

Alternative *in vitro* propagation methods if required for translocation or restoration.
Addresses Ministerial Statement 753 conditions 6/7-1-2.

5 RESEARCH TIMETABLE

The following table provides an indicative timeline for the research on *Darwinia masonii* and *Lepidosperma gibsonii*

Year	2007		2008				2009				2010	
Quarter	3	4	1	2	3	4	1	2	3	4	1	2
Activity												
Conservation Genetics:												
Phylogenetic analysis												
Population variation and differentiation												
Genetic threats												
Population demography												
Establish/remeasure pop monitoring plots												
Experimental fire and monitoring												
Fire history development												
Breeding Biology												
Phenology / seed production												
Seed germination cues												
Seed bank demography												
Pollinator exclusion												
AFLP outcrossing study												
AFLP seed and pollen dispersal												
Population Viability Analysis												
Model development and analysis												
Environmental interactions												
Abiotic associations												
Translocation study												
Drought study												
Response to environmental variation												
Plant health												
Below ground adaptations												
Biotic interactions												
Restoration and translocation												
Storage and propagation of materials												
Translocation methods												
***Ex-situ* Storage:**												
Seed storage research												
Germplasm into *ex situ* storage												

6. REQUIREMENTS OF THE MINISTERIAL CONDITIONS RELATING TO THE *Darwinia masonii* and *Lepidosperma gibsonii* RESEARCH PROPOSAL

Conditions 6.1 and 7.1 of Ministerial Statement 753 specifically relate to the preparation and implementation of a research plan for *Darwinia masonii* and *Lepidosperma gibsonii* respectively.

The Research Plan is also relevant to and addresses in part Conditions 6.2.2, 6.2.3, 7.2.3, 7.2.3, 8.1, 8.1.7, and 8.1.8.

The following tables detail the specific Ministerial Statement 753 requirements relating to research on *Darwinia masonii* and *Lepidosperma gibsonii*, and where the requirements are addressed in the Research Plan.

Condition 6

Condition No in Ministerial Statement 753)	Requirement	In BGPA Research Plan (this document)	Comment
6.1	Prior to the commencement of ground disturbing activities for the mine site, the proponent shall prepare a *Darwinia masonii* Research Plan to the requirements of the Minister for the Environment on advice of the Environmental Protection Authority and the Department of Conservation and Environment. The objective of this plan is to: "facilitate the continued in situ survival and improvement in the conservation status of *Darwinia masonii* over time through targeted research that assists development of a recovery plan for the species"	Y	This document incorporates the *Darwinia masonii* Research Plan
6-1	The Plan shall set out a timetable, objectives and methodologies for research and measures to	Y	Research timetable detailed in Section 5. Objectives and methodologies detailed in Sections 4.1 to 4.7
6-1-1	"monitor the numbers of individuals, their health, and reproductive success"	In part Also see	Monitoring for research purposes detailed in Sections 4.2 and 4.3.

		Interim Recovery Plan	Monitoring of entire population detailed in Interim Recovery Plan Recovery Action 3
6-1-2	"investigate the requirements for maintaining or improving viability of the population through genetic and ecological factors relating to the conservation, management, restoration, propagation and translocation of the species"	Y	Addressed in sections 4.1 Conservation Genetics 4.3 Breeding Biology 4.4 Population Viability Analysis 4.5 -Environmental Interactions and Plant Health, and 4.6-Restoration and Translocation
6-1-3	"provide a scientifically robust analysis of the habitat requirements of the species"	Y	Addressed in section 4.5-Environmental Interactions and Plant Health
6-1-4	"offset the direct impacts of the proposal on the local population of the species by regeneration, re-establishment or translocation of additional plants or subpopulations on suitable un-impacted areas of banded ironstone formations in the Mt Gibson area"	Y	Addressed in section 4.6-Restoration and Translocation
6-1-5	"provide information that (combined with the results of monitoring activities required by condition 8) assists in ensuring that mining and other activities of the proposal, particularly the generation of dust, do not lead to a further decline in the local population of the species"	Y	Addressed in section 4.5-Environmental Interactions and Plant Health (see especially 4.5e) and 4. 2-Population Demography Also See Recovery Action 3 in Interim Recovery Plan
6-2	Prior to the commencement of ground disturbing activities for the mine site, the proponent shall prepare an Interim Recovery Plan for *Darwinia masonii* to the requirements of the Minister for the Environment on the advice of he EPA and DEC. The objective of this plan is to maintain or improve the conservation status of *Darwinia masonii* during the development of the Recovery Plan required by Condition 6.3.	N See Interim Recovery Plan	See *D. masonii* Interim Recovery Plan
6-2-1	This Plan shall include a timetable and actions to "Locate and report any additional populations of the species"	N	See Interim Recovery Plan Recovery Action 8.
6-2-2	"enhance the survival of existing populations of the species	Y	See Interim Recovery Plan Recovery Actions 2, 3, 4 and 5

6-2-3	"expand the existing populations or establish new populations"	Y	The BGPA Research Plan Section 4.1-4.5 will contribute to knowledge of limiting factors and potential alleviation if required See Interim Recovery Plan Recovery Action 2-Implement research plan See BGPA Research Plan Section 4.6 Restoration and Translocation, which provides a timeline and actions
6-3	Within four years following the commencement of ground disturbing activities for the mine site, the proponent shall prepare a Recovery Plan for *Darwinia. masonii* to the requirements of the Minister for the Environment on the advice of the EPA and DEC. The objective of this Plan is : "to maintain, and ultimately improve, the conservation status of *Darwinia masonii* such that its conservation status is more secure in the Mt Gibson area" This plan shall identify	The findings of BGPA Research Plan will form the basis of the Recovery Plan	The Recovery Plan will be Based on findings of BGPA Research plan and review of Interim recovery Plan.
6.3.1	1. habitats that are critical to the survival of the species and the actions than needed to protect those habitats;		
6.3.2	2. threats to the species and areas and populations under threat;		
6.3.3	3. objectives to be achieved;		
6.3.4	4. criteria against which achievement of the objectives is to be measured;		
6.3.5	5. identify management actions to remediate the impacts of the project and provide a net improvement on the pre-mining conservation status of the species; and		
6.3.6	6. identify further research required into the management or recover of the species.		

Condition 7

Condition No in Ministerial Statement 753)	Requirement	In BGPA Research Plan (this document)	Comment
7.1	Prior to the commencement of ground disturbing activities for the mine site, the proponent shall prepare a *Lepidosperma gibsonii*[1] Research Plan to the requirements of the Minister for the Environment on advice of the Environmental Protection Authority and the Department of Conservation and Environment. The objective of this plan is to "facilitate the continued *in-situ* survival and improvement in the conservation status of *Lepidoseprma gibsonii* over time through targeted research that assists development of a recovery plan for the species"	Y	This document incorporates the *Lepidosperma gibsonii* Research Plan
7-1	The Plan shall set out a timetable, objectives and methodologies for research and measures to	Y	Research timetable detailed in Section 5. Objectives and methodologies detailed in Sections 4.1 to 4.7
7-1-1	"monitor the numbers of individuals, their health, and reproductive success"	In part Also see Interim Recovery Plan	Monitoring for research purposes detailed in Sections 4.2 and 4.3. Monitoring of entire population detailed in Interim Recovery Plan Recovery Action 4
7-1-2	"investigate the requirements for maintaining or improving viability of the population through genetic and ecological factors relating to the conservation, management, restoration, propagation and translocation of the species"	Y	Addressed in sections 4.1 Conservation Genetics 4.3 Breeding Biology 4.4 Population Viability Analysis 4.5 -Environmental Interactions and Plant Health, and

[1] Ministerial Statement refers to *Lepidosperma* sp Mt Gibson. Following the release of the Ministerial Statement, the species has been described as *Lepidosperma gibsonii* (Barrett, 2007). The species is referred to as *Lepidosperma gibsonii* in this table.

			4.6-Restoration and Translocation
7-1-3	"provide a scientifically robust analysis of the habitat requirements of the species"	Y	Addressed in section 4.5-Environmental Interactions and Plant Health
7-1-4	"offset the direct impacts of the proposal on the local population of the species by regeneration, re-establishment or translocation of additional plants or subpopulations on suitable un-impacted areas of banded ironstone formations in the Mt Gibson area"	Y	Addressed in section 4.6-Restoration and Translocation
7-1-5	"provide information that (combined with the results of monitoring activities required by condition 8) assists in ensuring that mining and other activities of the proposal, particularly the generation of dust, do not lead to a further decline in the local population of the species"	Y	Addressed in section 4.5-Environmental Interactions and Plant Health (see especially 4.5e) and 4. 2-Population Demography Also See Recovery Action 4 in Interim Recovery Plan
7-2	Prior to the commencement of ground disturbing activities for the mine site, the proponent shall prepare an Interim Recovery Plan for *Lepidosperma gibsonii* to the requirements of the Minister for the Environment on the advice of he EPA and DEC. The objective of this plan is to maintain or improve the conservation status of *Darwinia masonii* during the development of the Recovery Plan required by Condition 7.3.	N See Interim Recovery Plan	See *L. gibsonii* Interim Recovery Plan
7-2-1	This Plan shall include a timetable and actions to "Locate and report any additional populations of the species"	N	See Interim Recovery Plan Recovery Action 3.
7-2-2	"enhance the survival of existing populations of the species	Y	See Interim Recovery Plan Recovery Actions 2, 4, 5 The BGPA Research Plan Section 4.1-4.5 will contribute to knowledge of limiting factors and potential alleviation if required
7-2-3	"expand the existing populations or establish new populations"	Y	See Interim Recovery Plan Recovery Action 2-Implement research plan

			See BGPA Research Plan Section 4.6 Restoration and Translocation, which provides a timeline and actions
7-3 7.3.1 7.3.2 7.3.3 7.3.4 7.3.5 7.3.6	Within four years following the commencement of ground disturbing activities for the mine site, the proponent shall prepare a Recovery Plan for *Lepidosperma gibsonii* to the requirements of the Minister for the Environment on the advice of the EPA and DEC. The objective of this Plan is : "to maintain, and ultimately improve, the conservation status of *Lepidosperma gibsonii* such that its conservation status is more secure in the Mt Gibson area" This plan shall identify (i) habitats that are critical to the survival of the species and the actions than needed to protect those habitats; (ii) threats to the species and areas and populations under threat; (iii) objectives to be achieved; (iv) criteria against which achievement of the objectives is to be measured; (v) identify management actions to remediate the impacts of the project and provide a net improvement on the pre-mining conservation status of the species; and (vi) further research required into the management or recover of the species.	The findings of BGPA Research Plan will form the basis of the Recovery Plan	The Recovery Plan will be Based on findings of BGPA Research plan and review of Interim recovery Plan.

Condition 8

Condition No in Ministerial Statement 753)	Requirement	In BGPA Research Plan (this document)	Comment
8-1	Prior to the commencement of ground disturbing activities, the proponent shall prepare a Significant Flora Species and Communities Management Plan, to the requirements of the Minister for the Environment on advice of EPA and DEC. Note Significant flora species include Declared Rare Flora, Priority listed flora; geographically restricted flora and newly discovered and undescribed flora. The following species shall be addressed in the Plan: *Darwinia masonii;* *Lepidosperma gibsonii;* *Acacia cerastes;* *Grevillea aff. Yorkrakinensis;* *Cryptandra imbricata;* *Podotheca uniseta; and* *Psammomoya implexa.* Note Significant communities include Threatened Ecological Communities; Priority Ecological Communities; and geographically restricted ecological communities	N See EMP	See Environmental Management Plan
8-1-6	“outline the regeneration or revegetation strategies that may be required for significant flora species and components of communities, including completions criteria to be met”	Y	Addressed in part in BGPA research plan sections 4.4–Population Viability Analysis; and 4.6-Restoration and Translocation
8-1-7	“outline management or mitigation actions required to address any failure to achieve regeneration completion criteria arising from (8-1-6)”	Y	Addressed in part in 4.4–Population Viability Analysis; and 4.6-Restoration and Translocation
8-1-8	“outline further investigations into regeneration and reproductive	Y	Addressed in BGPA Research Plan

	ecology of affected significant flora species and components of communities, in order to determine regeneration methodologies, if the completion criteria are not being achieved”	including sections 4.4–Population Viability Analysis; 4.5-Environmental Interactions and Plant Health, 4.6-Restoration and Translocation; and 4.7-*Ex-situ* Conservation

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