THE GOVERNMENT OF WESTERN AUSTRALIA

BOTANIC GARDENS
& PARKS AUTHORITY

Darwinia masonii and *Lepidosperma gibsonii* Conservation and Restoration Research

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

Report to Sponsors
October 2010

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

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1 INTRODUCTION

This research report is based on the *Conservation and Restoration Research Proposal for 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.* August 2008. That proposal was developed by BGPA in response to the commitments of Mount Gibson Mining Limited (MGM) and Extension Hill Pty Ltd (EHPL) to fund a 3+ year research program on the declared rare flora species *Darwinia masonii* (Myrtaceae) and *Lepidosperma gibsonii* (Cyperaceae).

This research program is based on, and 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 proposal document development was also assisted through consultation with DEC Threatened Species and Communities and the EPA.

The project commenced in May 2007, and was described as having a ‘3 year plus’ duration, with the suggestion that the program may be extended subject to achieving requirements as detailed in Ministerial Statement 753.

Acknowledgements:

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2 EXECUTIVE SUMMARY

Summary of results

Conservation genetics

- *Darwinia purpurea*, and *D.* sp. Chiddarcooping are identified as the taxa most closely related to *D. masonii*.

- *Lepidosperma gibsonii* was described as a new species and formally named, with its Rare conservation status transferred from *L.* sp Mt Gibson and is most closely related to nearby populations of the *L. costale*.

- While between population genetic structuring in *Darwinia masonii* is low, some populations do not mate randomly with other populations – suggesting that there are some weak barriers to gene flow across the Mt Gibson range.

- There is very low genetic structuring between populations of *L. gibsonii*, but tests show that there are some barriers to complete gene flow across the Mt Gibson range system.

- The current population size of *L. gibsonii* is estimated to be 1.25 times greater than the current census, due to multiple genetic individuals within some clumps. Some measures of survival may over-estimate by up to 25% due to unobserved loss of genetic individuals from clumps.

Population Demography

- *Darwinia masonii* are long lived (likely to ca. 100 years) and fire-killed. Most individuals recruit from long-lived soil-stored seedbanks in a single cohort following fire. Limited inter-fire recruitment may occur in older populations.

- Plant size data and known population ages suggest that *D. masonii* stem diameter growth averages 0.4 mm.yr^{-1} and height growth averages 2.9 cm.yr^{-1}. Negative height growth recorded for tagged mature plants reflects poor growth conditions in measured years.

- Post-fire seedling recruitment is high in *D. masonii*, with as many as 3.2 seedlings per pre-fire adult, although ~90% of seedlings died over their 1st summer (albeit in a dry season).

- While mortality is rare among mature *D. masonii* plants, drought over the winter of 2010 contributed to a significant level of mortality (>10% in one site). Mortality among 4-6 year old seedlings was recorded at 2.5-15% per year.

- Reproduction commences in *D. masonii* seedlings as young as six years, but increases with plant size, in both proportion of plants flowering, and flowers per plant.

- *Lepidosperma gibsonii* individuals recruit from long-lived soil-stored seedbanks in a single post-fire cohort. There is no evidence for inter-fire recruitment. Plants are long-lived (perhaps to ca. 100 years) and about half of plants exposed to fire appear to survive and resprout.

- Plant size data and known population ages suggest that *L. gibsonii* basal diameter growth averages 2 – 2.5 mm per year for seedlings and adults. Surveys of tagged plants identified mean negative growth rates between 2007 and 2010, possibly reflecting growth conditions in these years.

- Post-fire *L. gibsonii* recruitment averaged 4.2 seedlings produced per pre-fire adult, but ~75% did not survive to 2 years. Mortality among 4-6 year old seedlings averaged 3% per year.

- Reproduction commences in *L. gibsonii* seedlings as young as six years, but increases in terms of proportion of plants flowering, and flowers per plant as plant size increases.

Seed production and seed biology

- *Darwinia masonii* is predominantly pollinated by a single species of Honeyeater. *D. masonii* is capable of producing low-viability, selfed seeds but the production of outcrossed seed is a critical requirement for self-sustaining populations, as there is weak evidence that selfed seed is less fit than outcrossed seed.

- *Darwinia masonii* flowering and seed production takes place over a long period in spring and early summer with the peak of ripe seed production occurring around mid November. Seed fill rates varied between years from 15 to 30% and predation rates from 6 to 22%. Seed dispersal occurs by ants. *Darwinia masonii* seed production is moderately low, varying between years from 9 to 59 seeds per plant in mature populations. Inbreeding and predation by moth larvae contribute to reduced seed quality.

- *Lepidosperma gibsonii* reproduction takes place over multiple years, with inflorescence production occurring in one year and flowering and fruit ripening occurring in the next. Pollination is via wind. If seed is produced, the period for which ripe seed can be collected from *L. gibsonii* plants is brief (one to two weeks in mid October) as good seeds fall soon after ripening.

Seed germination and dormancy

- Large scale production of seedlings of either species via germination of fresh or stored seed involves physical manipulation of small seeds for seed coat nicking or removal, or retrieval of seed buried for months or years.

- Germination of fresh *D. masonii* is low but can be improved by a combination of detailed physical treatments and smoke application. Germination rates of 90% have been achieved with filled seed exhumed after 9 months of burial and treated with smoke water.

- *Lepidosperma gibsonii* seed germination remains unsolved, however indications of small positive, effects of seed burial, fruit wall breakdown/removal, smoke and heat treatments are apparent. Manual seed coat removal followed by a heat treatment resulted in 60% germination.

- Seed bank demography trials established for both species are ongoing. Buried seed is in place with experiments designed to continue for up to 5 years. Results to date indicate complex germination / dormancy strategies for both species, combining a requirement for physical degradation of the seed coat, environmental (seasonal temperature) cuing – with seeds cycling in and out of dormancy, and heat- and smoke-related physiological responses.

Environmental adaptations

- *D. masonii* and *L. gibsonii* share with co-occurring species the drought avoiding strategy of closing down transpiration and photosynthetic function to enter a period of physiological dormancy through summer drought with the capacity to restore tissues as soils wet.

- Roots of both *D. masonii* and *L. gibsonii* have a capacity to enter large cracks, pores and fissures in regolith and may achieve considerable root depths (perhaps to >10m), but neither species showed root growth adaptations that were significantly different from close relatives from non-BIF habitats.

Threats

- Grazing – presumed to be by goats and rabbits – can have a significant impact on growth and reproduction of *L. gibsonii* but has a negligible impact on *D. masonii*

- Predation of *D. masonii* seed (by larvae of an unidentified moth species) can be significant. This moth, and a gall forming insect observed on *D. masonii*, are both potentially specific to *D. masonii* and may therefore be rare and threatened species.

Propagation, Restoration and Translocation

- Techniques for the successful propagation of both *D. masonii* and *L. gibsonii* have been proven at both BGPA and an independent specialist nursery and involve greenstock production from cuttings (*D. masonii*) or separated clumps (*L. gibsonii*).

- Propagation from collected seed may be preferable for genetic diversity reasons and is possible for *D. masonii*, although at this stage requires some time for treatments to take place. As seed germination remains difficult for *L. gibsonii*, propagation from plant collections is the best viable option, however methods to grow plants from seed embryos in tissue culture have been developed, and provide another, more time-consuming solution.

- Species distribution models for *D. masonii* and *L. gibsonii* were able to provide good descriptions of the species' respective distributions and identified different habitat attributes for each. These models also identify localities for possible translocation sites.

- Distribution models indicate a preference of *L. gibsonii* for cooler sites and suggest that restoration surfaces should be sloped to minimise solar radiation receipt for this species. Similar models indicate broad habitat requirements for *D. masonii* (BIF rocky loam soils) but may possibly mask an association with unmapped sub-surface features.

- Translocation trials of both species utilised unmanipulated, naturally occurring substrate variation and demonstrated that both *D. masonii* and *L. gibsonii* have the ability to be planted and survive in restoration sites although this is effectively limited to BIF rock and BIF gravel substrate sites.

- Survival of transplanted *D. masonii* greenstock averaged under 40% (at 9 months) at the best performing site (BIF rocky loam). An additional pilot trial suggests that irrigation may improve *D. masonii* survival and growth rates.
- Transplanted *L. gibsonii* survival was greatest on BIF gravel sites (70%) but was also high on BIF rock sites
- Translocation sites differed in several soil properties, of which texture, gravel/rock content, patterns of moisture content and total Nitrogen may be the most critical.

***Ex Situ* Conservation**

- Batches of 1000 filled seeds of each of *D. masonii* and *L. gibsonii* have been deposited at three (Australian and international) conservation seed storage facilities. In addition genotypes of both species are stored as live plants at two locations off-site and as seed at three secure locations off-site.
- *in vitro* culture has been achieved with both *D. masonii* and *L. gibsonii* and cryostorage is an option for long-term storage of key clonal germplasm if required.

Summary of Recommendations

Conservation genetics

- Investigations requiring complete mapping of individuals (e.g. mating studies) for *L. gibsonii* requires exhaustive genetic sampling within clumps to identify all individuals.
- The precautionary principle should apply to avoid mixing genotypes in restoration between respective populations of *D. masonii* or *L. gibsonii*.

Population Demography

- Monitoring of tagged plants in plots established in this program, including post-fire plots should, continue. The monitoring program may require expansion in order to meet ministerial requirements for numbers of plants.
- Population survey should take place annually in October or November. All plants in selected, permanently marked plots should be individually tagged and measured.
- Key variables to measure in marked plots include: survival, recruitment (new plants should be tagged and recorded as found), health, herbivory, infructescence production (and seed production for *L. gibsonii*) and growth of seedlings and smaller plants.
- Annual collection of a sample of (>10) infructescences of both species from each major population to assess rates of seed predation and seed fill are also recommended.
- Established seed burial and retrieval trials with associated germination treatments should continue for at least several further years.
- Seed collection should be timed closely to ensure that collected fruits contain viable filled and un-predated seed. Ideal times appear to be mid November for *D. masonii* and mid October (in fruiting years) for *L. gibsonii*. Seed counts should take into account the low number of filled seeds per fruit, known selfing rates, and assess seed predation rates.

Environmental Interactions

- Mapping of soil or regolith data for the region is suggested to refine distribution models to improve understanding and predictions of the habitat and restoration requirements for *D. masonii*

- Root systems could be examined in mining pit walls as they are constructed, to determine rooting depth of *D. masonii* and *L. gibsonii* and their use of surficial versus deeper layers in the soil profile. Results would inform requirements for restoration substrates.

Threats and *Ex Situ* Conservation

- Manage populations of goats and rabbits, and monitor herbivory impacts of macropods on *L. gibsonii*.

- Ensure habitat requirements for key *D. masonii* pollinators are retained.

- Identify the seed-eating moth species and survey for its occurrence in co-occurring species and related *Darwinia* species.

- *Ex situ* collections of live plant and seed material and multiple (>100 for live plants) genotypes should be maintained, monitored and supplemented as required

Restoration / Translocation

- Samples of large numbers of filled seed can be assembled for *D. masonii* and *L. gibsonii* (in years in which it produces seed) with careful attention to timing of seed maturation, predation rates and seed screening and cleaning.

- Propagation of live plant material from wild collections and nursery stock are likely the most cost effective approach for the short-medium term storage and production of plants for restoration purposes.

- For *D. masonii*, further research into seedling production under lab, glasshouse or field conditions appears promising and may provide a preferable approach to providing a genetically diverse and numerous source of restoration plants.

- Experiments manipulating restoration substrates using mine waste components or other available and appropriate materials are recommended as mining construction commences. Record plant growth and survival.

- Sand and clay materials may not be effective restoration materials for *D. masonii* and *L. gibsonii*, although mixing clays with rock and/or gravel may be worth trialling. Final restored structure surface must incorporate a large proportion of BIF rock or gravel for successful restoration of both species.

- Trials in which *D. masonii* and *L. gibsonii* are translocated into restoration substrates designed and constructed with varying amounts of rock and gravels, and with rocks at varying depths are recommended.

- Restoration areas for *L. gibsonii* should be shaped as slopes or gullies oriented with lower radiation receipt. Restoration trials for *L. gibsonii* should include treatments varying shade and moisture.

- Restoration areas for *D. masonii* may not require particular topographies, but attention to soil requirements may be important. Restoration trials for *D. masonii* should include treatments varying degrees of soil depth and rockiness.

- Restoration efforts must include adequate community context e.g. ensuring adequate habitat for White-fronted Honeyeaters and seed dispersing ant species.

3 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 (BIF), 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. 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, which has since been described as *Lepidosperma gibsonii* R.L. Barrett (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 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 Environmental Protection and Biodiversity Conservation Act, 1999. The proponents are now developing an 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). Expansions of the project's footprint were approved 20 February 2008.

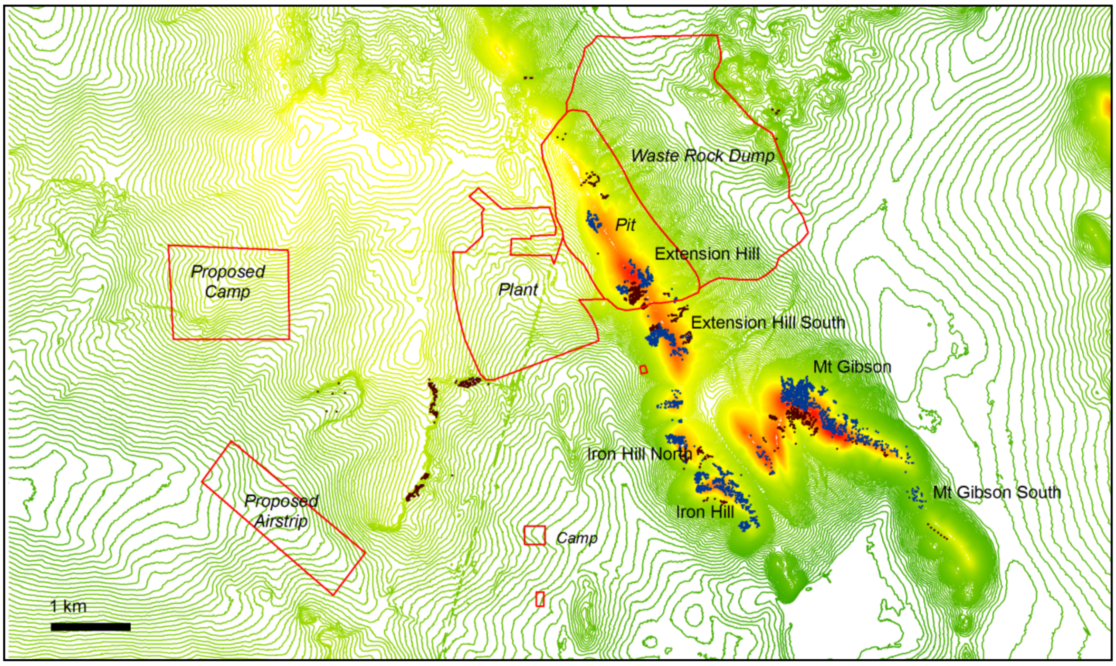

Figure 1 Major features of the study area including proposed (approved) mining project footprint, topography (1m contours), major peaks and the distribution of *L. gibsonii* (brown dots) and *D. masonii* (blue dots)

3.1 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.

3.2 OTHER RESEARCH

The research program reported here is devoted specifically to the Declared Rare Flora (DRF) species *Darwinia masonii* and *Lepidosperma gibsonii.* However, other research initiatives are required to meet Conditions 6.1.5 and 7.1.5 (other impacts on DRF, including from dust) and 14 (Closure) of Ministerial Statement 753. In response to these requirements, BGPA has prepared and submitted a **Proposal for research into the restoration of plant communities in the Extension Hill – Mt Gibson iron ore mining project** (submitted February 2008, updated February 2010) as well as a **Proposal for research into the affects of dust on rare plants** (submitted July 2008) to EHPL and MGM. Funding of a 2008 version of the restoration research plan was agreed by both EHPL and MGM subject to final approval of mining and the (subsequently delayed) commencement of operations. This community restoration research was specifically noted in the ***Darwinia masonii*** **and** ***Lepidosperma gibsonii*** **Conservation and Restoration Research Plan** (i.e. the plan outlining the research presented here and signed-off by DEC) as a necessary research component additional to the work covered in the plan.

The results of the research program presented here show that some research areas require ongoing investigation for satisfactory conclusion in relation to Conditions 6.1.1, 6.2, 7.1.1 and 7.2 of Ministerial Statement 753. In particular, the strong inter-annual variation observed in key population parameters (i.e. growth, survival and seed production rates) means that long-term population monitoring is required for determination of their average values and patterns of variability. This long-term monitoring is essential in order to determine whether future observed fluctuations result from mining impacts or natural variation. The same data are also essential for tests of population viability and extinction likelihoods in response to impacts such as fire, herbivory or continued drought (or similar climatic extreme), or mining impacts such as population loss, or augmentation through restoration. A program for continuation of the DRF research presented here – ***Proposal for continuation of Extension Hill – Mt Gibson DRF Research Program*** (January 2010, updated May 2010) – has been prepared by BGPA and submitted to EHPL and MGM.

Details of requirements for monitoring of the health of, and mining impacts on, DRF populations (including a specified proportion of individuals) are given in

Ministerial Statement 753. As this monitoring does not constitute research per se, it was never an objective of the BGPA research program to meet these requirements. Nevertheless, monitoring of these parameters for research objectives has been a component of the work presented here, although not to the requirements (in terms of number of individuals represented) of the Ministerial Statement in several details. Additional monitoring will be required by EHPL and MGM to meet these requirements.

4 FINDINGS

4.1 CONSERVATION GENETICS

4.1a Phylogenetic context

Darwinia masonii

Charles Gardner, when describing *Darwinia masonii* in 1964, noted the pendulous flower heads and long marginal bracts, and concluded that it was closely related to the Stirling Range species *Darwinia leiostyla*. No analysis of relationships within *Darwinia* has been undertaken since that time (other than speculations on the relationships of a few individual species).

In order to identify appropriate comparisons for the assessment of genetic diversity and comparative ecology in *D. masonii*, a thorough analysis of phylogenetic relationships in the genus *Darwinia* was undertaken, including most species of *Darwinia* in south-west WA, and several species of the disjunct "*D. fascicularis*-group" in New South Wales and South Australia. Two gene regions were used in order to detect incongruent signal resulting from gene trees vs species trees: the nuclear ribosomal External Transcribed Spacer (ETS), and the chloroplast trnK intron (including the *mat*K gene).

Analysis of chloroplast and nuclear genes showed significant incongruence at many of the upper nodes in the tree (suggesting either incomplete lineage sorting or ancient hybridisation), however most species-groups identified had identical composition. In particular, the position of *D. masonii* clearly resolved with neither the (monophyletic) Stirling Range Bells, nor the group around *D. helichrysoides*/*D. neildiana*, but rather with a group of unassuming *Darwinia* species of the WA Northern Sandplain and Wheatbelt regions, including *D. purpurea, D. acerosa*, and the undescribed species *D.* sp. Chiddarcooping (S.D. Hopper 6944). Each of these three species is a spreading shrub with upright to sub-pendulous flower heads and short bracts and styles, unlike *D. masonii* which is upright, with pendulous flower heads and long bracts and styles. Optimising morphological characters on the phylogeny, it is clear that both pendulous flowers and long styles and bracts have evolved several times independently in the genus *Darwinia*, presumably to improve pollination success by honeyeaters. *Darwinia masonii* has thus evolved its defining floral characteristics from less specialised forms like *D. purpurea*. Relationships between *D. masonii*, *D. purpurea, D. acerosa*, and *D.* sp. Chiddarcooping were unresolved by either phylogenetic marker, indicating that they are closely related, and possibly speciated allopatrically, through isolation and subsequent adaptation of a previously widespread species.

Summary

- *Darwinia purpurea*, *D. acerosa* and *D.* sp. Chiddarcooping were identified as the taxa most closely related to *D. masonii* and have subsequently been employed as comparison species for several studies in later sections, including genetic diversity analyses and root adaptations.

Lepidosperma gibsonii

Lepidosperma gibsonii was first identified from collected material from Extension Hill. From its discovery in 2006 the taxa was informally named as *Lepidosperma* sp. Mt Gibson (R. Meissner & Y. Caruso 3), but in late 2007 was formally described and published as the new species, *Lepidosperma gibsonii* R.L.Barrett, in a special edition of the journal *Nuytsia* devoted to new BIF associated species (Barrett 2007).

Lepidosperma gibsonii belongs to a morphologically depauperate group of grass-like plants, whose relationships are especially difficult to discern due to the lack of morphological variation. Genetic methods are therefore critical to identify related taxa. *Lepidosperma* species with similar morphology were sampled across south-west WA, focussing on populations within 200 km of MT Gibson. Two genes were used to assess relationships, the nuclear ribosomal External Transcribed Spacer (ETS) and the chloroplast *trn*L inton + *trn*L-*trn*F spacer. The latter proved to be less variable than ETS, and so sampling was less comprehensive for that region, with ETS being used only for subsets of taxa within clades.

The molecular analyses found that *L. gibsonii* was clearly closely related to a group of taxa around *L. costale*, and only more distantly related to the morphologically similar species *L. ferricola*. Further sampling by BGPA has identified populations of the *L. costale* complex from Mullewa and Mt Karara to Paynes Find, south to York and east to near Southern Cross. Both between- and within-population diversity in this species complex can be considerable, and further studies are required to full elucidate their taxonomy. Only *L. gibsonii* can be easily distinguished, being the only member of the complex with rounded, terete (or sub-terete) culms; all other populations have sharply angled stems. A separately-funded project at BGPA has investigated ploidy-level differences within the *L. costale* complex, demonstrating that most populations are tetraploid, some populations are of allopolyploid-hybrid origin, and that diploids are almost entirely restricted to the semi-arid interzone between Mt Gibson, Mt Karara and Wubin (Figure 2). Due to different breeding systems in the tetraploid populations (wholly or partly agamospermous, producing seeds without recombination), only the diploid populations are useful for comparative genetic diversity in *L. gibsonii*.

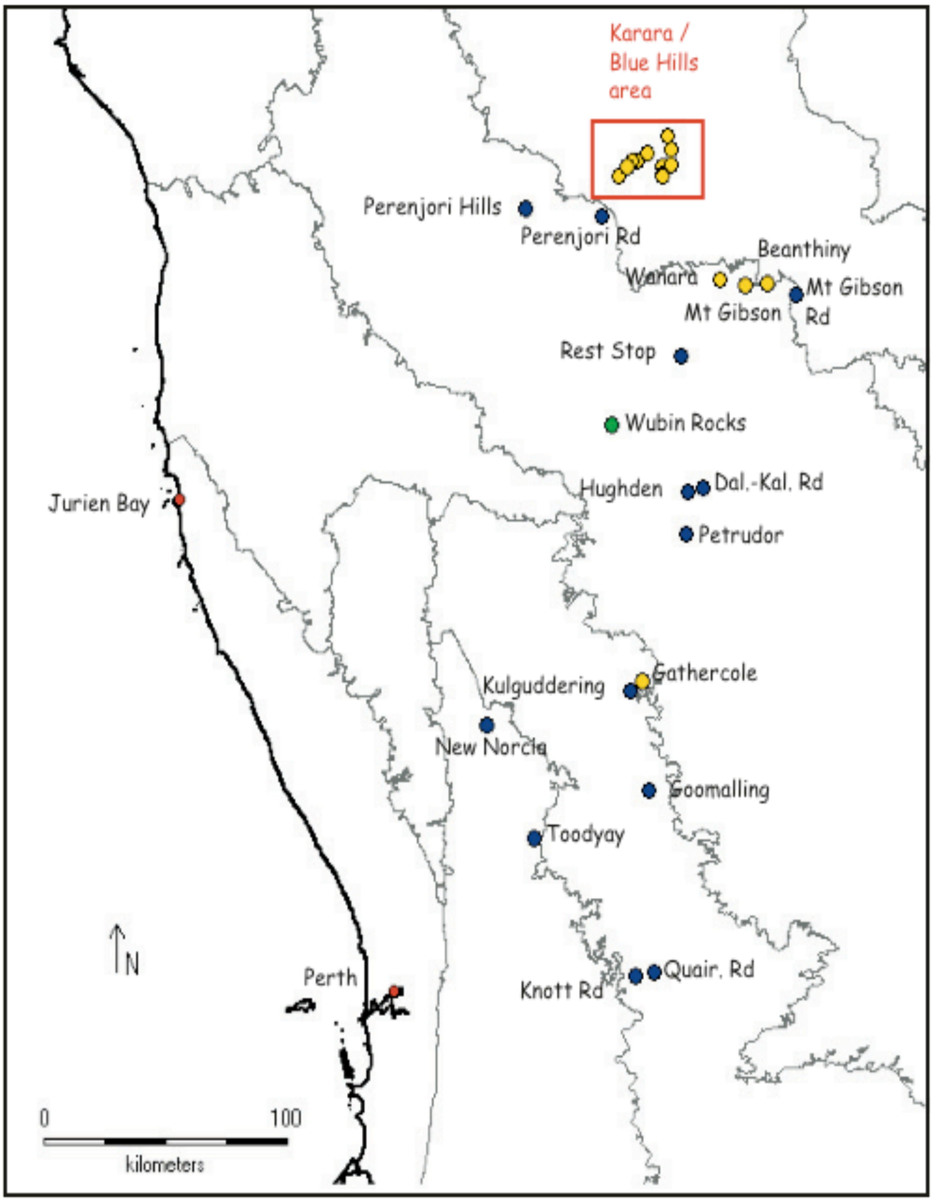

Figure 2. Map of genome content levels within the *Lepidosperma costale* complex. Yellow circles indicate populations with normal (diploid) DNA content. Blue indicates populations containing only individuals with double the normal DNA content per cell (tetraploid). The green circle (Wubin population) contains both diploid and tetraploid plants – extensive study of this population indicates that diploids and tetraploids are physically separated, and only a single triploid 'hybrid' was located. Diploid populations presumably gave rise to the now widespread tetraploid lineages, but are now almost confined to the driest area of the distribution of the species-complex. Some populations wholly or partly reproduce by agamospery (producing seeds without recombination). Other tetraploid populations are allotetraploid hybrids, with half the genome having come from a species outside of the *L. costale*-complex (at least two other species have contributed to this process in different locations).

Summary

- *Lepidosperma gibsonii* was described as a new species and formally named, with the conservation status of rare transferred to it from *L.* sp Mt Gibson.
- *Lepidosperma gibsonii* is most closely related genetically to populations of the *L. costale* complex around Mt Karara, Beanthiny Hill and Wubin. Comparisons of genetic diversity and adaptations were concentrated on these species.

4.1b Landscape scale genetic structure

Darwinia masonii

The initial genetic survey of *Darwinia masonii* (BGPA 2005, following a 6-month initial genetic survey) was based on 75 samples from four populations on the Mt Gibson range system, using the AFLP (Amplified Fragment Length Polymorphism) fingerprinting technique. This study found both limited diversity (with 50.6% of markers polymorphic, very low for AFLP), and low population differentiation (AMOVA found that 94% of genetic variation was contained within populations, and just 6% between populations). As recognised in the report, and also raised subsequently by reviewers from the Dept. of Environment and Conservation (DEC), the low variability observed in the AFLP markers lowered the power to test for population differentiation in this species, which was further hampered by insufficient sample size in the preliminary study, both in number of samples and number of populations. As a result, a more powerful genetic fingerprinting technique, simple-sequence repeats (SSR), more commonly known as microsatellites was employed following development of the marker system; this is the same type of genetic marker as used for *Lepidosperma gibsonii*.

SSR (Microsatellite) marker Development

Genomic DNA was extracted in bulk (100 ug of DNA total), and sent to Genetic Identification Services in the US for cutting, inserting into bacterial libraries, cloned, enriched for microsatellite-containing sequences, and sequenced. Microsatellite-containing sequences were then screened for the most appropriate microsatellite motifs (including a range of di- and tri-nucleotide motifs, while avoiding flanking regions containing long mono-nucleotide repeats as they can confound scoring). Primers were developed from the sequences to amplify the target loci, and these primers were screened for amplification reliability, copy number, and degree of stutter (an artefact that can prevent accurate scoring). A total of 14 microsatellite loci were finally produced, which were used in various analyses of genetic diversity and mating system in *D. masonii*.

Population genetic structure

A total of 179 samples from seven populations of *D. masonii* on the Mt Gibson range (Figure 3) were taken from young leaf tips and stored at -178°C in a liquid-nitrogen dry-shipper in the field, then placed in a -80°C freezer awaiting extraction. DNA was extracted following the Carlson-Qiagen extraction procedure as outlined in the Phase One study (BGPA 2005).

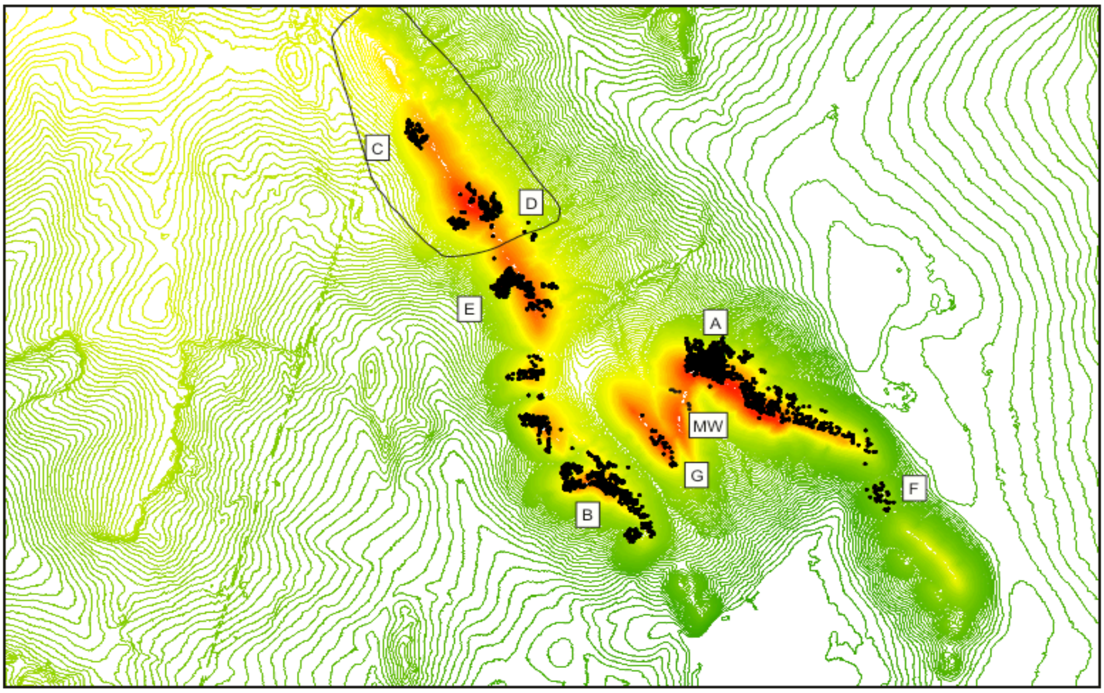

Figure 3. Distribution of *Darwinia masonii* (black dots) on the Mt Gibson range, and collection localities labelled by population code. Codes – A: Mt Gibson, B: Iron Hill, D: Extension Hill, E: Extension Hill South, F: Mt Gibson South, G: Iron Hill East, MW: between Mt Gibson and Iron Hill East. Black line shows approximate position of the pit.

Analysis of Molecular variance partitioned 94% of variation within populations, and 6% between populations, indicating weak population structure (as can be seen graphically in Figure 4, where samples from different populations do not group together, but are instead completely intermixed). This is the same result (6% between-population variation) obtained with AFLP data from fewer populations. In contrast to the previous AFLP study, which showed no significantly genetically distinct populations, pairwise permutation tests (

Table 1) between all populations show that two populations (on Extension Hill South and Mt Gibson South – E and F respectively in Figure 1) are statistically supported (at p< 0.001) as being genetically 'isolated' from each other and all remaining populations. In this context, 'isolated' means not mating randomly with other populations, with number of possible explanations. Aside from these two populations, other populations are scarcely significantly different from a single panmictic, interbreeding population.

Possible causes of divergence within populations: The population Mt Gibson South ("F") is somewhat disjunct and at the southern end of the range, as expected for a population diverging in isolation; however, the population has clearly not been burnt for > 50 years, unlike most other populations, and as a result the observed non-random mating could be an artefact of sampling different generations. The population on Extension Hill South ("E") is close to that the population on Extension Hill, occupies an intermediate position on the western ridge, and the observed result is surprising. The observed weak departure from non-random mating could be due to differences in population age, or some populations could be experiencing differential selection at loci linked to some microsatellite markers. Sampled plants came from a variety of plant ages, fire history, and substrate, as this population extends from almost bare BIF cliffs to laterite at the range base.

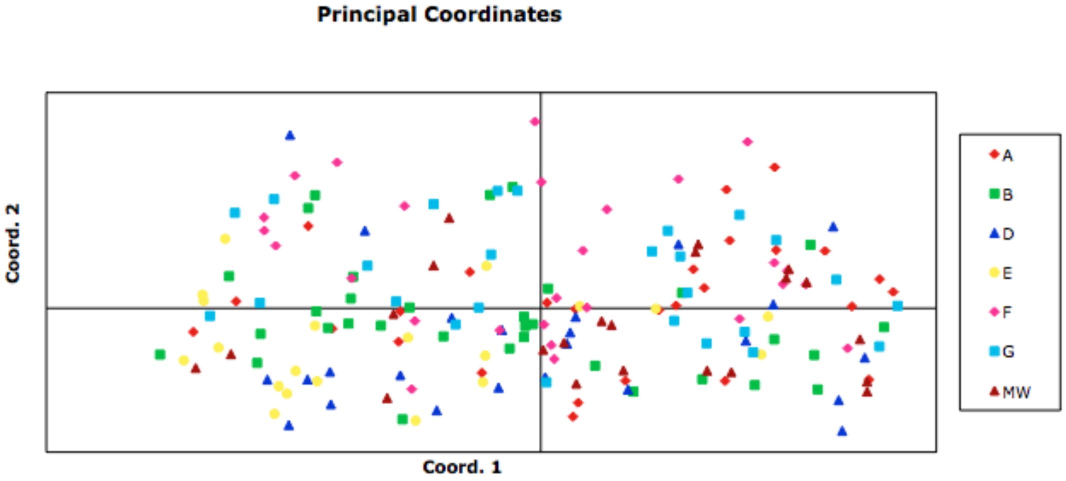

Figure 4 Principal Co-ordinates analyses of samples from seven populations of *D. masonii* showing weak differentiation between localities. Codes as previous figure. In this figure, samples placed close together are more closely related. The figure shows no strong clustering of individuals within populations; formal statistical tests demonstrated that only a few pairwise population comparisons are statistically distinct, with no geographic pattern.

Table 1. Pairwise permutation test of population differentiation. **Bold**: significant at p < 0.001, *italics* significant at p <0.005. Codes – A: Mt Gibson, B: Iron Hill, D: Extension Hill, E: Extension Hill South, F: Mt Gibson South, G: Iron Hill East, MW: between Mt Gibson and Iron Hill East. Populations E and F are significantly supported as departing from random mating with other populations; occasional other pairwise comparisons are also significant.

A	**B**	**D**	**E**	**F**	**G**	**MW**	
	0.001	*0.004*	**0.001**	*0.002*	0.076	0.425	**A**
		0.087	**0.001**	**0.001**	0.048	0.017	**B**
			0.002	**0.001**	*0.002*	0.119	**D**
				0.001	**0.001**	**0.001**	**E**
					0.004	**0.001**	**F**
						0.071	**G**
							MW

Population genetic diversity – within D. masonii

Expected heterozygosity, and fixation Index was estimated for all seven sampled *D. masonii* populations (Table 2). All were very similar in levels of diversity (Unbiased Heterozygosity estimates vary between 0.600-0.657). Fixation index for the species was 0.105 ± 0.024, indicating a low but significant level of inbreeding. This result is further corroborated below (section **4.3e Breeding and Mating systems**).

Table 2. Heterozygosity and fixation index estimates from populations of *Darwinia masonii*. Population codes as previous tables and figures.

Pop	**Ho**	**He**	**UHe**	**F**
A	0.487 ± 0.09	0.593 ± 0.115	0.605 ± 0.117	0.144 ± 0.041
B	0.59 ± 0.102	0.647 ± 0.109	0.657 ± 0.111	0.066 ± 0.048
D	0.474 ± 0.106	0.601 ± 0.123	0.614 ± 0.126	0.216 ± 0.057
E	0.657 ± 0.092	0.625 ± 0.088	0.642 ± 0.091	-0.067 ± 0.056
F	0.537 ± 0.094	0.629 ± 0.089	0.64 ± 0.091	0.172 ± 0.076
G	0.566 ± 0.126	0.63 ± 0.104	0.642 ± 0.106	0.149 ± 0.089
MW	0.533 ± 0.11	0.586 ± 0.117	0.6 ± 0.12	0.077 ± 0.031
Total	0.549 ± 0.037	0.616 ± 0.037	0.629 ± 0.038	0.105 ± 0.024

Population genetic diversity – comparison with other species

The preliminary genetic survey was unable to adequately reference the “low” AFLP variation due to the lack of a comparable study in *Darwinia*. It has been suggested that low chromosome number can decrease genetic variability (e.g. Diuris, Indsto et al 2009); since *Darwinia* species are known to show a dysploid chromosome-reduction series (Rye 1981, Rye & James 1990), this is one possible reason for the low observed diversity; the alternative hypothesis is a past low population size (population bottleneck) which caused loss of genetic diversity. The inability to distinguish between these scenarios was raised as a significant concern by DEC reviewers of the preliminary report. It was therefore necessary to reference the diversity in *D. masonii* with another species. Following the discovery through the phylogenetic research above, it was decided to use the closest relative of *D. masonii*, *D. purpurea*, as the comparative species. *Darwinia purpurea* is a relatively widespread species of sandplain and gravel soils, extending from near Perenjori and Mt Gibson South-East to about Warralakin (with a single disjunct population c. 80 km north of Mt Gibson at Kirkalocka Station on an isolated yellow sand lens). The potentially distinct species known by the informal phrase-name "*Darwinia* sp. Chiddarcooping (S.D. Hopper 6944)" was also included, due to apparent intergrades between it and *D. purpurea*, and the tendency for it to grow in isolated populations on granite rocks, and might therefore be a more appropriate comparison for *D. masonii* than the less habitat-constricted *D. purpurea*. The relative distribution of these species, and the related *D. acerosa*, is shown in Figure 5

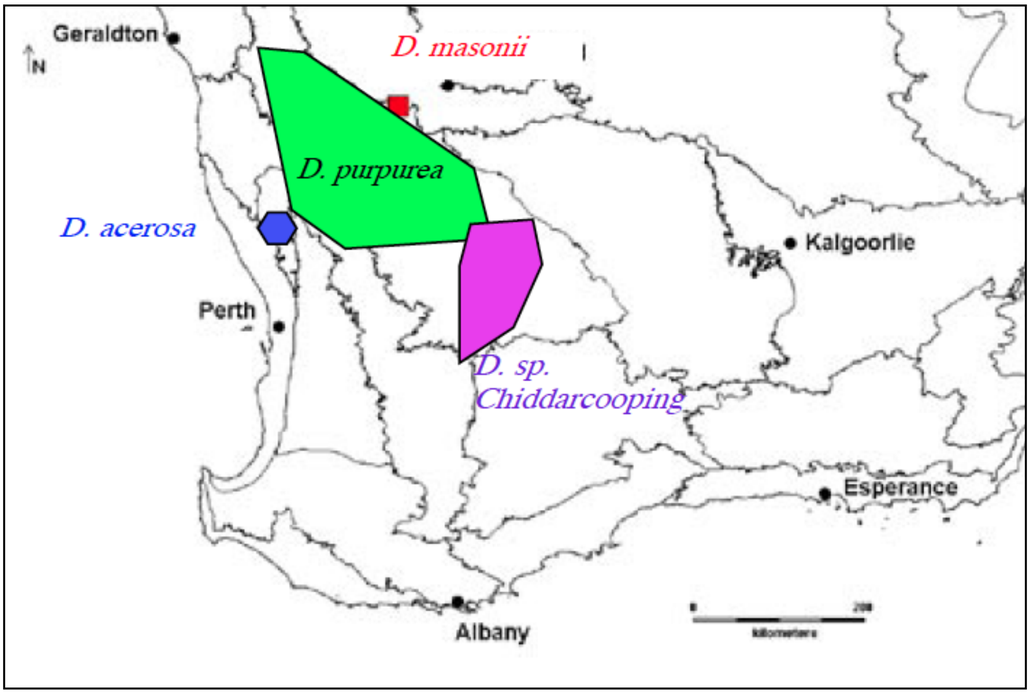

Figure 5. Distribution of *Darwinia masonii* and closely related species: *D. purpurea, D. acerosa* and *D.* sp. Chiddarcooping.

A total of 401 plants from thirteen populations of the *D. purpurea* / *D*. sp. Chiddarcooping species-group were sampled (Table 3), and genotyped using the microsatellite loci described above. Genetic diversity within and between these populations were compared to the diversity in all sampled *D. masonii* populations (as referred to above). Only one population of *D. masonii* (Iron

Hill) was used in the all-taxa population structure analysis (PCA) to allow comparisons of similar sampling numbers and sampling density (Figure 6).

Table 3 Average genetic diversity within sampled populations of *Darwinia masonii*, *D. purpurea* and *D. sp.* Chiddarcooping.

Population	Ho	He	UHe	F
***D. sp. Chiddarcooping* populations**				
Corrigin	0.111 ± 0.049	0.214 ± 0.093	0.217 ± 0.094	0.365 ± 0.116
Billy	0.693 ± 0.058	0.750 ± 0.055	0.762 ± 0.056	0.077 ± 0.028
Chidd	0.670 ± 0.063	0.661 ± 0.058	0.672 ± 0.059	-0.020 ± 0.054
Wara	0.498 ± 0.093	0.651 ± 0.047	0.662 ± 0.048	0.243 ± 0.128
***Darwinia purpurea* populations**				
Yorkrak	0.583 ± 0.059	0.670 ± 0.051	0.684 ± 0.052	0.109 ± 0.094
Burakin	0.722 ± 0.024	0.794 ± 0.024	0.807 ± 0.024	0.088 ± 0.035
Koorda	0.674 ± 0.093	0.762 ± 0.081	0.775 ± 0.082	0.103 ± 0.082
Korakadine	0.566 ± 0.086	0.577 ± 0.088	0.587 ± 0.090	0.007 ± 0.030
Ballidu	0.722 ± 0.027	0.775 ± 0.050	0.791 ± 0.051	0.056 ± 0.045
Dal-Kal	0.741 ± 0.069	0.758 ± 0.057	0.775 ± 0.058	0.029 ± 0.030
Bunjil	0.396 ± 0.090	0.434 ± 0.084	0.444 ± 0.087	0.184 ± 0.180
Latham	0.645 ± 0.080	0.684 ± 0.071	0.696 ± 0.072	0.066 ± 0.039
Wubin	0.806 ± 0.027	0.801 ± 0.034	0.822 ± 0.035	-0.008 ± 0.019
***Darwinia masonii* populations**				
A	0.487 ± 0.09	0.593 ± 0.115	0.605 ± 0.117	0.144 ± 0.041
B	0.590 ± 0.102	0.647 ± 0.109	0.657 ± 0.111	0.066 ± 0.048
D	0.474 ± 0.106	0.601 ± 0.123	0.614 ± 0.126	0.216 ± 0.057
E	0.657 ± 0.092	0.625 ± 0.088	0.642 ± 0.091	-0.067 ± 0.056
F	0.537 ± 0.094	0.629 ± 0.089	0.640 ± 0.091	0.172 ± 0.076
G	0.566 ± 0.126	0.630 ± 0.104	0.642 ± 0.106	0.149 ± 0.089
MW	0.533 ± 0.110	0.586 ± 0.117	0.600 ± 0.120	0.077 ± 0.031
Total	0.549 ± 0.037	0.616 ± 0.037	0.629 ± 0.038	0.105 ± 0.024

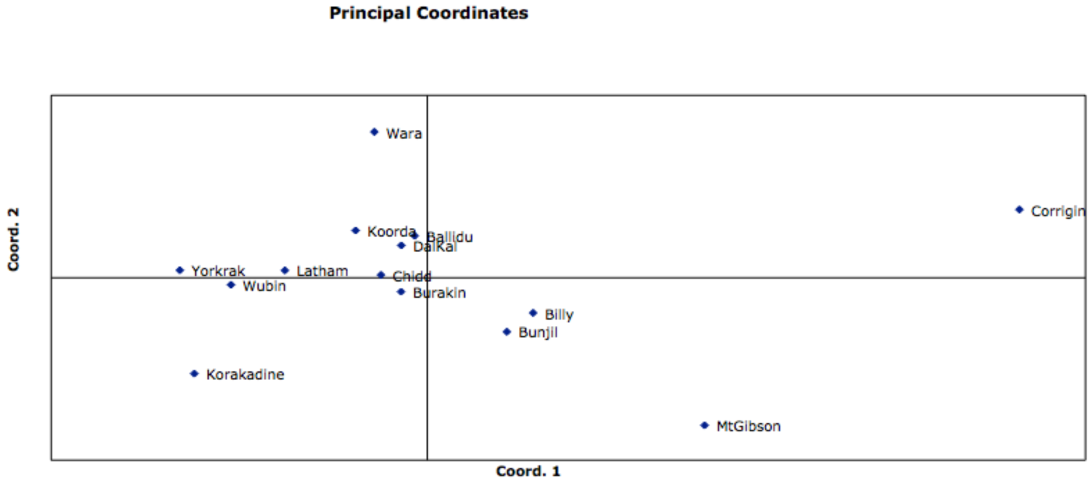

Figure 6 PCA analysis showing genetic relationships among sampled populations of *Darwinia purpurea*, *D. sp.* Chiddarcooping and *D. masonii* (as "Mt Gibson"), using Nei's genetic distance as a measure of divergence between populations. In this figure, sites located close together are more closely related. The two most divergent populations are the Corrigin population, which may represent a distinct species, and

D. masonii, with the *D. purpurea* and *D.* sp. Chiddarcooping populations forming a cluster, with little differentiation between the two species.

Results: Darwinia masonii contains comparable (but slightly lower than average) microsatellite diversity relative to other populations in this species-group (Table 3). For example unbiased heterozygosity estimates in *D. masonii* range between 0.600 and 0.657, compared with 0.587-0.807 for *D. purpurea / D.* sp. Chiddarcooping populations, (excluding two populations with abnormally low diversity discussed next). The only two populations showing a significant reduction in genetic diversity were the Corrigin and Bunjil populations. The Bunjil population is in a small, highly disturbed patch of remnant vegetation with only a few scattered plants, and has presumably lost some of its diversity through recent reduction in population size. The Corrigin population is restricted to a small area on a single granite rock, contains less than 100 plants, and is geographically disjunct from other members of the *D. purpurea / D.* sp. Chiddarcooping complex. It also shows morphological differences to all other populations and species of *Darwinia*, and may warrant recognition as a distinct species. In the context of this study it serves as a reference comparison as a population that has presumably lost much of its genetic diversity though inbreeding due to small population size (an alternative hypothesis is that a rare long-distance dispersal event occurred, and that the Corrigin population is showing the effects of a recent founder population; however, given the number of unique alleles in this population, and the divergent morphology of its individuals, it is more likely to be a in isolated relictual population).

Darwinia masonii shows no indication of recent inbreeding depression, unlike the Bunjil and Corrigin populations discussed above.

Summary

- The more powerful tests of population-genetic structure carried out here (relative to the earlier study) is mostly in agreement with the preliminary result using a different marker technique, especially in the level of population differentiation observed.
- In general, there appears to be little genetic structuring between populations of *Darwinia masonii*, (94% of genetic variation is partitioned within populations) . However pairwise tests show that some populations are statistically supported as non-randomly mating with other populations with the more powerful microsatellite analysis. This suggests that there are some barriers to complete gene flow across the Mt Gibson range system, and that the precautionary principle should apply to avoid mixing genotypes between populations without careful consideration of consequences.

Lepidosperma gibsonii

The initial genetic survey of *Lepidosperma gibsonii* (BGPA 2006, following a 3-month initial genetic survey using microsatellites developed for that study) was based on 145 samples from seven populations on the Mt Gibson range system. This study found high levels of microsatellite variation, and low population differentiation (AMOVA analyses partitioned 98% of genetic variation within populations, and just 2% between populations).

Subsequently to that report, several populations of *Lepidosperma* were found by BGPA staff, and surveyed by ATA environmental / Coffey Environments (populations EFN, EFS, WC, WD and MGS in Figure 7). These populations were off the Mt Gibson Range, except in the case of the population on Mt Gibson South, at the extreme southern end of the range; all were in habitats not initially recognised as being suitable for *L. gibsonii*, and so were not discovered in the initial, extensive but time-limited survey.

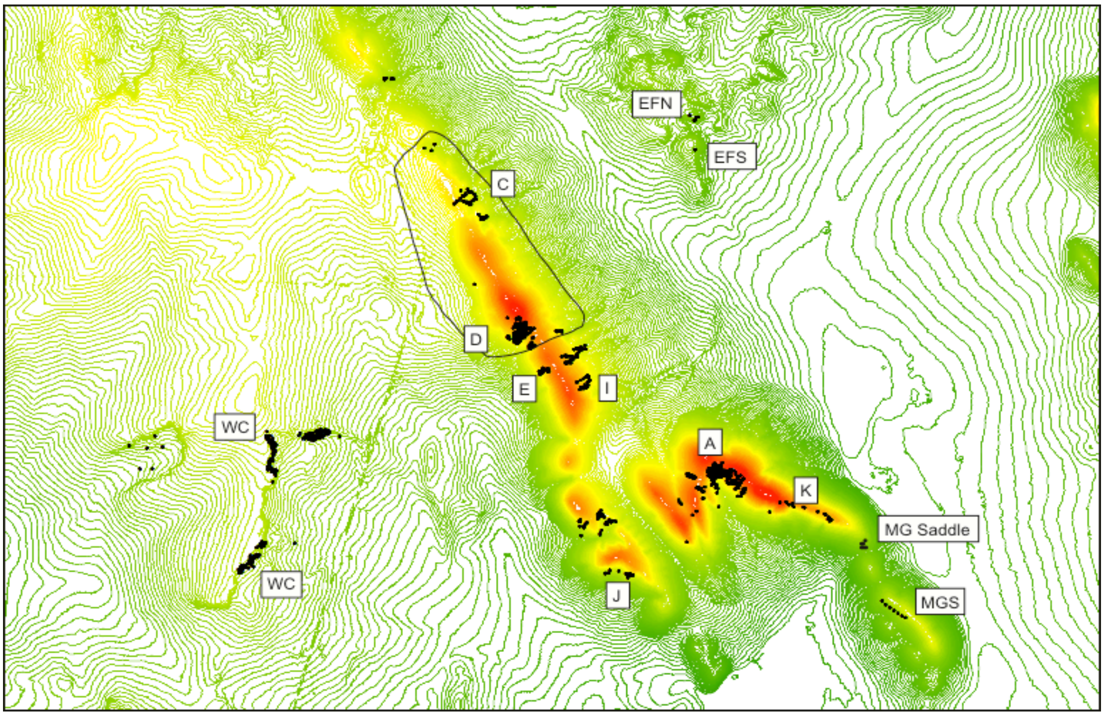

Figure 7. Distribution of *Lepidosperma gibsonii* (black dots), showing populations sampled in the genetic survey, and population codes: A - Mt Gibson, C- Extension Hill North, D – Extension Hill, E – Extension Hill South (west side), I - Extension Hill South (east side), J – Iron Hill, K – Mt Gibson (south end), MG Saddle – Saddle between Mt Gibson and Mt Gibson South, MGS – Mt Gibson South, EFN – Emu Fence North, EFS – Emu Fence south, WC – western breakaway north end, WD – western breakaway south end. Black line shows approximate position of the pit.

Samples from an additional six populations were collected, and genotyped using the procedure developed earlier. A total of 292 samples from 13 populations were analysed for population genetic structure (the degree of differentiation between populations, indicating the degree of dispersal of pollen and pores between populations), and diversity.

Population structure

Analysis of Molecular variance partitioned 96% of variation within populations, and 4% between populations, indicating weak population structure (as can be seen graphically in Figure 8, where samples belonging to the same population do not group together, but are instead completely intermixed, ie. individuals are just as closely related to individuals in other populations as they are to individuals in the same population). In contrast to the previous study, which examined half the number of populations and showed no significantly genetically distinct populations, pairwise permutation tests between populations (Table 4) show a few significant comparisons, in particular from that population on Mt Gibson Saddle, (MGSaddle respectively in Figure 7) which is statistically supported (at p< 0.001) as being genetically 'isolated' from nearly all remaining populations. In this context, 'isolated' means not mating randomly with other populations, due to a number of possible explanations. The most likely explanations are physical isolation, inbreeding in small populations, or strong selection at one or more linked loci. The population at the Mt Gibson Saddle is only moderately isolated from other populations, and geographically intermediate between populations that are genetically uniform. Neither of the populations (C and D) to be impacted by the pit on Extension Hill are supported as genetically distinct from other populations (except the Mt Gibson Saddle population as previously discussed, and also the population on the southern end of Extension hill from populations at the extreme end of the range (Mt Gibson and Mt Gibson South).

Table 4 Pairwise permutation test of population differentiation. **Bold**: significant at p < 0.001. Codes – MG - Mt Gibson, C- Extension Hill North, D – Extension Hill, E – Extension Hill South (west side), I - Extension Hill South (east side), J – Iron Hill, K – Mt Gibson (south end), MG Saddle – Saddle between Mt Gibson and Mt Gibson South, MGS – Mt Gibson South, EFN – Emu Fence North, EFS – Emu Fence south, WC – western breakaway north end, WD – western breakaway south end.

EFN	EFS	D	C	I	J	K	MG	MGS	MGSaddle	WC	WD	
0.001	0.012	0.002	0.004	0.109	0.165	0.257	0.038	0.005	**0.001**	0.007	0.050	**E**
	0.193	0.003	0.031	0.005	0.029	0.066	0.002	0.010	**0.001**	0.001	0.023	**EFN**
		0.004	0.007	0.022	0.038	0.098	0.082	0.020	**0.001**	0.010	0.079	**EFS**
			0.045	0.082	0.004	0.156	**0.001**	**0.001**	**0.001**	0.005	0.074	**D**
				0.055	0.026	0.282	0.025	0.008	**0.001**	0.044	0.239	**C**
					0.047	0.435	0.065	0.012	**0.001**	0.068	0.301	**I**
						0.435	0.422	0.374	**0.001**	0.289	0.430	**J**
							0.450	0.412	0.002	0.424	0.409	**K**
								0.138	**0.001**	0.127	0.288	**MG**
									0.001	0.023	0.085	**MGS**
										0.001	**0.001**	**MGSaddle**
											0.422	**WC**

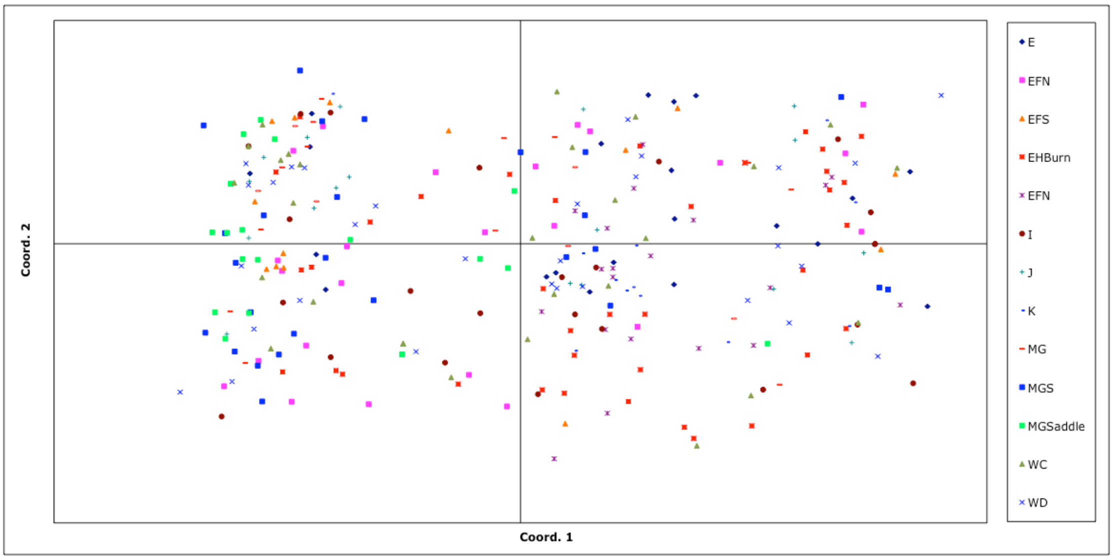

Figure 8. Principle coordinates analysis of samples from 13 populations of *Lepidosperma gibsonii*. Population codes as for the map in Figure 7. In this figure, samples placed close together are more closely related. The figure shows no strong clustering of individuals within populations; formal statistical tests demonstrated that only a few pairwise population comparisons are statistically distinct, with no geographic pattern.

Population genetic diversity and comparison with other species

Observed and expected heterozygosity, and fixation Index was estimated for all sampled *L. gibsonii* populations (Table 4). All were very similar in levels of diversity (Unbiased Heterozygosity estimates vary between 0.507-0.759). Fixation index for the species was 0.175 ± 0.026, suggesting a low but significant level of inbreeding.

In order to have a baseline comparison for the level of genetic diversity in L. gibsonii, several populations belonging to the *Lepidosperma costale* complex were sampled. A total of 88 plants from four populations of the *L. costale* complex were obtained, and confirmed as diploid in order to allow comparison of microsatellite data (tetraploids, with double the normal DNA content, are difficult to compare with diploid species, since they have four rather than 2 alleles per locus; in addition, tetraploid *Lepidosperma* have been shown to reproduce partly or wholly by agamospermy, and so show very different mating patterns to diploid populations). Several measures of heterozygosity and fixation index are reported in (Table 3). Genetic diversity within and between these populations/species is comparable with that observed in populations of *L. gibsonii*. Since sampled populations of both L. gibsonii and *L. costale* sens. lat. were sometimes quite small (e.g. populations EFN and Beanthiny Hill, where the c. 25 sampled plants represent most or a significant portion of the entire population), it is surprising that there is not greater evidence of inbreeding and population bottlenecks, suggesting either that gene flow is high over the scale of these population (quite possible given the wind-dispersed pollen), or that the current small populations are relicts of past populations, and their observed diversity is due to persistence of plants dating from a period of greater population size.

Table 5. Average genetic diversity within sampled populations of *Darwinia masonii*, *D. purpurea* and *D. sp.* Chiddarcooping.

Population	Ho	He	UHe	F
***Lepidosperma gibsonii* populations**				
E	0.547 ± 0.076	0.713 ± 0.043	0.731 ± 0.045	0.240 ± 0.079
EFN	0.590 ± 0.082	0.621 ± 0.070	0.635 ± 0.072	0.044 ± 0.072
EFS	0.535 ± 0.113	0.649 ± 0.045	0.673 ± 0.046	0.182 ± 0.163
D	0.528 ± 0.080	0.692 ± 0.038	0.701 ± 0.038	0.242 ± 0.090
C	0.541 ± 0.085	0.652 ± 0.058	0.668 ± 0.060	0.170 ± 0.095
I	0.638 ± 0.083	0.733 ± 0.033	0.749 ± 0.033	0.135 ± 0.096
J	0.583 ± 0.090	0.638 ± 0.052	0.657 ± 0.053	0.108 ± 0.097
K	0.625 ± 0.100	0.727 ± 0.039	0.759 ± 0.041	0.149 ± 0.116
MG	0.587 ± 0.090	0.643 ± 0.058	0.660 ± 0.059	0.102 ± 0.102
MGS	0.501 ± 0.085	0.642 ± 0.068	0.655 ± 0.069	0.244 ± 0.088
MGSaddle	0.407 ± 0.072	0.493 ± 0.076	0.507 ± 0.079	0.185 ± 0.034
WC	0.542 ± 0.069	0.688 ± 0.024	0.701 ± 0.025	0.212 ± 0.096
WD	0.508 ± 0.072	0.684 ± 0.037	0.696 ± 0.037	0.261 ± 0.098
Total	*0.549 ± 0.022*	*0.660 ± 0.014*	*0.676 ± 0.015*	*0.175 ± 0.026*
***Lepidosperma costale* complex**				
Blue Hills 1	0.454 ± 0.117	0.565 ± 0.098	0.576 ± 0.100	0.243 ± 0.114
Blue Hills 2	0.548 ± 0.159	0.626 ± 0.112	0.640 ± 0.114	0.088 ± 0.173
Beanthiny Hill	0.595 ± 0.107	0.699 ± 0.040	0.715 ± 0.041	0.176 ± 0.109
Wanara Rd	0.535 ± 0.134	0.609 ± 0.096	0.638 ± 0.101	0.239 ± 0.180

Clonality

Clumps of *L. gibsonii* are not necessarily a single genetic individual. The density of seedlings as reported elsewhere in this document raises the possibility of coalescence and intermingling of individuals as they clonally spread laterally by rhizomes. In order to determine the extent of clump coalescence, and the effect on census vs actual population size, a total of 213 samples were taken from 39 clumps of random sizes (excluding very small clumps clearly composed of ramets from a single individual), from 3 separate populations and genotyped using the microsatellite loci described above.

Results: A total of 49 individuals were detected amongst the 39 clumps, assuming correct assignment of individuals (which seems likely, as all ‘individuals’ recognised had at least 2 differences from other genotypes). Each clump was composed of between 1-3 individuals, with an average 1.25 individuals per clump.

Conclusions: The current population census of *Lepidosperma gibsonii* is approximately 1.25 times greater than currently estimated, assuming the observed ratio of intermingled clumps is similar in unsampled populations. Any investigations requiring complete mapping of individuals (e.g. mating studies) requires exhaustive genetic sampling within clumps to identify all individuals.

Partial clump death, after fire or drought, which has been observed in some individuals, may kill one or more genetic individuals, without complete death of the 'clump'. It is therefore likely that *Lepidosperma* death rates from fire and drought are underestimates (by 0-25%), since even a single resprouting ramet (and therefore a single individual) may be scored as survival of the clump, overlooking potential death of co-habiting individuals.

Summary and recommendations

- The increased population sampling (due to the discovery of more populations of *L. gibsonii* since the preliminary study) largely agrees with the preliminary results, although a slightly higher level of genetic population structure was observed (94% variation within populations vs 985 variation within populations).

- While there is little genetic structuring between populations of *L. gibsonii*, pairwise tests show that some populations are statistically supported as non-randomly mating with other populations. This suggests that there are some barriers to complete gene flow across the Mt Gibson range system, and that the precautionary principle should apply to avoid mixing genotypes between populations without careful consideration of consequences.

- The current population size of *L. gibsonii* is estimated to be 1.25 times greater than the current census, due to multiple genetic individuals within some clumps.

- Some measures of survival (e.g. from fire or drought) could be over-estimates by up to 25% due to unobserved loss of multiple genetic individuals from compound clumps.

- Any investigations requiring complete mapping of individuals (e.g. mating studies) requires exhaustive genetic sampling within clumps to identify all individuals.

4.1c Monitoring genetic threats

Baseline data on levels of genetic diversity within populations, relatedness among populations, the extent of inbreeding observed in populations, the processes contributing to inbreeding and the fitness costs of inbreeding was obtained and is described in sections **4.1b** and **4.3e**. These results do not indicate any level of inbreeding depression due to past bottlenecks. This data can be compared to future population (e.g. following translocation, regeneration after fire or catastrophic events) to detect departures from "normal" processes, e.g. pollinator effectiveness in reduced or translocated populations, or lowered outcrossing rate in small, isolated populations.

4.2 POPULATION DEMOGRAPHY

The demography of populations of *D. masonii* and *L. gibsonii* was studied by monitoring tagged plants in permanently marked plots. The initial tagging and measuring of plants for survey commenced in June/July 2007 and surveys were repeated in subsequent winters. Four extra plots were added in May 2009 within the boundary of a small experimental fire (12 May 2009) at the northern end of Extension Hill South. New seedlings observed in plots over the course of monitoring were mapped, tagged and measured in the same way as other plants. In each plot, plants were labelled with uniquely numbered aluminium tags and mapped to 1-5cm accuracy within an x-y system in each plot. Site factor data associated with plots was also recorded: GPS locality, estimated slope and aspect (later confirmed from GIS - DEM derived maps), estimated fire age (later confirmed from air photo analysis), landscape position (gully, ridge, slope..), mean vegetation height, community composition, estimated vegetation cover (subsequently confirmed by hemispheric photo image analysis), surface % cover of litter, rock, gravel, bare ground, soil crusts (lichen, algae, mosses). Soil samples were also collected for chemical analysis tests, performed at the WA Chem Lab, included electrical conductivity, pH, % organic Carbon, total N and 18 other major elements (of which, Mo, Cd, Se and As concentrations were at or below the limit of reporting and are therefore not presented)

Survey plots were located at six principal locations; both species were surveyed on Mt Gibson, Iron Hill and Extension Hill South, while plots for *Lepidosperma gibsonii* were additionally located at Iron Hill North and close to the Emu Fence (on duricrust; the only non-BIF locality surveyed) and three *D. masonii* plots were placed on Mt Gibson South (Figure 11).

The June/July census date was initiated to commence data collection soon after project startup and to enable 3 years of data collection within the project period, running from May 2007 to June 2010. In addition to the midwinter demography survey this program necessitated an early summer survey for seed production. However, as winter is the main growing season, a census in winter is not ideal as it means each census period, while incorporating one full summer, includes parts of two different winter growing periods. It is suggested that for future monitoring, the census date is moved to late spring. This would clarify the interpretation of the census year (to include all of one growth season not halves of two) and to reduce field survey effort (by combining seed and demography surveys). Three midwinter censuses have been completed from 2007, and the 2101 census is proposed for early summer.

Fire history

A fire history map was constructed for the region from air photo runs from 1968, 1974, 1990, 1996, 2000 and 2005. These were of varying scales, but mostly of high quality. Also utilised, were medium-low resolution satellite images from 1965-67, 1972, 1989, 2000 and 2004 and the high quality recent imagery from Google Earth (http://earth.google.com/). Other sources of fire history data used include Sentinel (MODIS hotpot data from Geosciences Australia, from 2003; http://sentinel.ga.gov.au), DEC preliminary fire mapping (1970-2005 – based on Landsat imagery), Landgate (from 1997: http://firewatch.landgate.wa.gov.au/), as well as personal observations of various workers, notes from literature and photographs. Each of these sources fails in at least one respect be either not recording all fires, accurately mapping boundaries or correctly identifying fire dates.

While these sources do not always agree, the best model of fire history since the mid 1960's (Figure 10) describes just four major fires on the Mt Gibson range and several others nearby. Scars for the two recent fires are clearly visible on images dating from 2004 and 2005, and these can be accurately dated from Sentinel to 7-10th February 2003 and from personal communications to December 2005. The two previous fires are attributed to 1972 and 1969 are visible on high resolution images up to the present, and dating back to 1972. Evidence supporting the dates of these fires include their absence from the 1968 photo, presence in 1972 and relative freshness apparent in the two fire scars in the 1972 and 1974 images (Figure 9). While it is recognised that this dating may be imprecise, variation of a year or two is relatively insignificant relative to the subsequent 40 years of growth of plants subsequently. In fact only one of these fires appears to have burnt surveyed populations of *D. masonii* or *L. gibsonii*, although the 1972 fire may have burnt populations of *L. gibsonii* to the west of the Mt Gibson range.

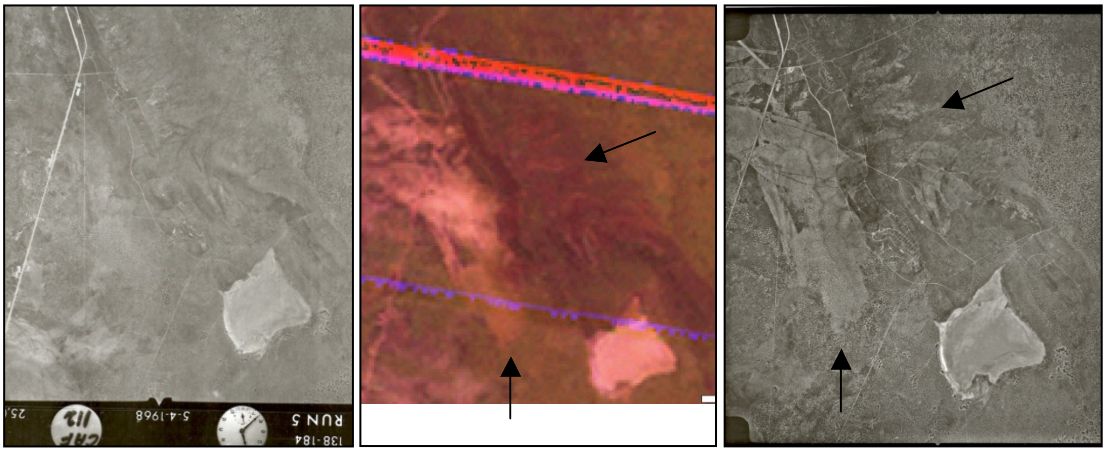

Figure 9. Air photo images from April 1968 (left) and December 1974 (right) with a LandSat image from 1972 (middle). Major identifiable features include the salt playa to the south of Mt Gibson in the bottom right of each image, the Great Northern Highway in the west, with the airstrip, built between 1968 and 1972 in the north west. No nearby fire scars are apparent on the 1962 image, but two are apparent on both the 1972 image: the '1972' fire is indicated by the lower arrow, and the '1969' fire scar by the upper arrow). The outlines of both fires are clearer in the 1974 image, with the '1972' fire most apparent.

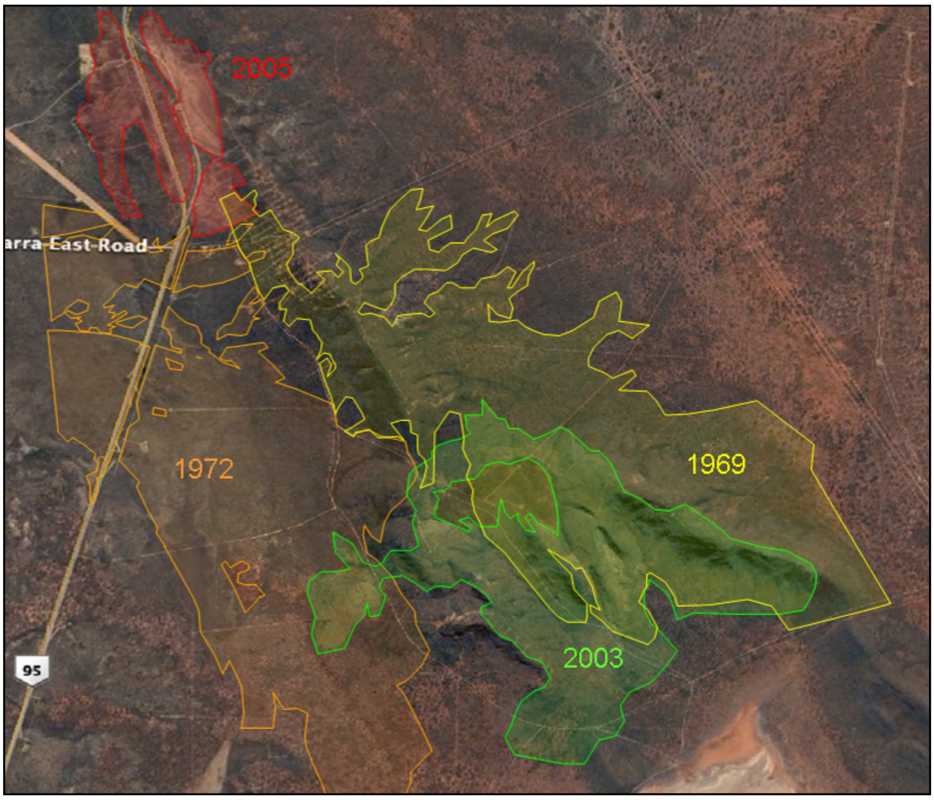

Figure 10. Mt Gibson-Extension Hill fire history; 1968-2010

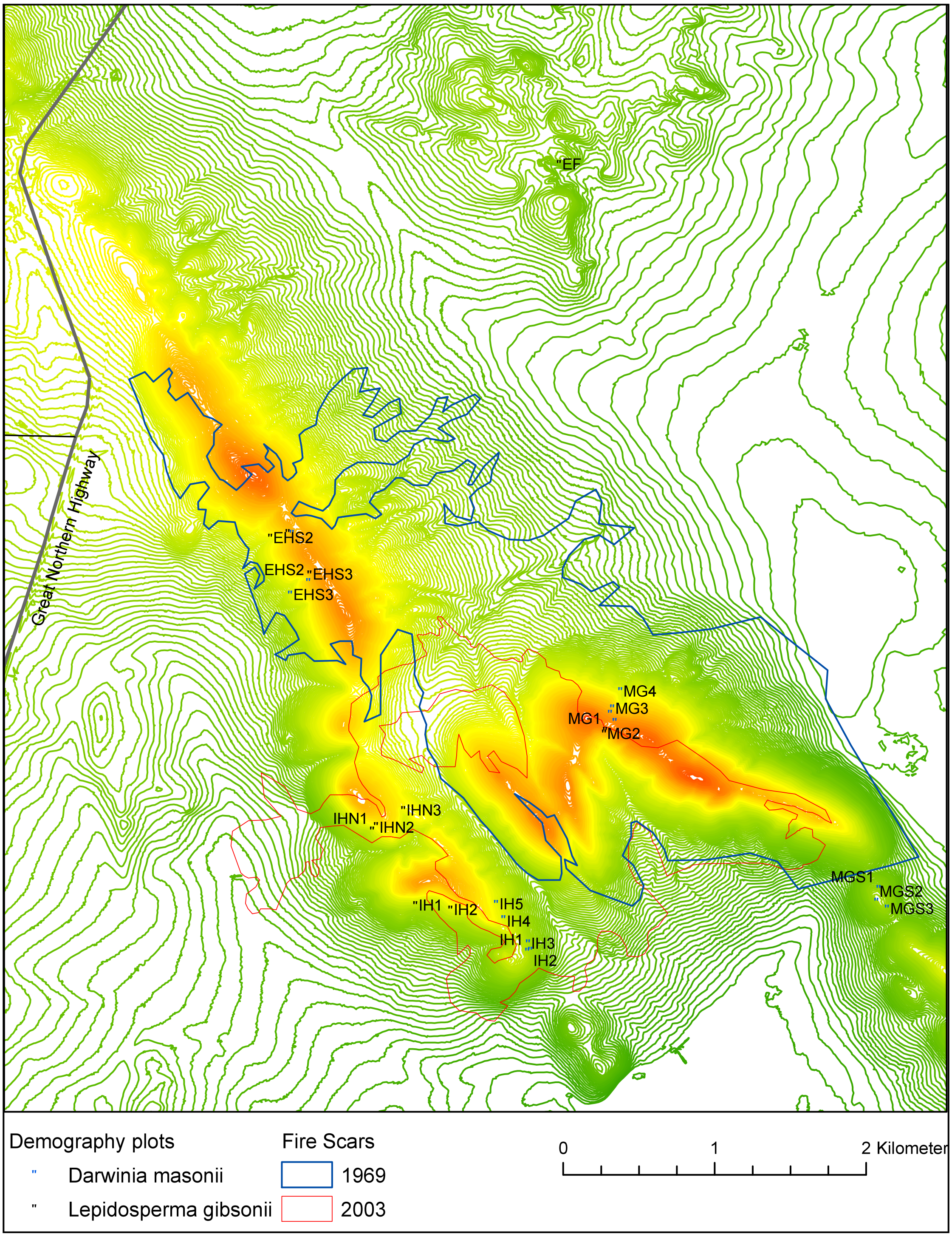

Figure 11 Location of permanent population demography monitoring plots in relation to fire history and topography (contours at 1m intervals).

Thus, fires on the Mt Gibson range fall into two periods, 'recent' (2003/2005), when the north of Extension Hill and the South West part of the range burnt, and older (1969/1972), when the central and western parts of the range burnt. As a result, most of the range was last burnt either 2 or 5 years prior to the project's start in 2007, or ~35-40 years prior, or long unburnt (parts of

Extension Hill and Iron Hill). As there is little overlap in fire scars, fire interval cannot be calculated for most of the range: the main exception is for a region between Mt Gibson and Iron Hill East which burnt in 2003 after an interval of ~ 34 years. All other areas have had fire intervals of not less than 42-35 years, but as no scars are visible on the 1968 image, and with a conservative estimate of the period for which scars are visible, this minimum previous interval estimate is more likely >50 years. Evidence from demographic studies (below) suggest a much longer period.

Some important issues in this fire history analysis must be noted. Firstly, the map focuses on the range itself and there is some uncertainty at the range's extremities, with evidence that some of the mapped fires may have extended to areas of Mt Gibson South and Extension Hill North that are not fully mapped. Some smaller areas within mapped fire boundaries are known or suspected to have not burnt. Finally, notes from various workers in the mid 1990s report inference of fires from the previous 1 – 10 years, but these inferences are not borne out in the 1996 (or subsequent) air photo series. These notes derive from observations of the state of development of vegetation, which our own experience reveals to be misleading – our initial fire age estimates were proven to underestimate fire dates by as much as 50%.Demographic results are described in terms of the fire histories as indicated in Figure 11. The fire history of the Emu Fence and Mt Gibson South plots is unknown, but presumably these sites are long unburnt. Similarly the *L. gibsonii* plots on Iron Hill are not known to have burnt since the late 1960s at the earliest (the LIH2 plot is located in a small, clearly unburnt patch embedded in the 2003 fire). These sites are described as 'old', or 'long-unburnt'.

All Extension Hill South and Mt Gibson plots occur within the 1969 fire boundary, but two of these – *D. masonii* DMG2 and DMG3 – have a population structure, including very large stem sizes, which are taken to indicate that these rocky and open sites did not burn in that fire. As well as burning in the 1969 fire, the two Mt Gibson *L. gibsonii* plots were additionally burnt in 2003. The five remaining *D. masonii* and three *L. gibsonii* sites (Iron Hill and Iron Hill North) were also all burnt in the 2003 fire and were therefore 4 years old at the time of their first survey (into their fifth growing season).

The two oldest fire ages (i.e. 1969 and <1968) are collectively described as 'older' or 'mature' sites, while the 2003 fire sites may be described as 'young' or (in the case of *D. masonii*, 'seedling') sites.

Climate history

Climate data (chiefly rainfall) exists for several weather stations in the region of the Extension Hill – Mt Gibson Range (Figure 12a). Several stations have very long records (e.g. Ninghan, to 1905), others have opened and closed at different times, and many have missing data for various periods (e.g. Ninghan in 1909, 1968, 1971 and 1972). One rainfall record operated between 1970 and 1972 at 'Mt Gibson', possibly at Iron Hill while the adit there was being worked. The closest operating rainfall records are from Ninghan Station, while Mt Gibson Station (distinct from 'Mt Gibson') records missed parts of 2009 and 2010. In 2009, BGPA purchased and installed an automated climate station near the summit of Mt Gibson close to monitored populations of *D. masonii* and *L. gibsonii*. The closest current (non-BOM) records are from the Mt Gibson gold mine camp, which indicate a mean of 240 mm in the decade from 1999. Regionally, rainfall averages between 350 mm (at Dalwallinu) and 284 mm (Paynes Find) - (Figure 12b).

Regional rainfall over the study period (Figure 13) included two years of average to above average rainfall (2008 and 2009) and two below average years (2007 and 2010). While 2010 still has some months remaining, rainfall totals to October are, for a number of nearby stations, amongst the lowest on record. By October 2010, BGPA's Mt Gibson weather station had recorded <100 mm since January, < 50% of the regional annual average. The study was preceded by four years of approximately average rainfall.

While the exact date of fire events around 1969 are not certain, it may be worth noting rainfall over the period 1967-1971 during which the fire and the post-fire population regeneration is likely to have occurred. Regional rainfall over these years was average, high, very low (1969 was the driest recorded year for 4 of the 6 then-active stations), above average, and average respectively. Thus, if fires did occur in 1969, they occurred during a period of severe drought and were followed by several years with good growing conditions.

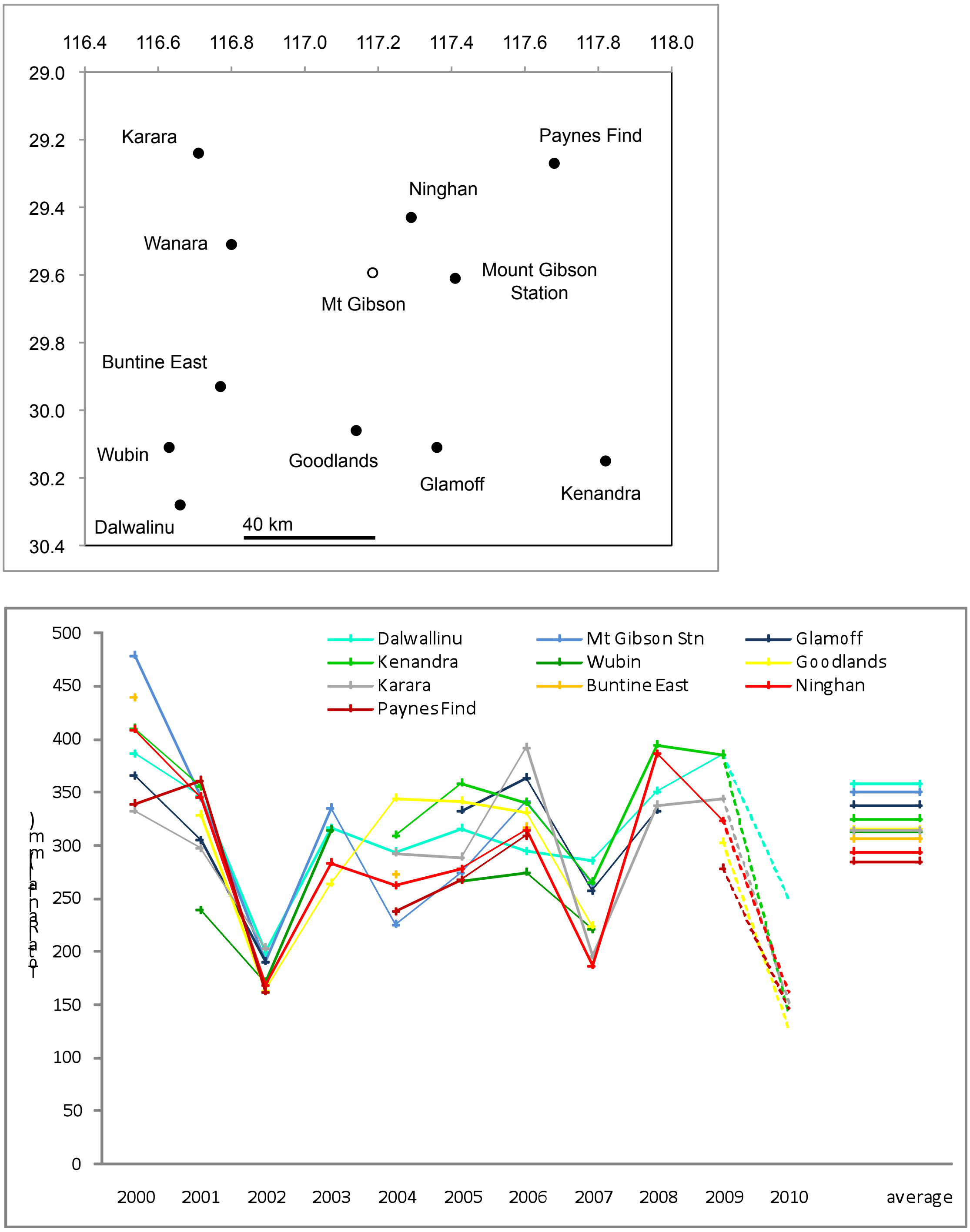

Figure 12 a) Locality of climate stations relative to BGPA's Mount Gibson climate station (open diamond). **b)** Annual rainfall for 2000 to 2009, to October 2010 (dashed) and for the long term average for nearby rainfall/climate stations (www.bom.gov.au).

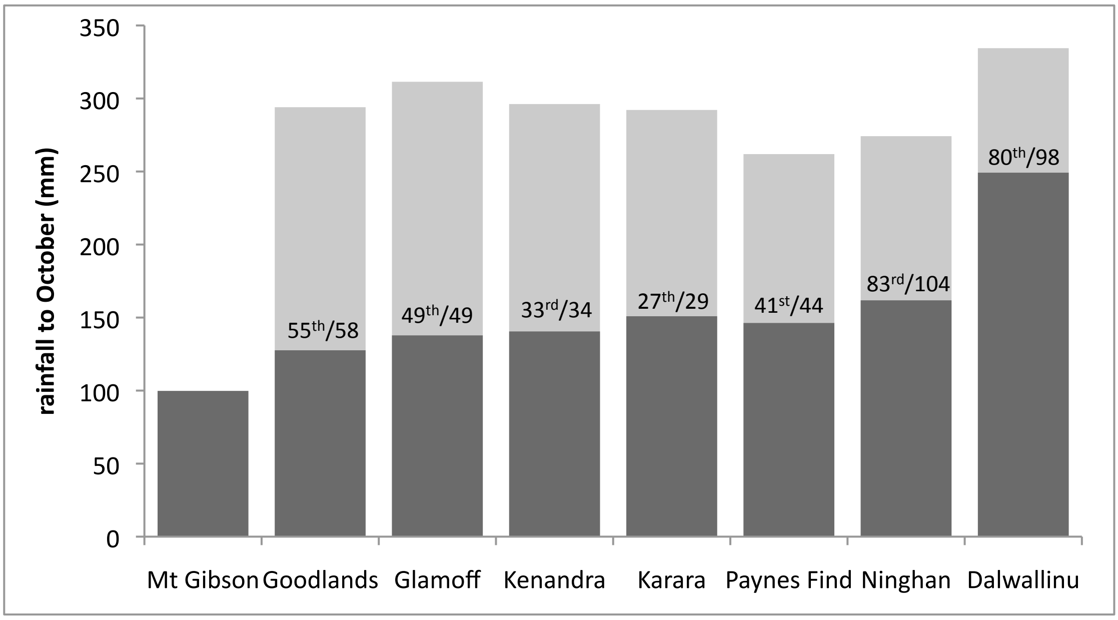

Figure 13 Cumulative rainfall to October, for 2010 (dark bars) and the long-term average (light bars) for the BGPA climate station on Mt Gibson (average not known), and for nearby stations with complete records (www.bom.gov.au). The rank of the 2010 record relative to the size of the record (number of years complete to October) also given. On average, rainfall to October accounts for 89% of annual rainfall for these stations.

Darwinia masonii

Darwinia masonii populations were monitored in 15 10×10 m plots on Extension Hill, Iron Hill, Mt Gibson and Mt Gibson South, with sites last burnt in 2003, 1969, or some time prior to 1968 (Figure 11, Table 6). In each plot, all live plants, including seedlings, were tagged, mapped and measured. Plots had between 13 and 57 plants each, and a total of 378 plants were measured. Stem density varied between 0.13 and 0.57 plants.m^{-2} and averaged 0.25 plants.m^{-2}. Plots were established in July 2007 and remeasured annually.

Table 6 Midpoints and fire histories of the *Darwinia masonii* 10×10m demographic survey plots.

Plot	Population	LAT	LONG	Last burnt
DEHS1	Extension Hill South	29°34.953	117°09.940	1969
DEHS2	Extension Hill South	29°35.124	117°09.999	1969
DEHS3	Extension Hill South	29°35.169	117°09.934	1969
DIS1	Iron Hill south	29°36.437	117°10.779	2003
DIS2	Iron Hill south	29°36.433	117°10.789	2003
DIS3	Iron Hill south	29°36.407	117°10.781	2003
DIS4	Iron Hill south	29°36.321	117°10.694	2003
DIS5	Iron Hill south	29°36.267	117°10.668	2003
DMG1	Mt Gibson	29°35.621	117°11.090	1969
DMG2	Mt Gibson	29°35.592	117°11.073	Older
DMG3	Mt Gibson	29°35.573	117°11.081	Older
DMG4	Mt Gibson	29°35.512	117°11.110	1969
DMGS1	Mt Gibson South	29°36.214	117°12.030	Older
DMGS2	Mt Gibson South	29°36.259	117°12.022	Older
DMGS3	Mt Gibson South	29°36.284	117°12.060	Older

For all tagged *D. masonii* plants in each plot height was measured as the vertical distance from the highest living tissue in the canopy to level at which the stem emerges from the ground. However, as plants were occasionally prostrate or reclining, and many grew on slopes, this measure often did not adequately describe plant size. In these cases, the equivalent ‘length’ was recorded as distance from the base of the stem on the ground, to the furthest edge of the canopy. ‘Canopy diameter’ was also measured for all plants as the longest horizontal distance across the canopy, and secondly (‘diameter 2’) as the horizontal distance across the canopy in the direction orthogonal to the first. Stem basal diameter also was measured in initial surveys for all plants. This was measured, using digital callipers, as the diameter of the stem and bark close to the plant base, but above any immediate swellings or corky areas. For non cylindrical stems, this was recorded in two horizontal dimension following the same principals as per canopy diameter measurements. Inflorescence numbers were counted, and a subjective 'health' score using a 5 point scale (Table 7) was also given for all plants. The health score was determined on the basis of relative foliage colour (noting that colour changes through the year – see **4.5e Plant Health**) and on inspection of vegetative bud activity.

Table 7 Qualitative health score for *Darwinia masonii*.

Score	Plant vigour	Canopy	Leaf colour	New growth
0) Near death	Dead or nearly	Absent or nearly	Yellow/ brown	absent
1) Very poor	Very low	Thin	Yellow / brown	absent
2) Poor	Moderate	Moderate-full	Grey - green some yellow-brown	absent
3) Fine	Good	Full	Blue green	present
4) Very good	Precocious seedling reproduction	Full	Dark green	vigorous

The slow, irregular and modular growth form of *D. masonii* means that neither plant height nor canopy diameter is ideal for measuring the 'growth' of mature plants at the year-to-year scale. In any year, branchlets at the top or furthest extent of a plant's canopy may die-back, while at the same time, strongly growing branchlets not at the canopy extremes would not contribute to an increase in measured plant size. Basal stem diameter may be a preferable measure of plant growth, but as plants (older ones particularly) have irregularly shaped stems – which may often crack, split or swell or lose or gain spongy bark – this measure also proved not ideal. However, seedling growth forms are more regular and their increase in height and diameter is also more amenable to meaningful measurement. As a result, data on plant population structures and growth rates are presented in a number of metrics.

Population structure

When surveyed in 2007, plant size ranged up to 240cm in height and 2.5m in canopy width and stem basal diameters varied from 1 to 74 mm.

Plant size varied in a clear pattern with population age (time since last fire) but stem densities did not (Table 8). The frequency distribution of plant size in plots (Figure 14, Figure 15) includes distinct peaks and narrow size ranges in the 2003 and 1969 plots and broader and flatter distributions in older sites. These patterns indicate that plants are killed in fire, and that the majority of plants arise in a single cohort following fire. Older site size distributions suggest that a small number of seedlings may recruit at infrequent intervals in the absence of fire.

Table 8. Attributes of *Darwinia masonii* populations and plants assessed in sites last burnt at in 2003, 1969, and prior to 1968 as assessed in 2007.

		Year last burnt		
		2003	1969	<1968
Sites (n)		5	5	5
Age (years at 2007)		4	38	>40
N		128	149	100
Density (stems.m^{-2})		26	30	20
Height 2007 (cm)	Min-Max	11-72	36-202	26-240
	Mean	37	110	119
Corresponding growth rate	(cm.yr^{-1})	9	2.9	
Canopy diameter 2007 (cm)	Mean	15	59	110
Corresponding growth rate	(cm.yr^{-1})	3.8	1.5	
Extrapolated age	years)			73
Stem diameter 2007 (mm)	Min-Max	1.1-9.2	2.9-37	2.1-120
	Mean	3.8	15	33
Corresponding growth rate	(mm.yr^{-1})	0.95	0.39	
Extrapolated age	(years)			85

Plants growing in sites burnt in 2003 (i.e. 4 year old seedlings), averaged 37cm in height, 15 cm in diameter and 3.8 mm in stem diameter when measured in 2007 (Table 8). These sizes represent mean growth rates of approximately 9 cm.yr^{-1} in height, 4 cm.yr^{-1} in diameter and 1 mm.yr^{-1} in stem diameter over their 4-year lifetimes. These growth rates are between 2 and 4 times greater than those observed in the next two years of survey (below), suggesting considerable year-to-year variation in growth rates.

Extrapolating the mean growth rates for canopy diameter and stem diameter from plants in sites last burnt in 1969 to the average dimensions of the older sites, suggests a mean ages of plants in these older sites of 73 and 85 years respectively. As well as assuming that the assessed mean canopy and stem diameters and their growth rates for this period are correct, representative and unvarying through time, this calculation assumes that all of the older plots were all burnt in the same previous fire, and that all plants date from the last fire. These assumptions suggest that the estimated age of these older populations 73-85 years may be underestimate their actual age. Taking the value of 85 years suggests a fire in these areas in 1922 (with 162 mm, 1922 was the 8th driest year on record at Ninghan, 1924 was almost identical).

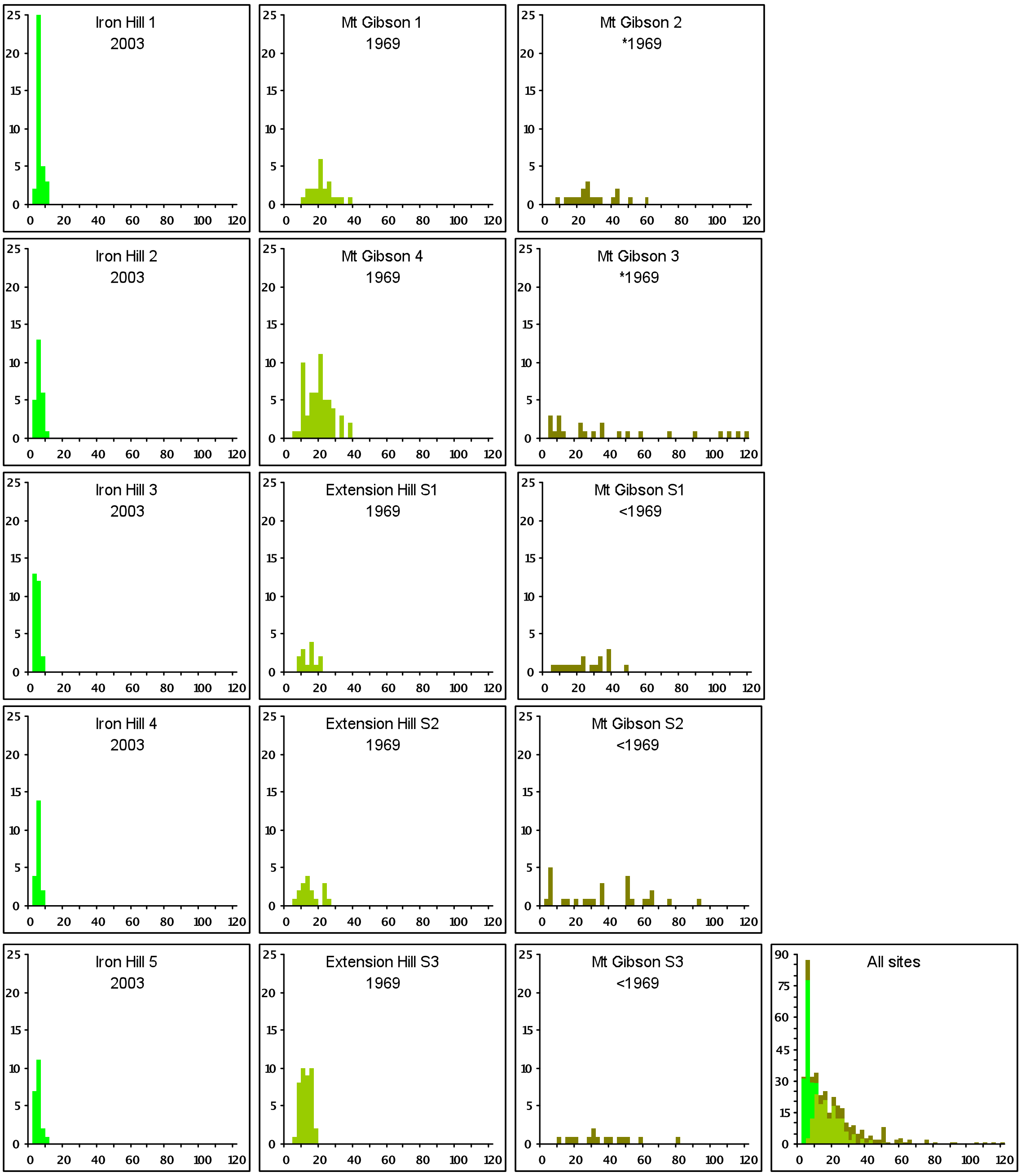

Figure 14. *Darwinia masonii* population structure: number of plants (Y-axis) classified by size (X-axis: Stem diameter close to ground level, 2.5 cm increments). Data from 2007 survey of fifteen 10×10m plots. Colour codes correspond to date of last fire (2003, 1969, <1969; *1969 = within the 1969 boundary but appears to have escaped that fire and hence is also <1969)

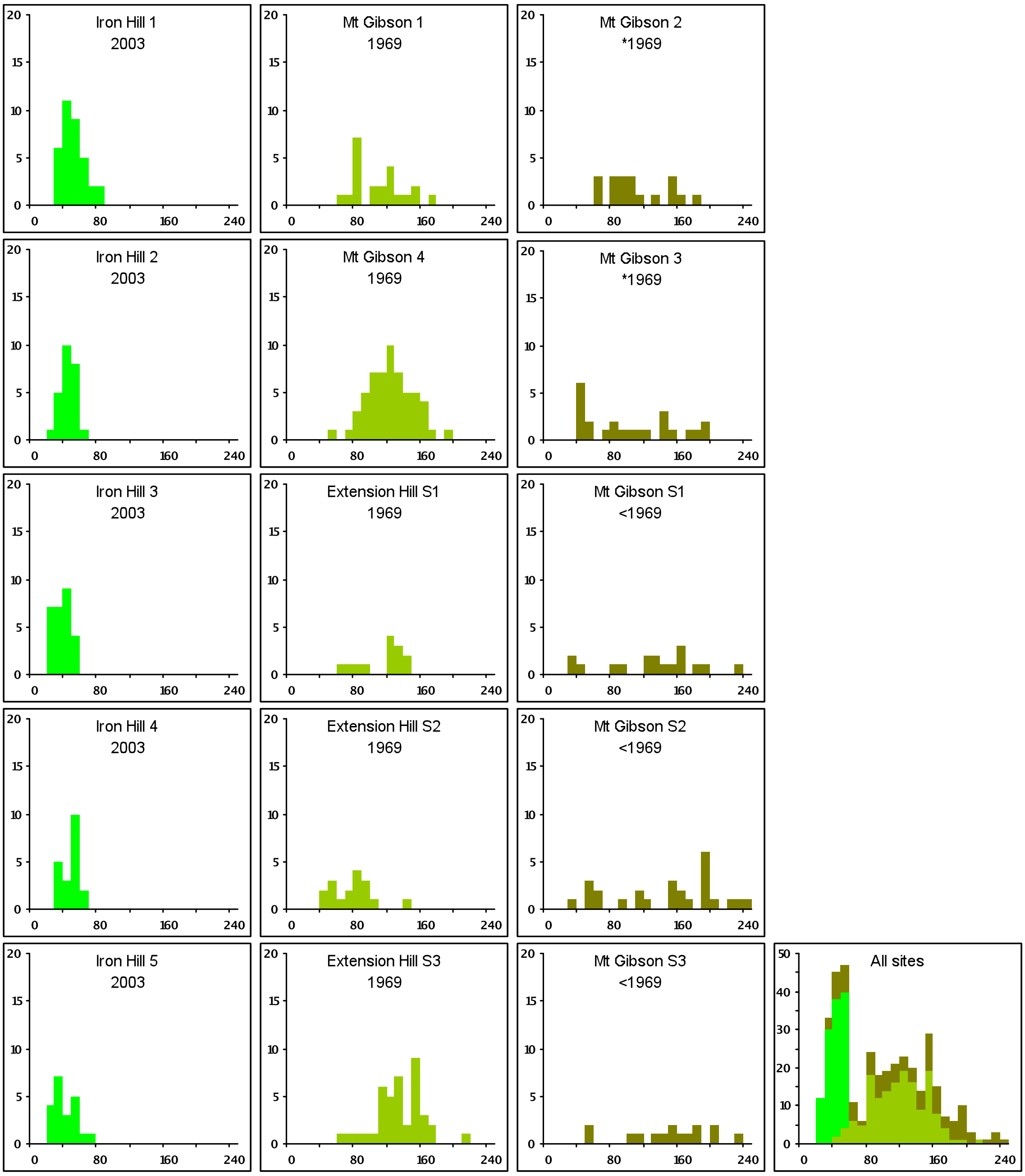

Figure 15. *Darwinia masonii* population structure: number of *Darwinia masonii* plants (Y-axis) classified by size (X-axis: plant height in 10 cm increments). Data from 2007 survey of fifteen 10 × 10m plots. Colour codes correspond to date of last fire (2003, 1969, <1969; *1969 = within the 1969 boundary but appears to have escaped that fire and hence is also <1969)

Recruitment

Just one new seedling was found in the 15 unburnt survey plots over the course of the study, this in July 2009 at the Mt Gibson South plot 2, a long unburnt site. The seedling was 9 cm tall, suggesting that it may have germinated in the previous winter (the preceding survey was July 2008). For what it is worth, this represents a mean interfire recruitment rate of 0.0023 new seedlings per adult per year in older plots.

In contrast, 233 seedlings were counted in the four plots (total area = 250 m^2) surveyed following the May 2009 experimental fire. This indicates a mean density of *D. masonii* seedlings of 0.9 m^{-2}. There were 93 pre-fire adults in the same area – equivalent to 0.37 m^{-2} and 1.2-1.9 times the density of adults in the unburnt survey plots. This would represent an average seedling production rate of 2.5 seedlings per pre-fire adult. However, the experimental fire was patchy and did not burn the entirety of the four plots, such that 20 (22%) of the 93 pre-fire adults did not burn. If we use this same proportion to represent the area of plots that did not burn, and assume that seedlings only germinated in burnt areas, it may be more appropriate to record a seedling density of 1.2 seedlings.m^{-2} of burnt area and 3.2 seedlings per adult.

Survival / Mortality

Of the 277 seedlings tagged at the end of the winter following the May 2009 experimental fire, just 9% were refound and alive one year later in October 2010. In addition, 79% were refound and dead, and 11% could not be found. Thus mortality rates of *D. masonii* seedlings over their 1st summer was 88-91%. This low survival rate may partly result from the drought experienced over the 2010 winter at Mt Gibson, as well as a likely high failure rate of establishing young seedlings.

Three of the 373 *D. masonii* plants tagged in demography plots in July 2007 died over the following 12 months: all were seedlings, and two were from one plot (Iron Hill South 5: DIHS5). A further 14 seedlings from this plot died over the following 12 months as did two smaller individuals (53 and 62 cm tall) from long unburnt populations on Mt Gibson and Mt Gibson South. The 5 remaining DIHS5 seedlings were still alive in November 2009, but a further 16 seedlings from other 2003-fire area plots had died. These deaths indicate a mean seedling mortality rate of 9.8% per year over the study period (Table 9), but also significant spatial and temporal variation in seedling survival (Figure 16).

Table 9 Number and proportion of tagged *Darwinia masonii* plants dying in each year 2007-2010; comparing seedlings from 2003 fire and older plots.

	2003 seedlings		1969 + older	
	N	%	N	%
2007-2008	3	2.5	0	
2008-2009	14	11.7	2	1.6
2009-2010	16	15.2	0	
average	33	9.8	2	0.5

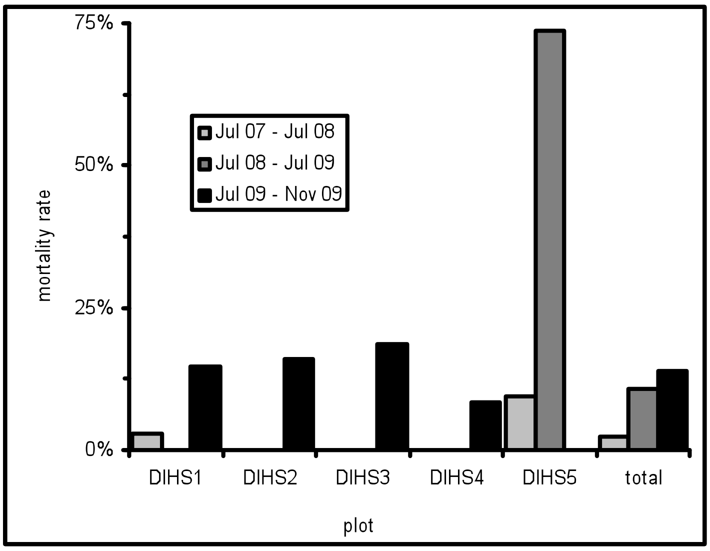

Figure 16 Proportion of *D. masonii* plants dying in five Iron Hill South seedling plots (burnt Feb 2003) at three sample times (the last not representing a full year).

The 16 deaths in DIHS5 represent a major population collapse for this locality over the two years to July 2009: in one year, 74% of remaining plants died. Skeletons of a further 21 seedlings were counted at the establishment of the plot, suggesting that poor survival is endemic at the site. It is unclear what the cause of this death is at this and other 2003 plots. The DIHS5 plot is the hottest of the five seedling plots in terms of solar radiation receipt (with an aspect of 65° versus 73-86°) and among the steepest (10° versus 8-10°). It is also mapped as a distinct geology: undifferentiated "white rock" versus cavernous Limonite and Breccia "(only hematite debris cemented by limonitic material)", although only a short distance down-slope from the mapped contact with these. These differences may well suggest important differences in soil water holding capacity and micro-climate. But the fact that *D. masonii* seedlings occur there at all suggests that a number of adult individuals must have survived and reproduced on the site or very nearby prior to the fire.

Whether the increase in mortality of seedlings in the remaining plots through spring of 2009 is an indication of the commencement of a similar period of mortality in the other sites remains to be seen.

With just two recorded deaths in the older plots, the mortality rate in these areas between 2007 and 2009 averaged 0.5% per year (Table 9). Tagged plants are yet to be resurveyed in 2010 at time of writing, but a sample of 261 plants adjoining plot DMGS1 on Mt Gibson South (last burnt <1969) and marked in the pollination study were re-surveyed. These plants had a population-wide mortality rate of 10.3%, much higher than that observed in previous years in survey plots. This mortality also seems likely attributable to drought, but is of concern both for its magnitude with a significant impact on population sizes, and if projected climate change does lead to increased drying, or increased frequency of dry years in the region.

Health scores

Most (75-99%) of the plants in older sites had good health scores (i.e. scores of 3 or 4, Figure 17). Just one and two individuals had low health scores (0 or 1) in 2007 and 2008 respectively (i.e. ≤ 1%). Each of these three plants had recovered to a score of 3 by 2009. The proportion of plants with a poor health score (2) varied from 0-25%. The only two plants from these older sites that died in the survey period (both by July 2009) had scores of 2 and 3 in 2008, and 3 in 2007.

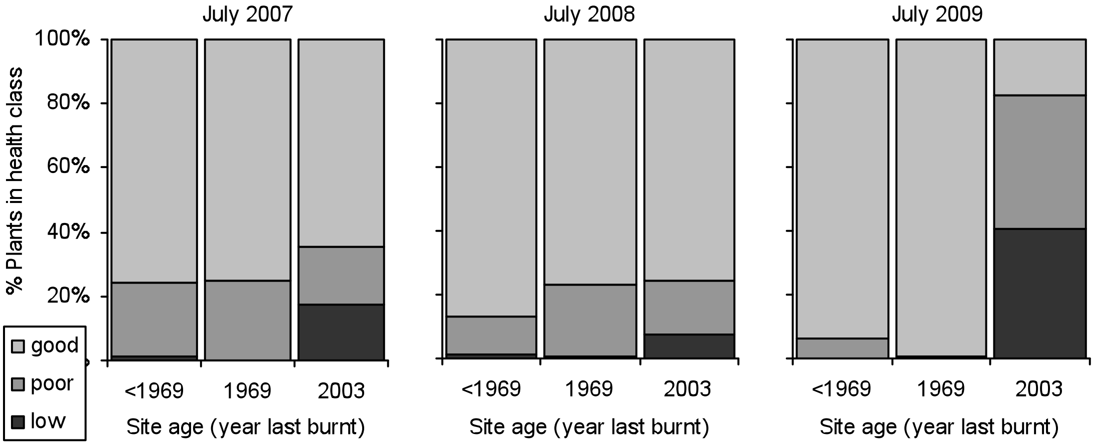

Figure 17 Variation in health scores for tagged Darwinia masonii plants varying between years and site age.

Examining patterns of annual change in health (Table 10) indicates greater fluctuation of apparent health among seedlings than in adults. Three quarters of plants in older plots did not change health score (most were 3), 20% improved in their health score and 4% declined, and very few moved more than one score class. In contrast, seedlings maintaining a constant health score across years were in the minority (18 - 51%). 82% of seedlings declined in health (26% by two or more scores) or died in 2007-2008, while 25% improved and 24% declined or died in the following year.

Averaging across years, 0.3% of seedlings with good health scores (3 or 4) died within the next 12 months, 20% of those with poor health (2) died and, 11% of individual seedlings with very poor or worse scores (1 or 0) were dead within 12 months. The proportion of live seedlings with low health scores has fluctuated between years and was highest in July 2009, suggesting that greater mortality of seedlings may be expected subsequently.

Table 10 Year to year variation in health status of *Darwinia masonii*

Site age	older			2003		
Period	2007-2008	2008-2009	2007-2009	2007-2008	2008-2009	2007-2009
improved	19%	14%	21%		25%	6%
>1 score	1%		0.4%		3%	
1 score	18%	14%	20%		21%	6%
no change	76%	75%	75%	18%	51%	19%
declined	4%	11%	4%	67%	22%	61%
1 score	3%	11%	3%	40%	22%	31%
> 1 score	0.5%	0.4%	0.4%	26%		31%
died	1%		1%	15%	2%	14%
Total	220	231	236	121	122	121

Live plants given a health score of 0 (near death) in 2007 mostly did eventually die, although all survived for more than one year before doing so (Figure 18). All but one of the 19 plants with a very poor health score (1) in 2007 were seedlings: four were dead by July 2008 and eight more (all seedlings) died by November 2009. Some of these plants regained health to a good standard within a year, but even so, some of these later died, and none had scores > 2 (poor) at last survey.

Figure 18. Two plants from plot DIHS4 photographed in July 2009. Both had experienced complete leaf loss and had health scores of 0, or 1 in the preceding two years, indicating that they had persisted with no canopy and only the leaf buds like those visible here on the stem for at least a few years. Their continued survival seems unlikely.

Growth

The growth of older plants appears to be slow relative to measurement errors and diffuse across their canopies – not necessarily resulting in an increase in maximum height – and confounded by both a pattern of shoot die-back and the datum problems described previously. Significant shoot die-back was recorded in 11% of measured *D. masonii* individuals. This occurred when the uppermost branchlet or shoot died such that subsequent measurement, from the ground to the highest growing point were lower than previous measurements, and resulted in reduced, or negative height growth. No specific factor appeared to be responsible for this process, other than the normal process of branchlet longevity interacting with overall poor plant vigour. The health score of plants that died back averaged 1.5, while the average score for the other plants in the plots where die-back was observed was 2.6.

Across all sites and sample years, growth averaged 0.6 cm.yr^{-1} among all plants (Table 11), but there was considerable annual and site-based variation, as well as between plants that died back or and those that did not. At 3.4 cm.yr^{-1}, mean seedling growth across both years was 6 times higher than the average for all plants. Seedlings which died-back grew at less than half this rate. In 2007-2008, when Iron Hill seedlings were five years old, their growth averaged 4.1 cm.yr-1, but this declined to 2.7 cm.yr^{-1} in the following year. Height growth of plants in older sites followed a similar pattern, being lower in 2008-09 than 2007-08, and lower in plants with stem die-back. Overall, height growth of older plants was slightly negative (-0.8 and -1.0 cm.yr^{-1}). Only 2007-08 growth in the oldest (burnt <1969) sites had a positive mean value (2 cm.yr^{-1}).

Table 11 Plant height growth rate (cm.yr-1; with ± SD and n) for tagged *Darwinia masonii* plants with varying time periods, time since fire and whether canopy die-back was recorded or not.

Year last burnt	2003	1969	<1968	Total older
2007-2008	4.1 ± 5.5 (121)	-0.1 ± 15.0 (129)	2.0 ± 6.6 (85)	0.6 ± 12.1 (228)
2008-2009	2.7 ± 9.5 (121)	-3.1 ± 13.8 (128)	-3.6 ± 7.7 (85)	-3.3 ± 11.7 (213)
mean 2007-2009	3.4 ± 5.0 (121)	-1.0 ± 4.1 (130)	-0.8 ± 2.8 (85)	-0.9 ± 3.7 (235)
Died back 07-09	1.5 ± 3.9 (21)	-2.5 ± 5.2 (12)	-4.1 ± 4.2 (4)	-2.9 ± 4.9 (16)
No d.b 07-09	3.8 ± 5.1 (100)	-0.8 ± 4.0 (118)	-0.7 ± 2.6 (81)	-0.8 ± 3.5 (219)

The difference between positive height growth in seedlings and neutral or negative height growth in mature plants reflects the details of their growth form. Seedling growth focuses on the extension of a single erect shoot, whose growth direction, and growing tip persists from year to year. This results in strong and sustained vertical growth – until plants reach maturity and flower, or the growing tip becomes damaged or dies. As flowering is terminal (occurs at the end of growing shoots) in *D. masonii,* reproduction means that shoot axes terminate and new growth develops laterally following reproduction. In older plants, new growth occurs via many dispersed branchlets growing in many different directions. This growth form does not encourage vertical growth, in fact, as branchlets die when they flower, and re-shoot laterally the canopy surface has a dynamism which may result in a fluctuating canopy height, with perhaps more net height growth in years of better growth conditions.

The pattern of variation in height with canopy width in *D. masonii* (Figure 7) shows increasing variation in canopy size with plant height once plants exceed 50-70cm in height, indicating the slower or more irregular nature of vertical growth in mature plants, as well as the propensity for older individuals to recline. That seedling growth was higher in 2007-08 than 2008-09 in seedlings and adults suggest better growth conditions in the earlier season.

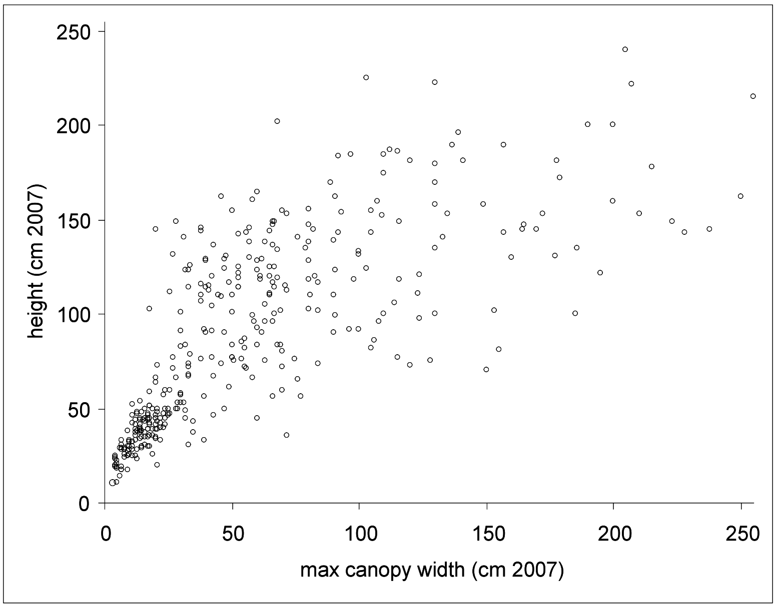

Figure 7 Maximum canopy diameter varying with plant height in tagged *Darwinia masonii* individuals (2007).

The 12 May 2009 experimental fire enabled assessment of seedling growth at their initial stages. The first rains after the fire occurred May 21-22 (with 22 mm recorded at the Mt Gibson Oroya camp), and seedling germination can most conveniently be dated from this time. Seedlings were measured after 4 months, 6 months and 17 months. In late September 2009, four months after the fire, seedlings averaged 2.8 cm in height, by November they averaged 3.3

cm. Assuming negligible growth over the summer period, this provides an average growth rate of 7.2 cm.yr^{-1}. By October 2010, surviving seedlings averaged 5.9 cm, suggesting a much lower 2nd year growth rate of 2.7 cm.yr^{-1}. As rainfall in the winter of 2010 was well below average, this low growth rate may reflect a water limitation of growth, as much as an inherently slow 2nd year growth.

The mean measured rate of growth of tagged plants is considerably less than the mean rate of growth derived from their population structure and known fire ages (Table 8). The population structure data suggest a mean height growth rate of 9 cm.yr^{-1} for seedlings up to 4 years old, and 2.9 cm.yr^{-1} for plants up to 38 years. Our results indicate annual variation in growth rates, suggesting that the sample size (in terms of numbers of years) is not sufficient to accurately assess mean annual growth rates for this species. The estimate of growth rate to 38 years includes a period of growth as a mature plant, but also a juvenile period of higher growth. As a result, 2.9 cm.yr^{-1} must be an overestimate of annual growth of mature plants. The length of the juvenile period is as yet unknown (but see *Fecundity* below).

Stem diameters were recorded for all plants in 2007 surveys, providing useful data on population structures, and then again for all plants in the 2003 fire areas and selected other individuals elsewhere in subsequent surveys. At the second survey, it became clear that slow growth rates, together with complications associated with remeasuring stems for older plants – due to bark swelling and sloughing, low branches and irregular cross-section shape – meant that the accuracy of stem diameter measurements was not sufficient for assessing individual growth rates on an annual basis. While 94 (of 247) of older plants were remeasured in 2008, remeasurement accuracy was such that 25% of these were not deemed adequate for comparison. Considerable variation in measured diameter growth rate was observed in the remaining 69 individuals (Table 12), and it is unclear to what extent measurement errors are responsible. The reported rate of 1.4 ± 2.6 mm.yr^{-1} should be treated with some caution.

Table 12 Plant stem diameter growth rate (mm.yr-1; with ± SD and n) for tagged *Darwinia masonii* plants with varying time periods and time since fire.

Year last burnt	1969	2003
2007-2008	1.38 ± 2.57 (69)	0.14 ± 0.55 (87)
2008-2009		0.55 ± 0.58 (86)
2007-2009		0.34 ± 0.43 (112)

Seedling stems have few of the problems listed for mature plants, and the measured mean rates and variation measured are more likely to reflect true growth patterns. Stem basal diameter growth of 4-6 year old seedlings averaged 0.34 mm.yr^{-1} over the study, and was four times higher in 2008-09 than in the previous year (Table 12). Mean measured stem growth in these seedlings in their 6 and 7th years is about two thirds less than their mean growth in the preceding 5 years – as suggested by their mean diameters in 2007 (Table 8). The stem diameter of seedlings 17 months after the experimental fire averaged 0.84 mm, indicating a growth rate of 0.58 mm.yr^{-1}.

Fecundity

No tagged seedlings growing from the 2003 fire flowered in 2007 or 2008, but 6 of 115 (5%) surviving plants flowered in 2009. With heights of 51 to 95 cm these plants were all above average for seedlings of this age (45 cm), but made up just 13% of plants on this size range. It seems reasonable to suggest that 6 years is therefore the minimum age of reproduction for *D. masonii*, but to note that the proportion of plants flowering increases with plant size. The proportion of flowering plants also varies considerably between years, with 62% of all plants in older sites flowering in 2007, 36% in 2008 and 90% in 2009. In 2009, 93% of plants over 1m tall flowered.

The mean number of inflorescences per flowering plant also varied between years: averaging 29 in 2007, 5.6 in 2008 and 33 in 2009 (the 6 flowering seedlings averaged 2.3 inflorescences; Figure 19). This pattern reflects less flowering among smaller than larger plants and variation in the number of inflorescences produced by plants of different sizes (Figure 20).

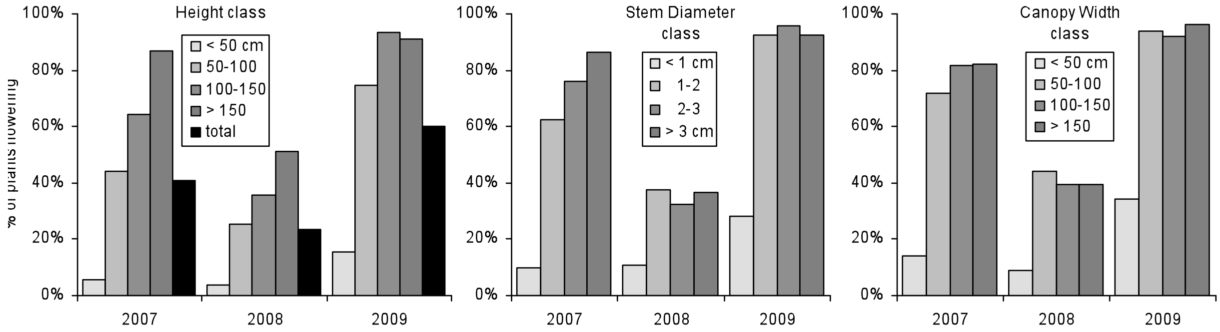

Figure 19 Proportion of *Darwinia masonii* plants flowering / fruiting varying by size class and year: Stem height (cm), Stem diameter (mm) and Canopy width (cm) as measured in 2007.

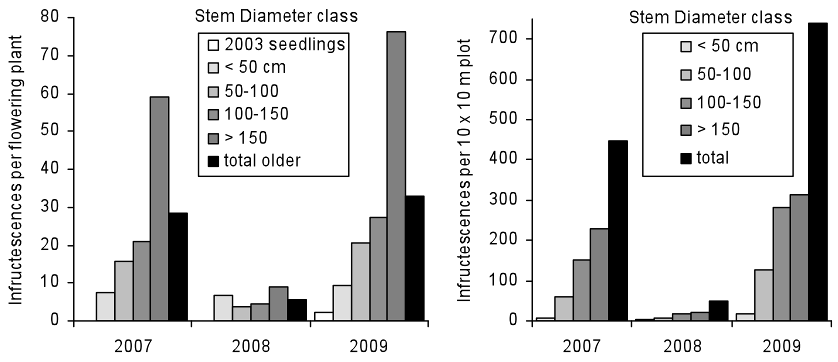

Figure 20. Inflorescence production per flowering plant (left) and per 10×10 m plot (right) varying by year and size class (canopy diameter) in older sites, as well as for seedlings from the 2003 fire.

The outcome of fewer flowering plants and fewer inflorescences per flowering plants is of course many fewer inflorescences per plot. In 2008, plants in the 10 older sites produced an average of 50 inflorescences per plot, but in 2007 and 2009 inflorescence production was 9 to 15 times higher (446 and 739 inflorescences per plot respectively; Table 13)

Table 13 Mean number of *Darwinia masonii* inflorescences per flowering plant and per 10×10 m plot varying between years and height class.

	Height	per flowering plant			per plot		
site age	class(cm)	2007	2008	2009	2007	2008	2009
1969 + Older	< 50	7.6	6.8	9.4	6.1	3.4	17.0
	50 - 100	15.6	3.9	20.7	59.2	8.6	128.5
	100 - 150	21.1	4.5	27.3	149.8	17.6	280.8
	> 150	59.1	8.9	76.2	230.4	20.4	312.5
	Total	28.6	5.6	33.0	445.5	50.0	738.8
2003	all	-	-	2.3	0	0	2.8

Summary – *Darwinia masonii*:

- The population structure of *D. masonii* indicates that most individuals recruit in a single cohort post-fire, with minimal inter-fire recruitment until populations age to (perhaps substantially) > 40 years. The oldest populations were more evenly structured, suggestive of infrequent inter-fire recruitment.

- Evidence from population structure suggests a mean stem diameter growth rate of 0.4 mm per year and a height growth rate of 2.9 cm.yr-1. Extrapolating these rates suggests that the oldest populations studied ('<1968') may have last burnt early in the 20th century, this estimation is crude, but suggests a fire around 1922.

- The survey of tagged plants confirms that interfire seedling recruitment is rare: just one new recruit was observed in the 15 survey plots over the 3 year period of the study, and that in an older site.

- Post-fire seedling recruitment is high, with as many as 3.2 seedlings produced per pre-fire adult. But mortality of these seedlings over the 1st summer following experimental fire was high, with 89-91% dying.

- Mortality among 4-6 year old seedlings was high, with 2.5-15% of seedlings dying each year.

- Death among 4-6 year old seedlings was highest among plants with low health scores, while the older plants that died were previously scored as healthy. Health scores varied considerably among seedlings, but little among older plants.

- Averaging 0.5% per year, mortality appears rare among plants in older sites in the absence of fire or extreme drought. The few deaths observed among plants in older sites were small plants, indicating an even lower mortality rate among older plants.

- Drought over 2010 appears to have contributed to a significant level of mortality of adult plants 10% in the Mt Gibson south population.

- Measured growth rates varied between years and younger and older sites. Negative height growth recorded for plants in older sites, may reflect poor growth conditions in measured years, but also difficulties in assessing plant size.

- Mean height growth averaged 7.2 and 2.1 $cm.yr^{-1}$ for 1 and 2 year old seedlings, 3.4 $cm.yr^{-1}$ for 4-6 year old seedlings. Mean height growth for older plants was close to zero or negative.

- Reproduction commences in seedlings as young as six years, but increases in terms of proportion of plants flowering, and flowers per plant as plant size increases.

- Total inflorescence production varied between years by more than an order of magnitude.

Lepidosperma gibsonii

Eleven 5×5 m plots were established for demographic monitoring of *Lepidosperma gibsonii* (Figure 11, Table 14), within which live plants, including seedlings, were tagged with uniquely numbered aluminium tags. All clumps found were tagged and measured in most plots, in plots where seedling density was very high, all plants were counted, but a large subsample of seedlings were tagged and measured. Plots had between 13 and 311 plants each, and a total of 549 plants were measured and 862 enumerated. Five plots contained seedlings, and 36% of tagged plants were deemed to be seedlings at the start of the survey. All of the plots with seedlings had been burnt in the February 2003 Mt Gibson-Iron Hill fire.

Table 14 Localities (midpoints) and fire ages of *Lepidosperma gibsonii* 5×5m demographic survey plots.

Plot	Population	LAT	LONG	Fire history
LEHS1	Extension Hill South	29°34.950	117°09.925	1969
LEHS2	Extension Hill South	29°34.967	117°09.863	1969
LEHS3	Extension Hill South	29°35.083	117°10.000	1969
LIH1	Iron Hill	29°36.272	117°10.380	Older
LIH2	Iron Hill	29°36.287	117°10.505	Older
LIHN1	Iron Hill North	29°36.007	117°10.226	2003
LIHN2	Iron Hill North	29°35.991	117°10.240	2003
LIHN3	Iron Hill North	29°35.934	117°10.337	2003
LMTG1	Mt Gibson	29°35.652	117°11.056	1969, 2003
LMTG2	Mt Gibson	29°35.662	117°11.052	1969, 2003
LEF1	Emu fence N	29°33.640	117°10.883	Older

For each tagged *L. gibsonii* individual, we measured ‘clump diameter’ using digital callipers as the distance across the collected live leaf and culm bases – at 0-1cm from ground level. For each clump this was measured for both the longest and perpendicular dimensions. Inflorescence counts were made for all plants, and health was assessed using a 3-point subjective ‘health’ scale (Table 15). This was largely determined on the basis of relative foliage colour (noting that colour changes through the year – see below), growth and reproductive activity.

Table 15 Qualitative health score for *Lepidosperma gibsonii.*

Score	Plant vigour	New growth	Reproduction	Leaf colour
0)	Dead or nearly	Absent	Little or none	Yellow-grey
1)	Poor	Little	Few inflorescences	Green-yellow

2)	Good	Vigorous	Many inflorescences	Green

Population structure

Population structure – the distribution of individuals across size classes – provides information on population processes and recruitment dynamics. The surveyed populations of *Lepidosperma gibsonii* varied considerably in their structure, with a number of distinct patterns distinguishable (Figure 21). Three of the five sites in areas burnt in 2003 showed a clear pulse of seedling recruitment following that wire, with the majority of individuals being seedlings. Seedlings were also found in the two remaining burnt plots, but not in abundance. No plant in any recently burnt area exceeded 20cm in diameter (Table 16). Two plots on Extension Hill South had a strong peak in plant sizes around 10cm and 14 cms respectively and both were probably last burnt in the 1969 fire, again few plants in these sites exceeded 20cm in diameter.

The remaining sites were last burnt at some time prior to 1968 and had fewer individuals but these did include all of the largest plants in the study. Two of these sites have a flat size distribution – indicating either a long period in which plants grew at varying rates or during which occasional recruitment contributed individuals at different times which now represent of a variety of ages. The smallest plants in these sites were 4-5cm and the largest 40-48 cm across at the base. The last site – Iron Hill N1 – had the lowest density of individuals and is therefore harder to interpret, however all individuals at this site were >9 cm and the largest was 32 cm.

Table 16 *Lepidosperma gibsonii* mean plant size in relation to fire history

plot	code	fire history	mean base diam (cm)	no of plants	Seedlings	Mature	
						<20cm	>20cm
Emu Fence	EF	<?1969	26	14	none	few	few
Iron Hill 1	IH1	<?1969	15	15	none	few	few
Iron Hill 2	IH2	<?1969	18	23	none	few	few
Extension Hill South 1	EHS1	1969	7	66	none	many	few
Extension Hill South 2	EHS2	1969	10	19	none	some	none
Extension Hill South 3	EHS3	1969	8	77	none	many	few
Iron Hill North 1	IHN1	2003	11	13	few	few	none
Iron Hill North 2	IHN2	2003	6	15	few	few	none
Iron Hill North 3	IHN3	2003	2	67	many	few	none
Mt Gibson 1	MG1	1969, 2003	2	45	many	some	none
Mt Gibson 2	MG2	1969, 2003	2	127	many	some	none

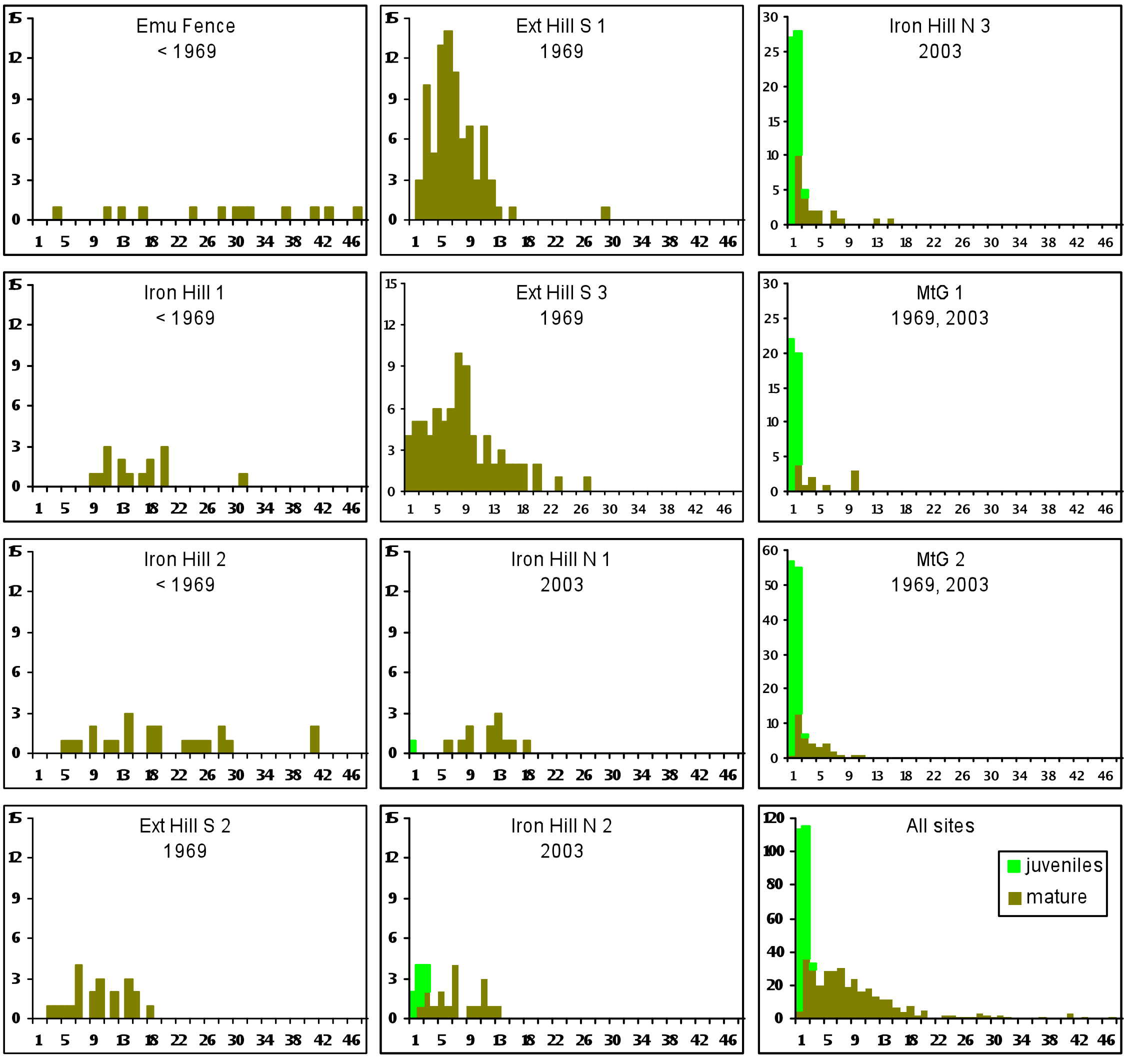

Figure 21. Population structure of surveyed Lepidosperma gibsonii plots. Number of Lepidosperma gibsonii plants (Y-axis) classified by size (X-axis: maximal clump diameter at ground level, 1cm increments). Data from 2007 survey of eleven 5×5m plots. Plots with seedlings were all burnt in a 2003 wildfire, other sites unburnt since prior to 1970 – year given.

These results indicate an important role for fire in determining the population structure and population dynamics of *L. gibsonii*. Firstly, the presence of both adults and seedlings in the most recently burnt sites indicates that at least some mature plants can survive wildfires but also that fires stimulate a large number of soil-stored seeds to germinate. The absence of seedlings in other sites suggests that fire is actually required for seedling recruitment, however the flat population structures of the oldest sites suggest that inter-fire recruitment might also occur. The high population densities, peaked population structures and smaller mean plant sizes in areas burnt only in 1969 suggest strong post-fire recruitment with few individuals surviving from the previous fire period. If that is so, with a mean plant size of 7-10cm and population age of 38 years would suggest a mean growth rate of ~ 2 mm per year. Seedlings deriving from the 2003 fire averaged 1.02 cm diameters and therefore a comparable 2.5 mm base diameter expansion per year. If these mean growth rates are consistent between sites, then the emu fence population could be 100 years old.

Recruitment

No *Lepidosperma gibsonii* seedlings were observed in study plots outside of areas burnt in 2003 or 2009. It is assumed that effectively all seedling recruitment occurs following fire. Seedling recruitment in areas burnt in 2003 was spatially very variable, with counts in 5 × 5 m plots burnt in 2003 of 1 to 261 seedlings (Table 17). This variation was equivalent to around 0.1 – 5.2 seedlings per pre-fire adult and post fire seedling densities from 0.04 to 10.4 per m^2. The extremes all occurred among Iron Hill North sites: two, both facing SW and respectively at the top and bottom of one shallow gully had very low recruitment rates, while the third site with high recruitment had a similar aspect but was in a protected site on the side of a deeper canyon. If mortality among pre-fire individuals (determined from counts of burnt clumps still visible as blackened leaf bases in 2007) is an indicator of fire intensity, the two sites with the lowest post-fire recruitment had both the highest and the lowest fire intensities.

Table 17 *Lepidosperma gibsonii* fire survival and recruitment data (2003 wildfire).

Plot	IHN1	IHN2	IHN3	MG1	MG2	all
pre-fire density (/m2)	0.5	2.8	2.0	0.4	2.0	1.5
% killed in fire	0	74	18	0	24	38
seedlings per pre-fire adult	0.1	0.1	5.2	3.5	2.0	2.1
seedling density (/m2)	0.04	0.3	10.4	1.5	4.0	3.2
Pop. growth (2003 - 2007)	1.1	0.4	6.0	4.5	2.7	2.7

In the spring following the May 2009 experimental fire, 229 seedlings were counted in three marked plots (Table 18). No new *L. gibsonii* seedlings were found in the fourth plot, which contained just one adult (and many *Darwinia masonii*). The density of seedlings ranged from 0.5 to 7.9 per m^2 and averaged 2/ m^2 (discounting plot four). The number of new seedlings per adult averaged 4.2 and varied from 1.3 to 12.8 among the three plots.

Table 18 *Lepidosperma gibsonii* fire survival and recruitment data (experimental fire in May 2009, seedlings assessed October 2009).

Plot	#	1	2	3	4	All*
	area m^2	100 m^2	25 m^2	25 m^2	100 m^2	250 m^2
Adults	no.	4	28	44	1	77
	% killed	50%	48%	56%	0%	54%
Seedlings	no.	51	197	53	0	229
	density /m^2	0.5	7.9	2.1	0	1.2
	per / adult	12.8	7.9	1.3	0	4.2

Survival / Mortality

Survival of *L. gibsonii* individuals was spatially and temporally variable, and differed between adults and seedlings. While plants are killed in fire, a proportion also survives fire. Mortality of pre-fire adults ranged from 0 to 74 % (Table 17) in the 2003 wildfire, while 54% of the 77 pre-fire adults burnt in the 2009 experimental fire were killed (Table 18). The mortality estimate from the 2003 fire is derived from counts of both resprouting individuals and observed burnt and non-resprouting plants. Burnt and non-resprouting plants are visible for some years post-fire as blackened leaf bases, but it is possible that a number of these were missed in the survey as they can be harder to find, and others may have degraded post-fire or were burnt to an extent that no evidence exists. Hence, the proportion given here of plants killed in the 2003 fire is likely an underestimate. Nonetheless, it is clear that this value is quite variable.

Mortality of older seedlings (i.e. of plants surveyed 2007-2010, emerging following the 2003 wildfire) averaged 3% per year (Table 19), but varied between years within sites. Among adult plants, mortality varied from 1 to 5% per year across all sites and also averaged 3% per year. However, most mortality occurred in a single plot. Of the 26 older plants observed to die over the course of the study 19 were in the EHS1 plot where 20% of plants died in one year (Table 19). This plot was first surveyed with 66 live plants: a comment noting 17 dead plants recorded at that time suggests a history of population decline at this site. Examination of dead plants revealed no clear cause of death. This site is not notable for any unusual environmental features. It quite high on the slope, and has relatively little catchment area above, although as *L. gibsonii* individuals do occur higher up the slope, it is not at the upper limit for the population.

Survival of seedlings that emerged following the experimental fire was markedly lower. Of the 230 seedlings marked in the first winter following the fire, the plant or tag of all but 12% was refound after the following winter. Of the 2020 refound plants 72% had died. If the not-found plants are assumed to have died, the mortality rate would be 76%. Wire mesh cage was placed over 37 seedlings to exclude vertebrate herbivores: 73% of these plants died.

Table 19 *Lepidosperma gibsonii* mortality data for plots burnt in 2003 (IHN, MG) and in older plots (EHS, EMN IH).

Plot	IHN1	IHN2	IHN3	MG1	MG2
count	1	7	257	38	99
% dying: seedlings 2007/08	0	14.3	0.8	7.9	7.1
% dying: seedlings 2008/09	0	0	4.7	0	0
count	12	18	41	11	38
% dying: adults 2007/08	0	0	0	0	7.9
% dying: adults 2008/09	0	0	0	0	0

Plot	EHS1	EHS2	EHS3	EMN1	IH1	IH2
count	103	21	87	16	15	23
% dying: adults 2007/08	1.2	0	0	0	0	0
% dying: adults 2008/09	20.2	0	2.3	0	0	0

Health scores

It proved difficult to determine a subjective, quantitative health score for *L. gibsonii* to more than three classes - based on apparent plant vigour, colour, and inflorescences production. With one of these classes representing dead or dying plants, the majority of scored individuals fell into the healthiest class, with very few or none scored as 0. The proportion of healthy plants varied from 66% of surveyed adults in 2008 to 99% of seedlings in 2009 (Table 20).

Table 20 Proportion of surveyed seedling and adult *Lepidosperma gibsonii* individuals with a health score of 2 (i.e. maximal).

	2007	2008	2009
Adults	89%	66%	95%
Seedlings	89%	85%	99%

Growth

The indeterminate and modular growth form of *L. gibsonii* clumps means that clumps can expand and contract in size, and this was observed (Figure 22). Older clumps often consist of live and dead sections, with measurements made across the longest dimension of the base between live parts. If one distant live section died then a clump could show a sudden large decrease in size. Over the period July 2007 – July 2009 the basal diameter growth rate of seedlings averaged 0.35 mm / year, while that of adults averaged -2.4 mm / year (Table 21). Seedlings averaged 6.3 mm growth in the first surveyed year and -5.0 mm in the next. Adult growth averaged -1.2 mm in -4.7 mm across consecutive years.

Table 21. Growth rate of *Lepidosperma gibsonii* clumps (of basal diameter, in longest dimension and perpendicular) in mm per year.

	2007-2008	2008-2009	2007-2009
Longest dimension			
adult	-1.2	-4.7	-2.4
seedling	6.3	-5.0	0.3
Perpendicular to longest dimension			
adult	3.0	-3.8	-1.8
seedling	5.3	-4.3	0.1

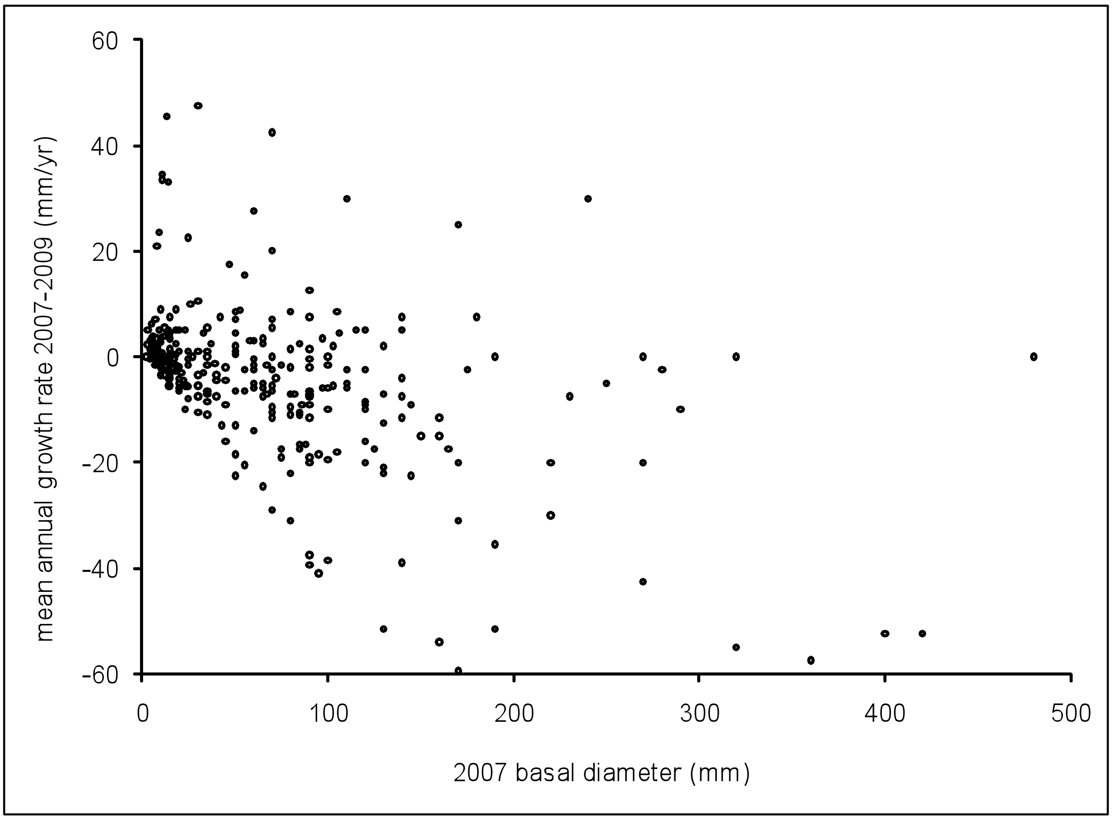

Figure 22 *Lepidosperma gibsonii* mean annual growth in relation to initial plant size (both of base diameter)

Fecundity

In 2007 57% of plants with a base diameter (bd) over 8 cm had infructescences indicating fruit production in 2006 and 39% of plants over 8cm bd had inflorescences ready for flowering in 2007. Flower and fruit production was observed among even the smallest individuals, with 7% of those < 1cm bd flowering and 4% fruiting. The proportion of reproductive plants increased through to 60 mm (for flowering) and 100 mm (for fruiting), and there was some evidence for reduced reproduction in the largest plants (Figure 23).

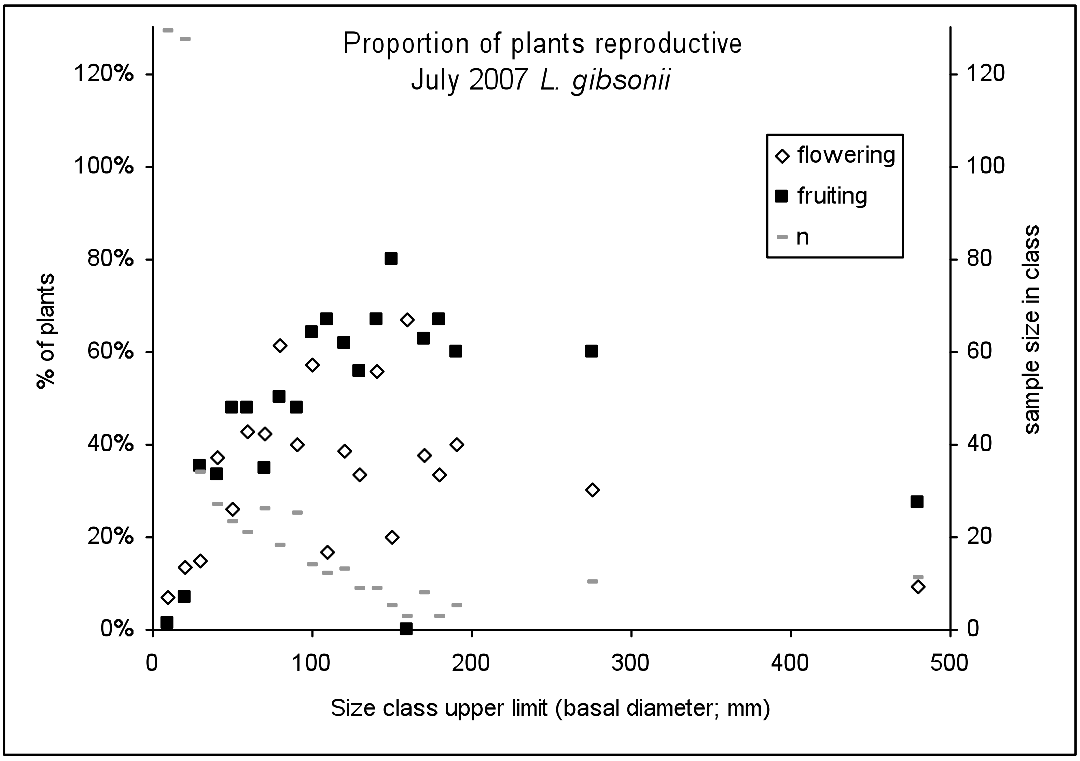

Figure 23 Proportion of plants in 2007 with flowers developing (for fruit production later in the year), or with evidence of fruits from 2006 varying with clump size.

Summary – *Lepidosperma gibsonii*:

- Population structure of *L. gibsonii* indicates that individuals recruit in a single cohort post-fire, with no evidence for inter-fire recruitment observed. That older populations were evenly structured, may suggest infrequent inter-fire recruitment, but are more likely to indicate varying growth rate and the coalescence and splitting of clumps through time.

- Evidence from population structure suggests a mean basal diameter growth rate of 2 – 2.5 mm per year for seedlings and adults.

- Extrapolating growth rates from population structure suggests that the oldest populations studied (“Emu Fence”) may have last burnt early in the 20th century, perhaps around 1910.

- Post-fire recruitment was higher, with an average of 4.2 seedlings produced per pre-fire adult.

- Considerable spatial variability in post-fire seedling recruitment was observed following both wildfire and experimental fires, with burnt sites recording 0.1 – 12.1 seedlings per pre-fire adult.

- Approximately 50% of plants are killed in fire, the remainder produce new leaves from buds surviving among burnt leaf bases

- Mortality is variable among plants in older sites, averaging 3% per year overall, but largely due to 20% mortality observed in one year in one plot.

- Mortality among 4-6 year old seedlings was similar, with an average of 3% of seedlings dying each year.

- Mortality among seedlings over the first summer and winters following fire (and germination) was much higher; 72-76%.

- Measured growth rates varied between years and smaller and larger plants. Negative growth recorded for many plants, reflects poor conditions in measured years, but also difficulties in assessing plant size.

- Mean measured seedling basal diameter growth averaged 0.3 mm / year, while mean growth for older plants was -2.4 mm / year.

- Reproduction commences in seedlings as young as six years, but increases in terms of proportion of plants flowering, and flowers per plant as plant size increases.

- Total inflorescence production varied between years by more than an order of magnitude.

4.3 BREEDING BIOLOGY

4.3a Phenology

We divided the reproductive cycle of each species into discreet stages based on non-invasive observable measures, and monitored numbers of inflorescences per plant at each stage over an entire reproductive season in 2009. For each species, five plants in each of four populations were visited at 2-3 week intervals, and the number of flowers at each developmental stage was recorded.

Darwinia masonii

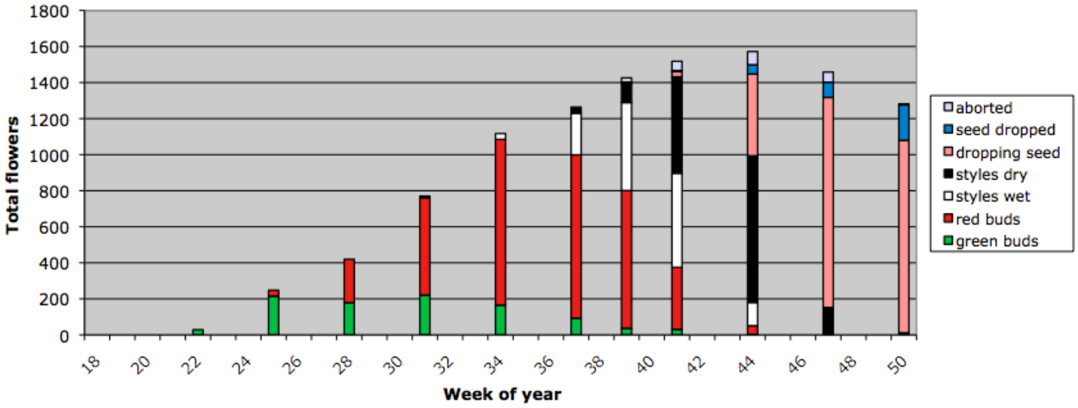

Figure 24 Phenology of developmental stages in *D. masonii*. Total of 20 plants assessed, 2009.

Table 22 Categories employed for assessing the phenology of *D. masonii* populations.

Green buds	New buds initiated (counted when clearly differentiated from leaves)
Red buds	Buds swollen but flowers not yet open (bud development stage)
Styles wet	Flowers at anthesis – actively donating and receiving pollen
Styles dry	Flowers with shrivelled styles but seed unripe (seeds maturing)
Seed ripe	Infructescences (flower heads) dropping seed
Seed dropped	All seeds dropped
Aborted	Development ceased at some stage prior to complete ripening

Floral initiation commences at the start of winter, with flowers opening from late August through to late October. Ripe seeds are first apparent in mid to late October and continue to ripen through to late November. Peak flowering (anthesis) in 2009 occurred about week 40 (first week of October). The 2009 season started late (no rain until late May), and was clearly extended by good late rains in October. In a more “normal” season plants have open flowers about 2 weeks earlier.

Optimal seed collection time is about when about number of infructescences yet to drop seeds = number with dropping seed. In 2009, this occurred around mid November. Note the broad initiation time (May-September) means that there are some flowers at all stages of development until the very end of the season, so the presence of *some* undeveloped seed is not a good indication of maximum seed availability. Seeds also drop rapidly, often with the bracts following quickly behind, so that it is then difficult to estimate the amount of seed already gone. Ants quickly remove seeds from around plants, so seed cannot be easily collected from the ground (see **4.3f Dispersal**). The most effective method of seed collection is from the plant, by hand during November – in most seasons at least some seed can be collected throughout the month (by agitating seeds within the drier flower-heads when still on the plant, and collecting seed that easily falls out). Note also that seed quality will probably depend on competing factors: earlier-developed seed is more likely to have been effectively pollinated (rather than inbred) because lower temperatures keep nectar liquid and attract more pollinators; styles are also less likely to be heat-damaged). However, predation by seed-eating moth larva (**4.3b Seed production**) may also be more prevalent earlier in the season, although this affect has not been quantified, and seems to vary considerably between population and season.

Seed drop is also progressive, with an average of around 10% of seed fallen from seed heads in late October, 50% by mid November and 90% fallen by December. A few (probably unfertilised) flowers remain attached to the inflorescence to late in the season, so the average percentage of seed dropped is a more accurate measure of seed maturity than number of heads with all seed dropped. Moth larvae in some infructescences prevent developed seeds from falling by attaching them to the infructescence-base (technically, the disk or receptacle) with silk threads. As fruits fall from un-infested infructescences through summer, the proportion of remaining infructescences that are predated by moth larvae increases: so some infructescences may appear to still be holding ripe fruits later in the season, but these may be predated and empty (Figure 25).

A small percentage of buds aborted, with a peak around late October as increasing daytime temperatures cause damage to buds and fresh flowers.

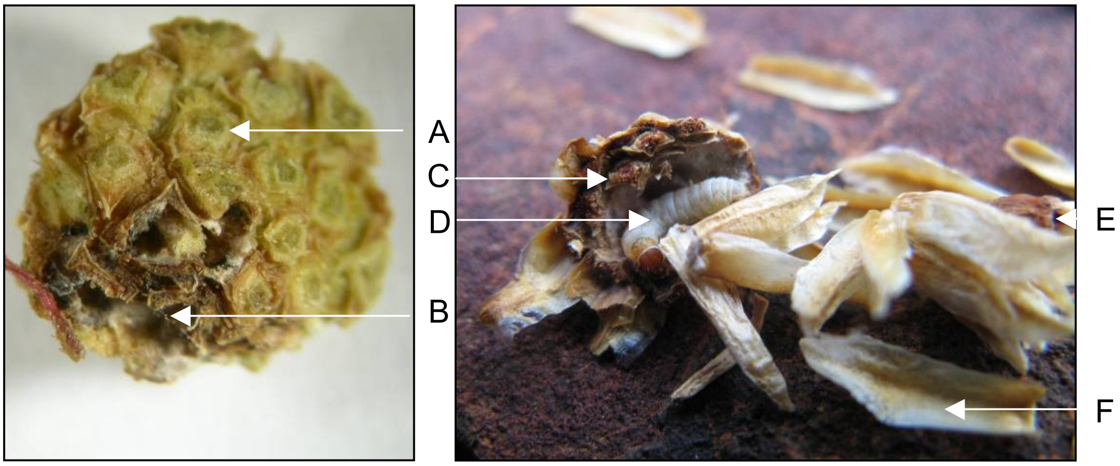

Figure 25 Left: The disk (or receptacle) of a *Darwinia masonii* inflorescence with flowers/fruits and bracts removed showing ~23 insertion sites for flowers/fruits (**A**), including a number which have been hollowed out by a moth larva (**B**). Right (April 2008): a receptacle (**C**) opened showing moth larva (**D**) with dried bracts (**E**) and fruit (**F**) retained.

Lepidosperma gibsonii

Flowering and fruit production in *Lepidosperma gibsonii* is a process which takes almost 18 months from initiation to seed release (

Table 23). Flower initiation commences with the initiation and extension of reproductive inflorescences or culms in late winter / early spring, culms cease development as they reach a length similar to or slightly longer than that of existing leaves, and then remain dormant over summer. Flower development is complete in April or May of the next year and the wind dispersed pollen is released in a synchronised burst correlated with stigma elongation, lasting only a couple of weeks (occasional flowers are still receptive for another couple of weeks; Figure 26).

Under ideal conditions, fruit development of pollinated flowers continues through winter and ripe seeds are released from infructescences during a brief period in late September-October (

Table 23). Undeveloped fruits and old infructescences usually remain on the plant over the following summer and into the next growing season. Thus successful seed development requires suitable conditions (i.e. sufficient soil moisture) for inflorescence development in spring of one year and suitable conditions again over the winter and spring of the next year. At any one time, evidence of two or three annual reproductive sequences may be visible on plants. Depending on the progress of their respective development and ageing, distinguishing between these is sometimes easy and sometimes difficult. It is particularly difficult to distinguish current year's inflorescences from the previous season's inflorescences after about July. It is therefore necessary to remove all inflorescence apices over summer to be able to score the number of new inflorescences produced each year (leaving the green part of the culm to prevent loss of photosynthetic area).

Table 23 Timing of reproductive events in *L. gibsonii.*

Event	**Time**	**Duration**	**Dependence**
Anthesis (pollen release and pollination)	late April to mid June	2-4 weeks	Presence and number of inflorescences depends on the previous season (may be very low). Timing of anthesis depends on heavy autumn dew and break of winter rains. Effective pollen release and stigma receptivity dependent on humid conditions. Anthesis is rapid and highly synchronised, with few flowers at anthesis outside of a 2-week period.
Gradual development of seed	Following anthesis to Nov	4 months	Depends on onset of anthesis and spring rains (which prolong development)
Emergence of new inflorescences for the break of the following season	August -November	1-4 months	Prolonged and increased numbers with spring rainfall
Seed release of viable seed (inviable seeds held on plant)	September-November	1-2 months	Seeds released more rapidly in drier conditions
Optimal seed collection (only 1 year observed with sufficient seed production)	Late September - Early October	c. 2 weeks	Seeds released more rapidly in drier conditions. NOTE: Post seed-release, inviable seeds are held on the plant and give false impression of seed availability.

By mid October in 2009, previously green seed (actually the fruit, a tiny thin-walled nut that contains one seed) had become brown and could be caused to fall by lightly running one's hands along the inflorescence. This was later confirmed, via X-ray analysis, to be the ideal time for seed collection – in terms of % of seeds filled. Examination of X-ray photographs of seeds in a Faxitron X-ray Corporation v1.2 (exposure 21kV for 10 seconds) camera reveals wether fruits contain filled seeds or not (Figure 27). *Lepidosperma gibsonii* fruits may be retained on infructescences for some time, but it the proportion of filled seed among retained fruits is much lower than among fruits which fall from the plant (Figure 28).

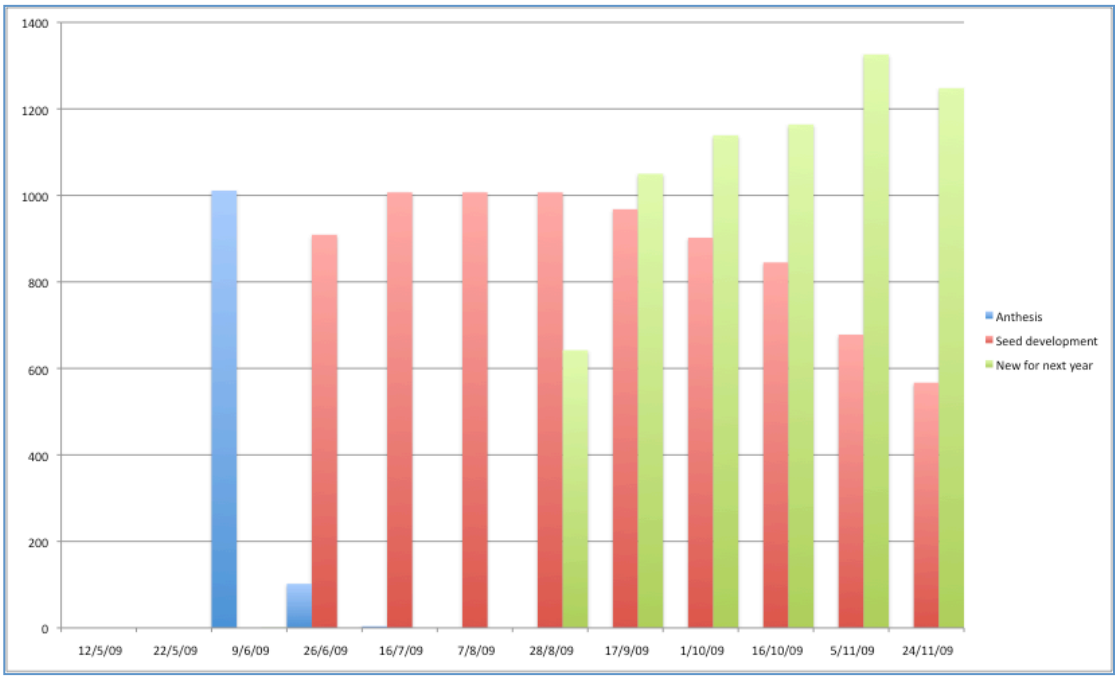

Figure 26. Relative number of inflorescences at differing development stages during 2009, showing rapid burst of anther release (and stigma receptivity), inflorescences developing seed, and production of new inflorescences for the following year. The y – axis is a total count of inflorescences at each stage, summed over 40 plants from 4 populations, as observed every 2-3 weeks over the growing season.

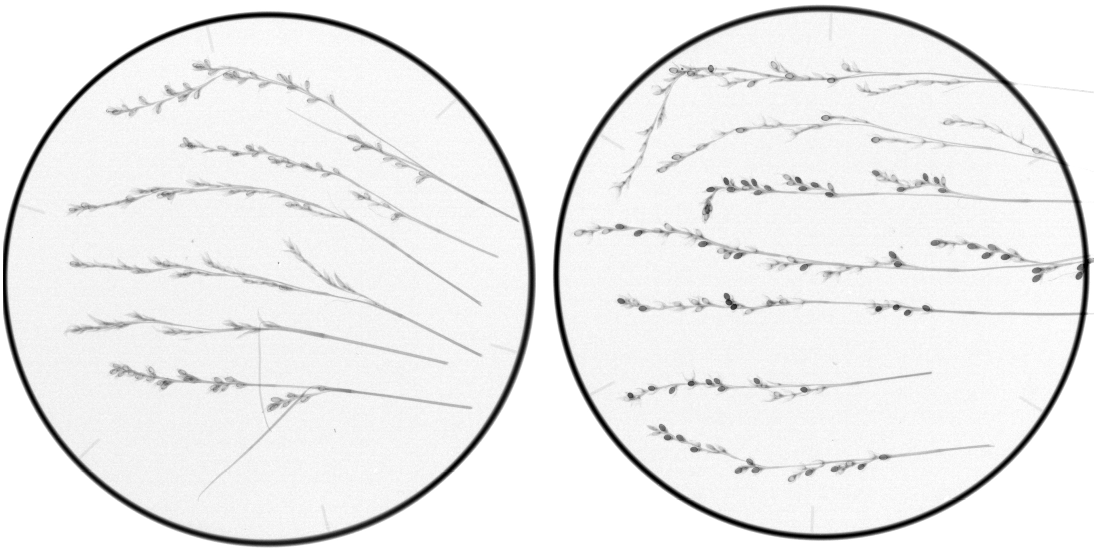

Figure 27. X-ray images of *L. gibsonii*, showing (left) developing seed from earlier in the season, unfilled and x-ray transparent; (right) developed seed from early October clearly showing developed seed (dark and x-ray opaque), and non-viable seed (pale, x-ray transparent. The darker seeds in the second image are ready to drop, while the non-viable seeds will remain on the plant, presumably to confound seed predators (and seed collectors).

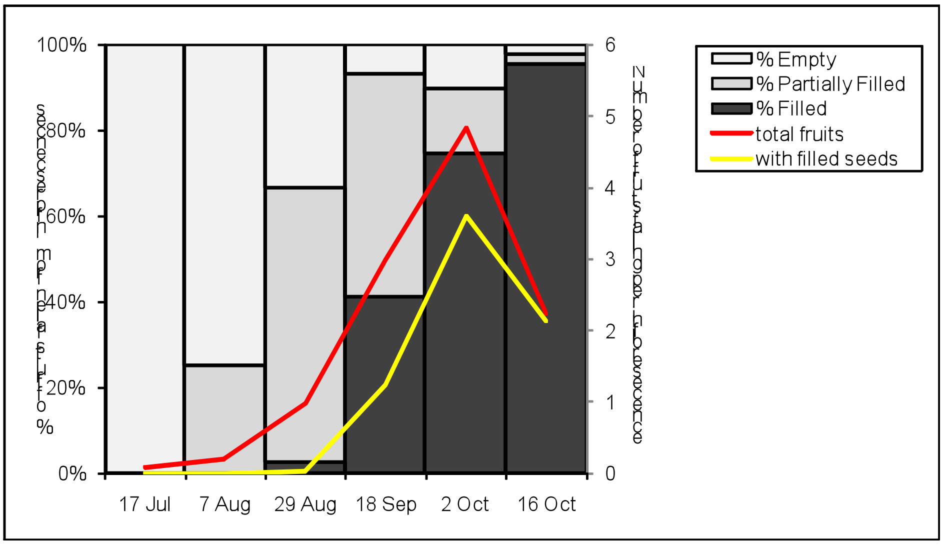

Figure 28. Proportion (bars) and number (lines) of filled fruits falling from *L. gibsonii* inflorescences (mean of ten inflorescence from each of four sites). Proportions are of fruits fallen at the sample time. Seed collection during late Sept-early Oct is possible, but the peak seed production (yellow line) is relatively short, as filled seed drop rapidly. In the field ripe seed can be detected by lightly running fingers along an inflorescence and counting the number of seed thus released.

Summary

- *Darwinia masonii* flowering and seed production takes place over a long period in spring and early summer.

- The peak period for *D. masonii* seed collection depends on the relative rates of seed development and seed drop, and may vary between years and localities, but in 2009 occurred around mid November.

- *Lepidosperma gibsonii* reproduction takes place over multiple years, with inflorescence production occurring in one year and flowering and fruit ripening occurring in the next.

- The seed collection window for *L. gibsonii* is brief (one to two weeks) as filled seeds fall soon after ripening. In this study mid October was found to be the ideal time for seed collection.

Recommendations

- Seed collection is timed closely to ensure that collected material contains viable filled seed.

- Ideal collection times appear to be mid November for *D. masonii* and mid October (in fruiting years) for *L. gibsonii*.

4.3b Seed production

Darwinia masonii

Seed production was assessed by counting the number of fruits in each of 30 infructescences collected from plants in the vicinity of each survey plot in each year. Seed fill rate was assessed by X-ray photography, and fruits with visible signs of external damage were counted as predated. Seed production per plant was calculated using these numbers together with the data on mean infructescences per plant and proportion of flowering plants reported previously (Table 13).

Table 24 Number of *D. masonii* fruits produced, number of filled seeds and number of predated seeds per infructescence (infr), as well as total seed production per plant for 2007-2009.

	2007	2008	2009
Flowers / infructescence	–	–	22.1
Fruits / infructescence	17.3	14.7	10.4
Filled seeds / infr (% of total)	2.6 (15%)	4.5 (30%)	2.0 (19%)
Predated seeds / infr (%of total)	1.1 (6%)	1.8 (11%)	2.3 (22%)
Uneaten seeds / flowering plant	75	25	66
Uneaten seeds per plant	47	9	59

Each flower in a *D. masonii* inflorescence has the potential to develop into a single fruit, each of which may in turn hold a single seed. However, when assessed in 2009, the number of developed fruits per infructescence was just under half the mean number of flowers per inflorescence (Table 24). Slightly fewer fruits developed per infructescence in 2009 (10.4) than in previous years (14.7 in 2008 and 17.3 in 2007). Externally indistinguishable, empty fruits (i.e. not containing developed seed), outnumbered filled fruits (containing developed seeds) in each year surveyed. With 70 - 85% of developed fruits not containing filled seeds, and the rate of seed predation varying between 6 and 22% per year, the mean number of good seeds that escaped predation varied from 2 to 4.5 per infructescence. Multiplying seeds per infructescence by the number of infructescences produced per flowering plants and the proportion of flowering plants in each year indicates that on average, between 25 and 75 good seeds are produced per flowering plant. Including non-flowering plants, this means an average of 9 – 59 seeds per plant per year in older plots.

Predated seeds are almost entirely all eaten by larvae of an unknown moth species. The moth prevents fruits from dispersing from infructescences by sewing them together with silk, and these are then retained on the plant for months after the fruits of non-predated infructescences have dispersed. The moth appears to survive summer in its larval stage, and has been observed in April living inside the hollowed out floral disk or inside a sewn-on fruit.

In 2008, filled fruits weighed an average of 6.4 ± 0.6 mg each (n = 976).

Lepidosperma gibsonii

The production of inflorescences and infructescences varies between plants in *L. gibsonii* as well as between plots and years. As described above (4.3a) *L. gibsonii* inflorescences develop over winter, become dormant over summer and flower and ripen seeds in the next winter. These inflorescences (which have green stems, i.e. photosynthesise) may also be held for a third year following seed production with the old reproductive parts still attached. Thus at any one survey time, plants may support evidence for reproduction across three years. At most seasons it can be difficult to distinguish old from developing infructescences.

As seed production requires two consecutive years of good rainfall for both culm initiation and development in one year, and flowering and fruit ripening in the next, and although it is not known what the threshold rainfall requirement is for these processes, it is possible to model seed production through time. With the exception of 3 years (1968, 1971 and 1972) the Ninghan annual rainfall record is continuous back to 1905. (Regionally, 1968 was above average, 1971 was average and 1972 below average). Seed production was observed in 2009, and rainfall at Ninghan in 2008 and 2009 was 130% and 110% of the average respectively. 2006 and 2007 had 63% and 107% of average rainfall, but seed production was not observed in 2007, suggesting that the minimum rain must be >63%. Thus the rainfall threshold is likely to be between 63% and 110% of the annual average.

Modelling of the sequence of years with rainfall receipt above a threshold percentage of the Ninghan long term average and reporting the years which are themselves both above the threshold and preceded by an equal or better year indicates that potential years of seed production are infrequent and clustered. If the limiting threshold for seed production is the same for both years of development and equal to (i.e. 100% of) the long term average rainfall, then seed production could occur in 22% of years, if this threshold is 110% of the mean rainfall, then fruit production would only have occurred in 10% of years. While these scenarios indicate an average of one year of seed production every 4.5 years and one per 10.5 years respectively, the actual run of years without seed production is very different. The 106-year Ninghan record indicates one period of 17 years without seed production (1944-1960) under a mean rainfall threshold, and a period of 49 years (1935-1983) without seed production if the threshold is 110% of average rainfall. If the threshold is as low as 65% (i.e. just exceeding that of 2007) then reproduction may occur in as many as 60% of years with 2 years being the longest run of consecutive years with reproductive failure.

This modelling does not account for possible effects of the seasonal distribution of rainfall (e.g. cyclonic summer rainfall may not assist in production of fruits if the following winter is dry), nor the possibility that inflorescence production and seed production have different minimum rainfall requirements.

Summary

- *Darwinia masonii* seed production is moderately low, varying between years from 9 to 59 seeds per plant in mature populations.
- Variation in production results from (in declining order of importance) variation in inflorescence production per flowering plant, seed predation rate, % of plants flowering, and % of fruits containing seed.
- *Lepidosperma gibsonii* seed production is limited by a requirement for sufficient rainfall in consecutive years. The amount of this rainfall is unknown but appears to lie in the range of 65-110% of the average. Applying these thresholds to the Ninghan rainfall record suggests that the frequency of *L. gibsonii* reproduction may vary between 60% and 22% of years, with likely historic runs of no seed production varying from 2 to 49 years.

Recommendations:

- Seed counts should take into account the low number of filled seeds per fruit.
- Seed collection should focus early in the season (November) before moth predation and seed dispersal lead to the loss of most seeds. Later collections will likely contain few uneaten seeds.
- Studies of the identity and habits of the *Darwinia* seed eating moth are recommended.
- Ongoing monitoring of *L. gibsonii* seed production each year, together correlation with rainfall data, will enable refinement of estimates of reproductive frequency.

4.3c Seed germination requirements

Darwinia masonii

Experiments on 2,700 *Darwinia masonii* stored and X-ray screened seed, collected in three different years (2004, 2007 and 2008) and using a variety of physical and chemical treatments were performed in 2009. Samples included 5 replicates of 10 or 20 seed (depending on availability) treated with smoke water or fresh water, light and dark storage, and excision (seed manually removed from fruit coat), nicking of fruit coats (allows water penetration to seed) or no physical treatment. In this experiment, the highest germination rate, 30%, resulted from dark storage of nicked seed, collected in 2007 and treated with smoke water. Insufficient seed was available to test all combinations, so physical treatments were tested only for dark treatments. In light treatments no germination was observed with H_2O, but 3% germinated with smoke (Table 25). In dark treatments, the best germination resulted from nicking or removal of seed coats. For excised seed, H_2O was as effective as smoke water, but in nicked fruits and those with no physical treatment, smoke water had a positive effect. The age of stored seed (up to 5 years old) appeared to have no clear (positive or negative) effect on results.

Table 25 Percent of fresh *Darwinia masonii* seed germinating from samples stored in with light or in dark conditions, treated with smoke or filtered water (SW v. H_2O) and with seed excised, from fruit coats, coats nicked, or no physical treatment (results include different seed batches pooled). nt = not tested.

	Dark		Light	
	H_2O	SW	H_2O	SW
Excised	18%	18%	nt	nt
Nicked	4%	19%	nt	nt
None	0%	6%	0%	3%
Grand Total	3%	13%	0%	3%

These results suggest that *D. masonii* seeds have both physical and a physiological dormancy processes, the former requiring the removal or breakdown of fruit walls, and the latter indicating a stimulatory effect of smoke chemicals. That smoke had limited effect on germination of fresh seeds also indicates an increased sensitivity to smoke with age. Further details on *D. masonii* germination are given in **4.3d Seed bank demography**.

Lepidosperma gibsonii

Due to the absence of seed production in previous years, experiments with fresh *L. gibsonii* seed were delayed until after late 2009. Results from germination experiments with fresh, X-rayed nuts (i.e. known to contain filled seed) using ± heat × ± smoke/ TC water × ± GA treatments, each with 5 replicates of 25 seeds, resulted in zero germinants. A small number of germinants were observed following burial for 3 and 6 months however (see 4.3d below).

Experiments with *L. gibsonii* seeds manually manipulated under a microscope to remove their external (i.e. fruit wall or nut) casing resulted in levels of germination: up to 60% of excised seeds treated with a heat (100°C) pulse germinated (4.7b see Germplasm storage).

Seed of *Lepidosperma* species are generally difficult to germinate. Recent work at BGPA by Shane Turner and others on other *Lepidosperma* species report the absence of germination without nicking of fruit coats or excision of seed (e.g. Kodym *et al*. 2010, Panaia *et al*. 2009). Turner (unpubl.) also reports a positive role for 80° and 100°C (but not 120°C) heat treatments in germination of previously buried *Lepidosperma leptostachyum* seed. Smoke water and gibberellic acid had independent and interactive positive effects, but maximal germination still did not exceed 25%.

These collected results indicate a physical dormancy process in *Lepidosperma* which may require the break-down of the fruit coat over time through weathering in the soil, and which can be replicated by the somewhat onerous process of seed excision.

Summary

- *Darwinia masonii* seed germination is low in experimental treatments on fresh and stored seed, but can be improved by a combination of physical treatments and smoke application.
- *Lepidosperma gibsonii* seed germination remains unsolved, however indications of positive, but still small, effects of seed burial, fruit wall breakdown (or removal), smoke and heat treatments can be derived from related studies and early results from burial experiments.

Recommendations

- Large scale production of seedlings of either species via germination of fresh or stored seed is possible, but inefficient, and involves physical manipulation of small seeds for seed coat nicking or removal, or retrieval of seed buried for months or years.

4.3d Seed bank demography

The significant soil seedbank germination response observed following experimental fire, described under ‘recruitment’ in section 4.2 above, indicates the presence of a significant soil seedbank for both species.

An experimental program was established to investigate the longevity and seasonal and longer-term patterns in germinability of seed buried in the soil. The *D. masonii* burial trial commenced in January 2009, and that of *L. gibsonii* as sufficient seed became available a year later. Collected seed was initially x-ray screened to ensure that only filled fruits were used in experiments. These were then counted into nylon mesh bags, with a total of 55 bags of 250 *D. masonii* seeds and 70 bags of 120 *L. gibsonii* seeds created. These were buried in 5 caches each for *D. masonii* and *L. gibsonii*, located on 100m transects running down the north (*D. masonii*) and south (*L. gibsonii*) slopes of Mount Gibson. Soil temperature sensors attached to battery operated data-loggers were placed with each collection which was covered with 2-5 cm of soil. Rocks and litter removed prior to burial was replaced, and a wire cage to exclude vertebrate disturbance was positioned over the top.

The experimental design incorporated provision for retrieval of one bag from each site, i.e. 5 replicate bags per sample period, for 11 (*D. masonii*) / 14 (*L. gibsonii*) sample periods. The retrieval schedule planned was 3 month intervals for two years, followed by annual (*D. masonii*) and biannual (*L. gibsonii*) intervals up to 5 years. This design allows for flexibility in sampling number or interval if early results suggest it may be required.

Prior to treatment, retrieved seeds were x-rayed and scored for internal and external signs of germination, predation or degradation. The five groups of intact seeds (one per retrieval site) were then split into groups of 100 (*D. masonii*) and 25 (*L. gibsonii*) for treatment. On retrieval, as well as (for control samples) at the time of burial (i.e. at time = 0) the five replicate samples were treated, plated out on filter paper in petri dishes and stored in the dark at 15°. Seeds were examined and the number of germinants counted fortnightly until germination ceased at approximately 150 days. *Darwinia masonii* seeds were treated with either smoke water or filtered water, while *L. gibsonii* seeds were treated to a crossed design of smoke water or filtered water and application of heat / no heat. Heat treatment was 100° for 10 minutes. Heat treatments were not applied for *D. masonii* as preliminary tests showed that heat (applied for 10 minutes or 30 minutes) did not enhance germination.

Darwinia masonii seed responded to smoke water (SW) at all periods, improving germination by 20-70% over fresh water only (Figure 29a).

Germination was low to negligible with fresh water except in spring (at 9 months) when it peaked at 29%. Smoke-water treated seeds germinated at all times, but at a much higher rate in spring and the second summer. The largest germination response (90%) was to smoke water after 9 months of burial (i.e. in spring). Germinability at 15 months was close to identical with that observed at 3 months and 6 months, suggesting no significant decline in seed viability with age over 2 years, and tight control over the germination process.

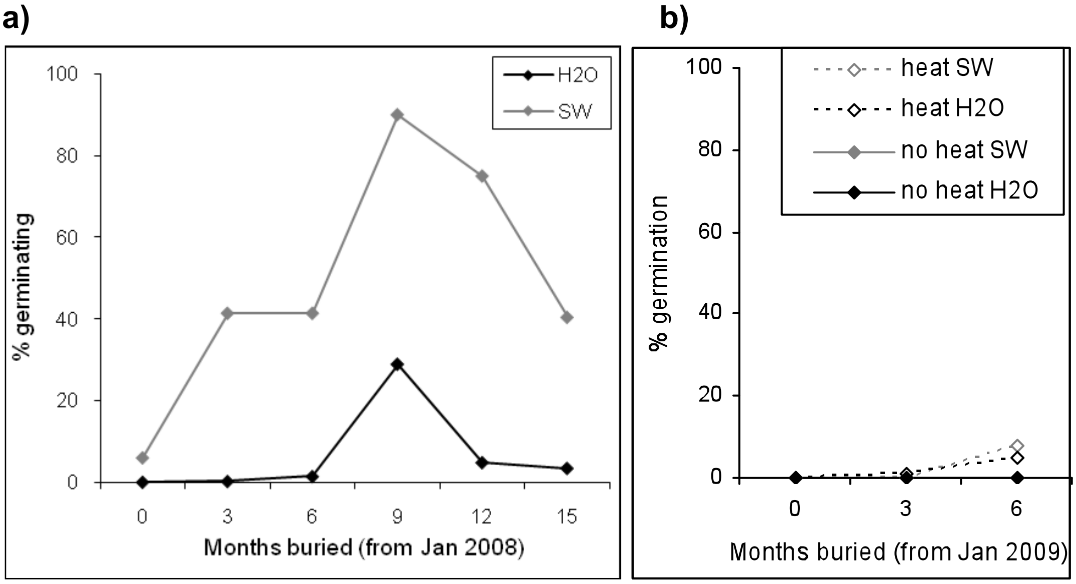

Figure 29. a) Germination rate of *Darwinia masonii* seed buried in the field and retrieved after 3-15 months. Results show average of 5 samples of 100 seeds, as well as smoke (SW) and filtered (H_2O) water treatments. **b)** Germination rate of *Lepidosperma gibsonii* seed buried in the field and retrieved after 3-6 months. Results show average of 5 samples of 25 seeds, as well as smoke and filtered water treatments crossed with ± heat (100° C for 10 minutes) treatments.

No seeds germinated for *L. gibsonii* in the absence of physical treatment, just one germinated after 3 months of burial (heat + SW treatment) and 16 germinated after 6 months of burial (Figure 29b). These were all in the heat treated samples and represented 8% of the SW and 4.8% of the H_2O only treated samples.

The seasonal pattern observed is common in species with long-lived seedbanks from areas with seasonal climates and indicates that seeds are cycling in and out of dormancy in relation to environmental cues. Soil temperature and moisture are likely to control the induction and loss of dormancy. The incomplete germination with smoke also shows that smoke does not break dormancy, but rather it acts as an additional cue.

Summary

- Seed bank trials have been established for both species, and are ongoing. The lack of availability of seed in previous years meant that *Lepidosperma gibsonii* trials had only had 6 months to run by the end of the project.

- Buried seed is still in place with experiments designed to continue for up to 5 years.

- Preliminary results indicate complex germination / dormancy strategies for both species, combining a requirement for physical degradation of the seed coat, environmental (seasonal temperature) cuing – with seeds cycling in and out of dormancy, and heat- and smoke-related physiological responses.

- Germination rates peaked for *D. masonii* at 90% with seed which had been exhumed after 9 months of burial and treated with smoke water

- Smoke water treatments of *L. gibsonii* seed buried for 6 months and exhumed in winter showed a small, but non-zero rate of germination. For this notoriously recalcitrant genus this result is encouraging.

Recommendations

- Established seed burial / retrieval trials should continue for at least several further years.

4.3e Breeding and mating systems

For most species, self-sustaining populations require both a large pool of genetic variation, and the ability to breed successfully with a wide pool of mates. Patterns of mating determine the level of homozygosity in the next generation, and thus affect reproductive success, fitness of offspring, genetic diversity and genome evolution. Relatively few inbreeding species have evolved mechanisms, or sufficiently purged their genome to withstand the deleterious effects of inbreeding over evolutionary timescales (hundreds of generation).

Knowledge of breeding and mating systems in rare plants is important for several reasons: (1) it gives basic information on the critical factors in maintaining mating patterns and seed production, (2) it gives baseline information which can then be monitored over time to detect changes in population sustainability (e.g. detecting lowered genetic variation in the seed rain before a fire event that might irrevocably kill off diversity not maintained in the seedbank), and (3) provides information on the critical factors for creating a self-sustaining population in translocation and restoration efforts.

Following Neal & Anderson (2005), Breeding system refers to the physical and physiological aspects of plant mating: (e.g. sex of flowers, relative timing of development of different organs, self-compatibility mechanisms etc). Mating system refers to the relatedness of mating gametes, and spatial relationships of parents (e.g. inbreeding, outcrossing, correlated paternity).

Darwinia masonii

Breeding system

Like most other species in Myrtaceae tribe subtribe Chamelaucineae (sensu Rye in press), the flowers of *D. masonii* exhibit *pollen presentation*. This specialised mechanism facilitates more accurate deposition and removal of pollen, or in some cases increase rates of self-fertilisation (for specific discussion of pollen presentation in Myrtaceae see Slater & Beardsell, 1991 and Beardsell et al., 1993). The pollen of *D. masonii* exudes from the anthers while the flower buds are still closed, it then becomes soaked in an oily pollenkitt which is excreted from a terminal gland on the anther. This pollenkitt and pollen attaches to a band of hairs just below the tip of the central style. As the flower opens and the enclosing bracteoles are pushed away, the style rapidly elongates to its full length, carrying the fresh, wet pollen with it just below the apex of the style. The timing of stigmatic receptivity has not been studied in *D. masonii*, however in the related species *Chamelaucium uncinatum*, the stigma is initially small and unreceptive at anthesis, but increases in size and becomes fully receptive 7 days after anthesis. (O'Brien 1996). In other species of *Darwinia* studied at Kings Park, the stigma is also initially unreceptive for many days after anthesis, but the stigma does not enlarge on becoming receptive; the only indication that the stigma has become receptive is a slight "wetting" of the style as a sugar-rich solution is released to simulate germination of pollen tubes. *D. masonii* is almost certainly very similar to other WA *Darwinia* species in its stigmatic development.

Pollen presentation has the potential to be very efficient at depositing and collecting pollen at a single area on a pollinator's body, but also increase the likelihood of *self-pollination* due to the close proximity of pollen to the stigma. An assessment is therefore necessary to determine (1) whether plants can and do self-pollinate, (2) what percentage of inbred seeds are produced, (3) do plants preferentially select outcross pollen, and (4) do outcrossed seeds germinate and survive better than selfed seeds? These factors are critical to allow accurate population-viability measures to be recorded and modelled; for instance, if most seeds are selfed, but selfed seed survive significantly worse than outcrossed seed, then effective seed production may be far lower than that measured crudely by seed fill rates. These questions are addressed below.

Pollinators: A total of 20 hours (x 2 people) was spent bird watching, initially at 2 hour intervals per day over 3 days, subsequently at times of peak bird activity between 0830 and 1100. Each observation point had a more-or less unrestricted view of 12 or more reproductive *D. masonii* plants. A total of 10 hours was spend watching insects on 1-3 plants at a time, initially in 15-minute blocks at 4-hour intervals over 3 days, subsequently at times of peak insect activity between 0930-1100. Each observation consisted information on: species, time spent feeding, number of plants visited, number of flowers visited per plant and destination after initial visitation.

Results:

A total of 26 bird visitations was observed, with all identifications (n=18) of a single species, the White-fronted Honeyeater (*Phylidonyris albifrons*). At least five other species of honeyeater have been observed at Mt Gibson, but none were observed visiting *D. masonii*. White-fronted Honeyeaters landed on the branches of *Darwinia* plants (or rarely the ground) and probed upward into the flower head to reach the copious nectar produced by recently-opened flowers. Birds were observed physically contacting styles. On one occasion a bird was seen vigorously wiping its beak on branches immediately after a visit to *D. masonii*, presumably to remove a build-up of sticky pollenkitt received from styles. Birds fed for 10 seconds to 2.5 minutes at a time, and visited single flowers on single plants, up to numerous flowers on at least 6 plants; most consecutive visits were between neighbouring plants, before flying away out of sight. Pollen longevity is not known, neither are honeyeater movements on larger temporal and spatial scales.

Although native bees and wasps were observed visiting other plant species around *Darwinia* plants, the only insect activity seen on *D. masonii* was very rare (n=2; total of 10 flowers) visitations of introduced honeybees, and one of a large native wasp. Visitations involved bees/wasps attempting to reach the nectar from the base of flower heads, and not attempting to collect the (wet) pollen held on styles [In contrast, numerous bees were observed combing plants of *Calycopeplus collinus* nearby for their dry pollen]. Most attempts by bees / wasps to reach nectar of *D. masonii* failed due to the angle of the head, and the insects usually left without touching the styles. Only twice was an insect (1 bee, 1 wasp) observed to reach the nectar, on a flower head held laterally and by the insects reaching in from the perimeter past the red bracts, without contacting pollen or the stigma. Only once was a bee observed contacting the style and stigma, during an unsuccessful attempt to squeeze through the mass of styles.

Conclusion: while both insect and bird visitation both occurs, by far the dominant (potential) pollinators are White-Fronted Honeyeaters, with insect visitation at best rare and ineffectual.

Pollinator dependence and inbreeding rate: The effective dispersal of pollen in most plants relies on the activity of pollinators. Some plants, however, have evolved strategies to avoid the need for outcrossing, and instead self. Other plants show a mixed mating system, with the ability to self-pollinate in the absence of pollinators. In this study, the ability of *D. masonii* to produce seed, and the effect of types of pollinators on total seed production was studied.

Sixteen plants in a single population were divided into three groups:

- Complete pollinator exclusion (caged with fine mesh to exclude all insects and birds); N=4.
- Partial exclusion (with 1 cm gap mesh to allow insect pollination but exclude birds); N=4.
- Open pollinated (uncaged); N=8.

Cages were erected to be self supporting and completely enclose plants, but provide minimal shade, and were placed over plants prior to anthesis of the first flowers (Figure 30).

Figure 30. Exclusion cages for *D. masonii* pollination study. Left: Bird and insect exclusion, Right: Bird exclusion.

Mature fruits (each fruit contains a sing (rarely two) seeds) were X-rayed in Faxitron X-ray Corporation v1.2 (exposure 21kV for 10 seconds) to determine whether a viable seed had developed. Seeds with filled ovaries (from the enlarged hypocotyl which forms the bulk of the seed in *D. masonii* (Prakash, 1969) were recorded (Figure 4). Seeds that are non-viable just show a cavity. A percentage of viable seeds out of the subset X-rayed were determined.

Mating system parameters was estimated using the MLTR program v3.2 (Ritland, 2002). MLTR estimates from progeny arrays the following parameters: 1) multilocus population outcrossing rate, 2) bi-parental inbreeding rate (mating among relatives) and 3) correlated paternity (fraction of siblings that share the same father).

Results: When all pollinators were completely excluded, seed set (6.6%) was significantly greater than zero (P<0.05; Figure 31). When birds were excluded but insects were allowed access to the plants, there was an increase of seed set to 14.8%, although this was not significantly greater than that following all pollinator exclusion (P>0.05). In contrast, plants given full access to pollinator showed a significant increase (23%, P<0.05) in seed set compared to complete pollinator exclusion; this result was however not significant when compared to the treatment excluding birds only (allowing insects).

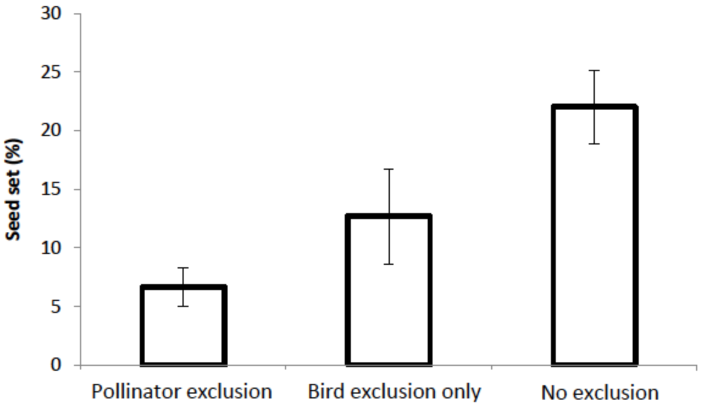

Figure 31. Percentage of flowers with filled seed from D. masonii plants allowed access to different classes of pollinators. In the control (no exclusion), seed set was relatively high (23%), compared to caged plants which excluded birds. The caged plants that excluded all pollinators (birds and insects) had the lowest seed set (7%), while the plants caged to exclude birds but not insects had an intermediate seed-set (13%). This data suggests that (1) plants can set a lowered level of self seed in the absence of pollinators, (2) that birds are significant pollinators, almost doubling the seed-fill rate compared to insect-only pollination, and (3) insect activity appears to have a weaker contribution (however the last result is not significantly different from zero).

Conclusion: *D. masonii* is able to self-pollinate at a low rate (6.6%) in the absence of pollinators, however pollinator activity significantly increases seed set. The study was not powerful enough to unambiguously separate the actions of pollinator classes, however the trend agrees well with pollinator observations: rare insect visitation increases the outcrossing rate (and seed set), however birds are much more effective pollinators and more common visitors, resulting in a higher seed set.

These results were further corroborated by an assessment of mating system parameters using MLTR (Ritland, 2002). Assignment of paternity using microsatellite genotypes (comparing maternal-only markers vs presence of non-maternal and therefore outcrossed markers) showed a multilocus outcrossing rate (tm) for the open pollinated plants (control) of 0.57 (0.09) (Table 26). This was greatly reduced, as expected, when all pollinators were excluded (tm = 0.17 ± 0.17, i.e. not significantly different from zero, as expected for complete selfing). The presence of insect pollinators only however increased the multilocus outcrossing rate to intermediate levels (tm = 0.45 ± 0.19; $P<0.05$), suggesting that insects can supply some pollen dispersal service in the absence of birds.

Table 26. Mating system parameters for pollinator exclusion experiment in *Darwinia masonii*. Multilocus outcrossing rate (tm), bi-parental inbreeding rate (tm-ts), and correlated paternity (rp) were estimated using MLTR (Ritland, 2002).

Treatment	tm (SD)	tm – ts (SD)	rp (SD)
Complete exclusion	0.17 (0.17)	0.08 (0.03)	-0.16 (0.71)
Bird exclusion	0.45 (0.19)	0.14 (0.04)	0.32 (0.43)
Control	0.57 (0.09)	0.03 (0.02)	0.09 (0.12)

Assessment of pollen limitation: The benefits of outcross mating over selfing can be expressed at several stages of development, including differential pollen germination, pollen tube growth rates, pollen tube growth in the style, fertilisation success (all pre-zygotic barriers to selfing), differential embryo development (in this case 2 ovules, usually only 1 develops), differential seed ripening, germination rate, seedling emergence, seedling survival and adult reproductive capacity (post-zygotic barriers). In this study we examined the effect of self vs outcross pollen up to seed maturation, by supplementing pollen over and above that received by plants from normal vectors. The three treatments were:

- Open pollinated plants with no supplemented pollen (control)
- Supplemented pollen from a known outcrossed plant, to determine whether there is an increase in reproductive success (seed fill rate)

over and above that observed from natural pollination – ie. is fertilisation *pollen limited* through scarcity of pollinators?

- Supplemented self-pollen (from other flowers on the same plant) to determine whether any observed increase in seed production is the result of *any* pollen limitation (e.g. physical or temporal separation of self-pollen transfer between the style hairs and the stigma), or whether it is *outcross-pollen* limited, implying pollen source is the controlling variable

Results: There was a significant increase of average percentage seed set over the control when outcross (+external) pollen was introduced but not when self-pollen was introduced (Figure 32). An ANOVA test showed $P<0.05$ when comparing no extra pollen (control) and external source of pollen. However, there is no significance increase of seed set when pollen was introduced from the same plant (+self pollen) compared to a control group.

Conclusion: Outcross pollen provides a significant improvement in seed-set rates over self-pollen; the addition of self pollen alone does not increase seed fill rates, suggesting that pollen presented near the style is able to self-fertilise plants effectively. The presence of external pollen, however implies a selection mechanism for outcross pollen – i.e. *D. masonii* is capable of selfing but is *preferentially outcrossing*, the same pattern reported in many eucalypt species.

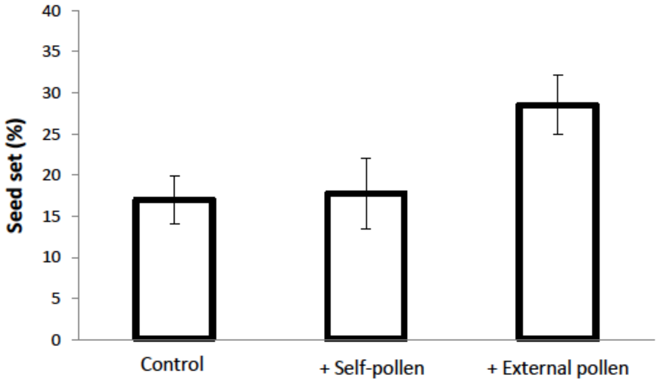

Figure 32. Seed fill rates (% of flowers with filled ovary cavity) when given access to natural pollinators (control), supplemented with self-pollen, and supplemented with outcross pollen.

Genetic diversity at different stages of the lifecycle:

Selfing is expected to lower the genetic diversity (especially observed heterozygosity and Fixation indices) of offspring relative to their parents. If left unchecked, this will result in a gradual decrease in genetic diversity, and loss of alleles until populations become almost uniform in genotype. Selection against homozygotes at different developmental stages can however result in maintenance of genetic diversity in the face of inbreeding.

We assessed genetic diversity at three stages of the lifecycle of *D. masonii*: adults, seeds and seedlings, by genotyping using microsatellite markers. Due to access constraints and the need for an experimental fire to generate seedlings under selection for their environment, two separate populations had to be used, (1) the adults and seedbank in the experimental burn are on Extension Hill, and (2) adults and their seed progeny in the Mt Gibson South population. A total of 220 adults and 113 seeds were analysed from Mt Gibson South, while a total of 77 adults and 146 seedlings were analysed from the Extension Hill experimental burn site.

As expected from the presence of inbreeding in *D. masonii*, seeds display reduced observed heterozygosity (Ho, table 27) compared to their parents (although the result is not statistically significant), and increased Fixation Index (a measure of homozygosity). Assuming both breeding system and selection processes are common between the two sites, the fact that parameters for seedlings are close to parent values is evidence for selection against inbred seeds / homozygotes, possibly through the action of lethal alleles.

Table 27. Comparative average diversity estimates for 2 adult populations of *D. masonii*, and their respective offspring: Pre-fire adults and post-fire seedling recruits at Extension Hill, and Adults and their seeds from Extension Hill south. Diversity measures are I (Information index), Ho (observed heterozygosity, He expected heterozygosity, and F fixation index; all values are means ± standard error). Seeds show a distinct decrease in observed heterozygosity and an increase in Fixation index (consistent with a percentage of inbreeding), while seedlings are more similar to adults, suggesting selection against inbred seeds in this species.

Averages:	**I**	**Ho**	**He**	**F**
EH Adults pre-burn	1.245 ± 0.290	0.557 ± 0.145	0.601 ± 0.128	0.106 ± 0.106
EH post-fire seedlings	1.251 ± 0.287	0.515 ± 0.133	0.597 ± 0.124	0.139 ± 0.099
MGS Adults	1.309 ± 0.307	0.571 ± 0.083	0.619 ± 0.094	0.070 ± 0.021
MGS seeds	1.209 ± 0.306	0.479 ± 0.095	0.587 ± 0.113	0.154 ± 0.047

A mating system in which plants can inbreed, but select against inbreed seed, ultimately producing mostly outcrossed offspring is termed *preferential* *outcrossing*, and is known to be the dominant system in *Eucalyptus* (also a member of the family Myrtaceae, like *Darwinia*) (House 1997).

Summary:

- *D. masonii* is predominantly pollinated by a single species of bird, the White-fronted Honeyeater.
- *D. masonii* is capable of selfing but selection for outcrossed seeds occurs at several levels, greatly reducing the number adult plants resulting from self-pollination, and the production of outcrossed seed is therefore a critical requirement for maintaining a self-sustaining population.
- Any restoration or translocation efforts must include the community context for *D. masonii*, especially in regard to ensuring adequate habitat for White-fronted Honeyeaters.

Lepidosperma gibsonii

Lepidosperma gibsonii is wind pollinated, and so does not have complex pollinator interactions as seen in *D. masonii*.

The mating system in *L. gibsonii* was investigated by sampling inflorescences from 12 plants and collecting the seed produced (total 48 seedlings). Embryos were extracted from seed using the protocol presented here, and left to grow into small seedlings on agar. Seedlings were then removed, DNA extracted, and genotyped using 11 microsatellite loci. Assignment of paternity using microsatellite genotypes (comparing maternal-only markers vs presence of non-maternal and therefore outcrossed markers) showed a multilocus outcrossing rate (tm) for the open pollinated plants of 91.7 %, demonstrating a very high rate of outcrossing compared to selfing, as expected for a wind-pollinated species, and as expected by the high genetic diversity and lower level of population structure observed in *L. gibsonii* (see section 4.1).

Population size and weather conditions are the likely factors affecting pollination success. Further research on the effect of population size (and therefore pollen abundance) on inbreeding rate would be beneficial.

Summary:

- *L. gibsonii* appears to have widespread, wind-assisted pollen dispersal and high rate of outcrossing.

4.3f Dispersal

Studies of dispersal took two forms: 1) inferences of possible seed dispersal patterns, vectors and distances from observations and experimental studies of dispersal agents and 2) measurements of patterns of actual dispersal of genetic material in both pollen and seeds, by using molecular techniques to identify the parents of seedlings observed following experimental fire.

Darwinia masonii

Pollen and seed dispersal

Pollen dispersal was studied by parental assignment of seed genotypes with known mothers to determine their most likely father (pollen donor). The program CERVUS (Kalinowski et al. 2007) was used to assign paternity to genotyped seeds collected from known, genotyped mother plants. A total of 200 seeds were genotyped and the most likely sire estimated from among 200 possible surrounding adult plants using likelihood assignment techniques. Effective Seed dispersal was studied by genetic assignment of seedlings within the surrounding pool of prospective parents. Since *D. masonii* seeds only rarely germinate in the absence of fire, an experimental fire was carried out in an area within the approved clearing footprint that contained a population of reproductively mature *D. masonii* (likely dating from the 1969 fire). Since adult plants are typically killed by fire, all adult plants in this population were genotyped prior to burning. The experimental fire was carried out in May 2009, just prior to the onset of winter rainfall, and seedlings were sampled at the end of spring, i.e. after the first season of growth but prior to the first summer. A total of 80 adult plants were genotyped, and a total of 230 seedlings were recovered and genotyped. Only the lowest leaves (senescing cotyledons) were sampled in order to track seedling survival (and genetic correlates with survival) through time. The program CERVUS (Kalinowski et al. 2007) was used to select the most likely parents (maternal and paternal) from among the 80 adult plants present before the fire. Results of this work are being prepared for publication.

Identity and behaviour of seed dispersal agents

Fruits of *Darwinia* species from NSW are reported by Auld (2009) as being dispersed by ants, and studies at Extension Hill confirm this behaviour with respect to *D. masonii*. Seed removal by ants was assessed in a baiting experiment, in which 14 piles of 5 *D. masonii* seed were observed between 9 am and 3 pm on December 2 2009, with seeds in baiting stations refreshed if any were seen to have been removed. The number and time of seed removal events was recorded and specimens of ant species observed removing seed were collected for identification (by Brian Hederick, Curtin University). The foraging behaviour by *R. violacea* ants (previously reported to be a key seed-dispersing species in SW WA; Gove *et al*. 2007) was examined on Extension Hill south (in December 2007 and May 2009). Foraging distances were assessed by offering randomly observed individuals food morsels (muesli bar fragments) and recording the distance back to their nest. Finally, to identify the interest of ants in *Darwinia* seeds, individually marked, weighed and photographed fruits were offered to captive *R. violacea* ants, which quickly removed the fruits below ground, but then later returned them to the surface. Returned seeds were rephotographed and weighed.

Six species of ant were observed removing *D. masonii* seeds in December 2009: *Iridomyrmex chasei*, *I. gracilis minor*, *Melophorus turneri perthensis*, *Rhytidoponera crassinoda*, *R. metallica* and *R. violacea*. Seed was removed from all 14 observed stations with an average of between 0.3 and 7.3 removals per station per hour, although at one station 41 seed were removed in a 1 hour period, including 18 in one 10 minute period, by *M. turneri perthenis* to a nest 2.5 m away.

A total of 30 *R. violacea* foraging distance observations were made at Extension Hill these indicate an average foraging distance of 3.7 m and a maximum of 10.8 m.

Auld (2009) suggests that ant dispersal of *Darwinia* fruits results from the attractiveness of *Darwinia* petals, however observations of *D. masonii* fruits collected and then returned to the surface by captive *R. violacea* ants with intact petals contradicts this idea. Instead, we suggest that ants are attracted to the highly concentrated but still-liquid nectar which coats the outside of *D. masonii* fruits. After processing by ants fruits weighed 10% less and had intact petals (n= 12 fruits). Before and after photographs also clearly show the removal of the external liquid coating.

Most offered fruits were disposed above ground by captive *R. violacea* ants, and 12 of 30 *R. violacea* nests discovered in the field had collections of up to 50 *D. masonii* fruits scattered at their entrance. However two observations of *D. masonii* seedlings emerging in groups of 4-6 individuals from buried ant garbage chambers at Extension Hill and Mt Gibson confirms the role of ants in the effective dispersal (and burial) of seed. It is possible that this dispersal process is responsible for the phenomenon of two or more *D. masonii* individuals growing in immediate proximity, with stems frequently observed abutting at their base.

Figure 33 An ant (*Melophorus turneri*) depositing fruits of *Darwinia masonii* on the soil surface close to a nest (left). (right) Four *D. masonii* seedlings germinating from a below-ground garbage chamber – note other seeds and parts of insects, including ants (identified as *Rhytidoponera violacea*).

Lepidosperma gibsonii

Pollen dispersal

Population genetic analyses of *Lepidosperma gibsonii* have shown that pollen dispersal must be extensive across all populations within the Mt Gibson area, due to the extremely low genetic differentiation between populations. This is almost certainly due to wind-dispersed pollen in this species (confirmed by field observations of dry pollen released in clouds at anthesis).

Seed dispersal

The small size, and abiotic dispersal vectors of *L. gibsonii* seed means that their dispersal is difficult to physically track – poor seed production also excluded the possibility of dispersal experiments which would be costly to limited seed stocks. The best method that could be constructed to measure seed dispersal was mapping actual dispersal distance by assignment of seedling genotype to its source plant. Since *L. gibsonii* seed only germinate after fire, this experiment made use of the May 2009 experimental fire to stimulate germination of seedlings. Three 5 x 5 m quadrats were marked out prior to burning, within which all adult plants were mapped, and then sampled and genotyped with 10 microsatellite loci. Plants were sampled exhaustively, with up to 11 samples per clump, since *Lepidosperma* clumps were previously shown to contain multiple intertwined clonal genotypes within larger clumps.

The experimental fire was carried out just prior to the onset of winter rainfall, and seedlings sampled at the end of spring, i.e. after the first season of growth but prior to the first summer. A total of 200 seedlings was collected and assigned to their most likely parents using the program CERVUS (Kalinowski et al. 2007). Results of this work are being prepared for publication.

Identity of seed dispersal agents

Insufficient seed was available for comprehensive studies of dispersal in *Lepidosperma gibsonii*; however seed have no apparent external dispersal adaptations. A small sample of fruits offered to captive seed-dispersing ants (*Rhytidoponera violacea*) collected from Extension Hill did not result in fruits been removed. The location of *L. gibsonii* seedlings, concentrated below rocks, in spouts and flow points on rocky slopes suggest that at least some seed is moved and concentrated by gravity (perhaps stimulated by scratching birds – e.g. Mallee Fowl), or flowing water.

Summary

- Effective pollen and seed dispersal distances were determined for both species.
- Experiments confirm the key role of ants in dispersal of *D. masonii* seed. Ants appear likely to collect and move nearly all fallen *D. masonii* seed, concentrating undamaged seeds in below-ground garbage chambers or surficial garbage piles. Predation of seeds by ants was not recorded.
- Observation suggests that water may be the primary dispersal vector of *L. gibsonii* seed.

Recommendations

- If collection of seed of *D. masonii* or *L. gibsonii* from the ground is to be attempted, allowance should be made for their dispersal processes i.e. – specifically where seeds might be concentrated.

4.4 PVA MODELLING

Population viability analysis (PVA) modelling of demographic processes in both species was proposed to assess population growth rates, and population (and species) extinction likelihoods, taking into account impacts of the loss or augmentation of populations, as well as variation in climate and fire regimes. This modelling would be based on demographic data derived from the demography and seed longevity programs (incorporating plant survival, growth, seed production, seed bank dynamics, fire response). The input data is required to be representative of the range of annual variation in each trait and associated with measured variation in climate (e.g. rainfall). With such data it is possible to model variation in population behaviour in relation to realistic climate data variation – manipulating the frequency or sequence of years of different types. The impact of fire can similarly be modelled by inserting fire years at varying intervals, varying the age and number of populations burnt. Soil seedbanks are an essential part of population dynamics of both DRF species, but add considerable complexity to models, and require accurate data to populate them.

The patterns of annual variation demonstrated through the period of the survey indicate that the data collected is not sufficient to construct worthwhile PVA analyses for either species. A greater number of years of data are required in order to capture sufficient natural variability for such models to make sense.

Examples of key processes inadequately represented by the three-year survey period include: growth rates of older *D. masonii* plants - which averaged negative growth; *L. gibsonii* seed production which occurred in only one of four observation years; and episodic mortality of *D. masonii* adults as observed in 2010. The longevity of seed in soil seed-banks of both species is equally important. Clearly *D. masonii* growth rates are not negative in the long term, the sample years have not captured anything like the mean rate of growth. Similarly it is unreasonable to assume that *L. gibsonii* reproduction occurs precisely once every three years. PVA modelling cannot be reliable without reasonable values of these (and other) parameters, including reliable estimates of both rates averages and variability (including correlates of this variability). It is clear that a longer period of sampling is required before confidence in the values for these two parameters (and many others) would be sufficient. For *L. gibsonii* two or three seasons with effective reproduction, and for *D. masonii*, enough years to give an average rate of plant growth that is at least positive, and ideally not dissimilar to the long term average derived tentatively from analysis of population structures. Soil seed-bank seed longevity is currently being examined in a program that was designed to monitor survival for ~5 years. For *L. gibsonii* this still has 4 years to run and for both species even a five year sample period may not fully indicate seed bank longevity. These complexities are inherent in species with slow and / or episodic growth dynamics, which are unfortunately common in semi-arid systems.

Without such data, PVA could be performed, but its results would be unreliable, unrealistic and likely unreasonable.

Summary:

- This component is incomplete due to the scale of annual variation in key demographic parameters relative to the project's running period.

Recommendations:

- Continued monitoring of plants in permanent plots and maintenance of seed burial experiments, including attention to regularity and timing of monitoring and adequate quality control and management and storage of data until confidence in key demographic parameters is confirmed and PVA can be performed.

4.5 ENVIRONMENTAL INTERACTIONS AND PLANT HEALTH

4.5a Abiotic associations

Two approaches to determining environmental associations of *D. masonii* and *L. gibsonii* were taken, one, an analysis of site factors assessed at locations where plants were surveyed, and the other modelling of species distributions against spatially mapped environmental data.

Site factors assessed at each demographic and physiological monitoring site are listed in Table 28. Canopy openness was assed via analysis of fish-eye photographs taken at 3 locations in each plot using a 180° angle lens adaptor to take full-sky hemispherical images (e.g. Figure 34). The camera was mounted on a tripod at 40 cm above the ground, levelled with a bubble level with the lens pointing directly upwards and oriented with north at the top of the image. Images were analysed using Gap Light Analyser (v 2.0, 1999) image analysis software for % canopy openness – the proportion of the vertical hemisphere that is not obscured by plants or surrounding hills. Site surface attributes estimated on the ground included % surface area covered by soil crusts (e.g. lichens), litter, gravels, outcropping rock, etc. and mean vegetation canopy height. Altitude, slope and curvature, together with solar radiation receipt were derived from a 1 m interval contour map (see this section below for details). Soils were collected from each site and analysed for pH, electrical conductivity, organic content and major plant minerals and other elements at the WA Chemistry Centre.

Several differences were determined between sites with *D. masonii* and sites with *L. gibsonii* (Table 28). Almost all soil elements analysed were less abundant in *D. masonii* than *L. gibsonii* sites, but only Ca, K and Ni were significantly lower. Sites with *D. masonii* also had significantly lower slopes and significantly greater solar radiation receipt at several times in the year, as well as large, but non-significant differences suggesting greater canopy openness and rock cover relative to sites with *L. gibsonii*. These results suggest that *D. masonii* typically occurs in flatter, hotter (drier), rockier and more open locations with poorer soils than does *L. gibsonii*.

Figure 34. Examples of hemispheric images used in estimation of % canopy openness at each site. Note the horizon visible around much of the perimeter of the right-hand image.

Table 28. Site factors assessed for demography survey, physiology survey and translocation sites, mean ± SE (n) within localities with *D. masonii*, *L. gibsonii* or neither (translocation and comparator species physiology sites). Asterisks indicate significant differences between *L. gibsonii* and *D. masonii*: sites * <0.005, ** <0.0005. To minimise type I errors due to the large number of tests an α of 0.005 is employed. Near-significant tests, *P* <0.05 are indicated '+'.

species	units	*L. gibsonii* sites	**sig.**	*D. masonii* sites	neither
canopy openness	%	62 ± 4 (7)	+	74 ± 4 (11)	
crust cover	%	13 ± 7 (10)		6 ± 3 (12)	
gravel cover	%	32 ± 5 (11)		27 ± 5 (15)	
rock cover	%	28 ± 6 (11)		44 ± 6 (16)	
bare soil	%	22 ± 3 (11)		19 ± 4 (16)	
litter cover	%	20 ± 6 (11)		19 ± 4 (16)	
debris >1cm cover	%	1.4 ± 0.5 (11)		1.8 ± 0.3 (16)	
vegetation cover	%	43 ± 5 (11)		36 ± 3 (16)	
vegetation height	m	2.5 ± 0.5 (11)		2.7 ± 0.5 (15)	
altitude	m asl	385 ± 7 (13)		385 ± 7 (19)	377 ± 6 (15)
slope	°	16.7 ± 1.9 (13)	**	9.4 ± 0.9 (19)	7.9 ± 1.2 (15)
curvature-profile	concave +	1.44 ± 0.59 (13)	+	0.01 ± 0.26 (19)	0.12 ± 0.11 (15)
curvature-plan	convex -	1.36 ± 0.72 (13)		-0.54 ± 0.44 (19)	0.24 ± 0.19 (15)
Solar Radiation					
July 10 am	w.m^{-2}.hr^{-1}	0.33 ± 0.05 (13)	**	0.83 ± 0.05 (19)	0.69 ± 0.04 (16)
July 12 pm	w.m^{-2}.hr^{-1}	0.91 ± 0.06 (13)		0.95 ± 0.03 (19)	0.93 ± 0.02 (16)
July 2 pm	w.m^{-2}.hr^{-1}	0.59 ± 0.05 (13)	**	0.82 ± 0.03 (19)	0.79 ± 0.03 (16)
July 4 pm	w.m^{-2}.hr^{-1}	0.24 ± 0.04 (13)		0.33 ± 0.04 (19)	0.27 ± 0.02 (16)
Equinox 10 am	w.m^{-2}.hr^{-1}	0.67 ± 0.05 (13)	**	1.11 ± 0.05 (19)	1.00 ± 0.03 (16)
Equinox 12 pm	w.m^{-2}.hr^{-1}	0.78 ± 0.05 (13)	**	1.12 ± 0.02 (19)	1.08 ± 0.03 (16)
Equinox 2 pm	w.m^{-2}.hr^{-1}	0.96 ± 0.04 (13)		0.97 ± 0.03 (19)	1.07 ± 0.02 (16)
Equinox 4 pm	w.m^{-2}.hr^{-1}	0.74 ± 0.05 (13)		0.67 ± 0.05 (19)	0.76 ± 0.02 (16)
December 10 am	w.m^{-2}.hr^{-1}	0.99 ± 0.06 (13)	+	1.17 ± 0.03 (19)	1.07 ± 0.02 (16)
December 12 pm	w.m^{-2}.hr^{-1}	0.79 ± 0.04 (13)	*	0.89 ± 0.01 (19)	0.90 ± 0.01 (16)
December 2 pm	w.m^{-2}.hr^{-1}	1.09 ± 0.03 (13)		1.03 ± 0.04 (19)	1.13 ± 0.01 (16)
December 4 pm	w.m^{-2}.hr^{-1}	1.07 ± 0.05 (13)	+	0.84 ± 0.05 (19)	1.01 ± 0.02 (16)
EC	(1:5) mS/m	7.6 ± 1.2 (12)		5.5 ± 0.5 (18)	6.6 ± 0.9 (20)
pH	(CaCl$_2$)	5.1 ± 0.2 (12)		4.9 ± 0.1 (18)	4.8 ± 0.1 (20)
OrgC	(W/B) %	3.2 ± 0.3 (12)	+	2.4 ± 0.2 (18)	1.5 ± 0.2 (20)
N	(total) %	0.15 ± 0.01 (12)		0.11 ± 0.01 (18)	0.08 ± 0.01 (20)
P	PRI mL/g	41 ± 5 (12)		55 ± 6 (18)	44 ± 8 (20)
B	mg/kg	0.91 ± 0.08 (12)		1.01 ± 0.05 (18)	0.58 ± 0.09 (20)
Ca	mg/kg	1025 ± 169 (12)	*	578 ± 53 (18)	525 ± 79 (20)
Co	mg/kg	0.17 ± 0.02 (12)		0.19 ± 0.02 (18)	0.23 ± 0.06 (20)
Cu	mg/kg	1.33 ± 0.13 (12)		1.38 ± 0.12 (18)	0.89 ± 0.11 (20)
Fe	mg/kg	98 ± 21 (12)	+	59 ± 5 (18)	63 ± 5 (20)
K	mg/kg	151 ± 11 (12)	*	108 ± 8 (18)	86 ± 8 (20)
Mg	mg/kg	171 ± 17 (12)	+	110 ± 13 (18)	67 ± 10 (20)
Mn	mg/kg	72 ± 10 (12)		68 ± 5 (18)	30 ± 4 (20)
Na	mg/kg	34 ± 6 (12)		24 ± 3 (18)	15 ± 2 (20)
Ni	mg/kg	0.38 ± 0.05 (12)	*	0.20 ± 0.03 (18)	0.2 ± 0.02 (20)
S	mg/kg	12.7 ± 1.1 (12)		15.4 ± 1.6 (18)	15.9 ± 1.6 (20)
Zn	mg/kg	1.5 ± 0.2 (12)	+	1.0 ± 0.1 (18)	5.0 ± 2.3 (20)
Pb	mg/kg	0.79 ± 0.08 (12)		0.84 ± 0.04 (18)	1.19 ± 0.21 (20)

Modelling of species distributions was undertaken using presence records from our own data, together with that of consultant's surveys (ATA 2004, 2006) and some provided from Extension Hill Pty Ltd. These data were combined into a single GIS layer totalling 2534 presence records for *D. masonii* and 912 for *L. gibsonii*. Variation in the numerical resolution represented by the *L. gibsonii* data was extreme, with many location records representing single plants, other representing several or many plants, and a small number representing several thousand individuals each. This data was therefore transformed to a 20m grid, where a single point was located on the grid if one or more plants were recorded within the surrounding 20×20m area. Large populations delineated with two or more point on their margins only were extrapolated appropriately to include more grid points.

Models were constructed using MaxEnt software (V3.3.1; Phillips *et al*. 2006, 2008) which compares environmental data for points where species presences are recorded with equivalent environmental data a large sample (10,000 in this case) of randomly selected 'background' points from the sampled landscape. The MaxEnt routine then select the simplest set of transformations and interactions of the environmental layer data that provide a best fit to the recorded localities. This can then be reprojected onto the spatial maps of the environmental data to produce a map of presence likelihoods. Other MaxEnt outputs include response curves for the model, which show how modelled presence likelihoods change with each environmental variable, and a table indicating the relative significance of each variable to the total model fit. Finally, model fit is tested using AUC / ROC statistics. MaxEnt is increasingly used in ecological research and studies comparing this and other approaches generally find the performance of MaxEnt to be among the best, and often actually the best approach (e.g. Elith *et al*. 2006).

The environmental layer data employed in this study were derived from three sources: Mt Gibson Iron Deposit Geological Plan (Pickands 1967) for geology, satellite and air photo imagery from 1969 to 2007 for fire history (see Figure 10 and Figure 11 for the derived fire history), and the 1m interval contour map available from the region received from Extension Hill. A number of parameters were derived from the contour data – Solar radiation receipt, Aspect, Slope, Curvature, and Elevation – these were calculated (by Sauter Geological consulting) with a 5 m horizontal resolution. Solar radiation (SR) was calculated (following Coleman *et al*. 2009) for the specific latitude of the site on three specified dates (solar equinox and the winter and summer solstices), and for four times on these days (10 am, 12, 2 and 4pm). SR results take into account site shading from nearby topography, assume a constant solar influx and are expressed in $w.m^{-2}.hr^{-1}$. Slope, aspect and curvature were calculated from the 1m contour data in mapinfo’s Discover>Surfaces utility.

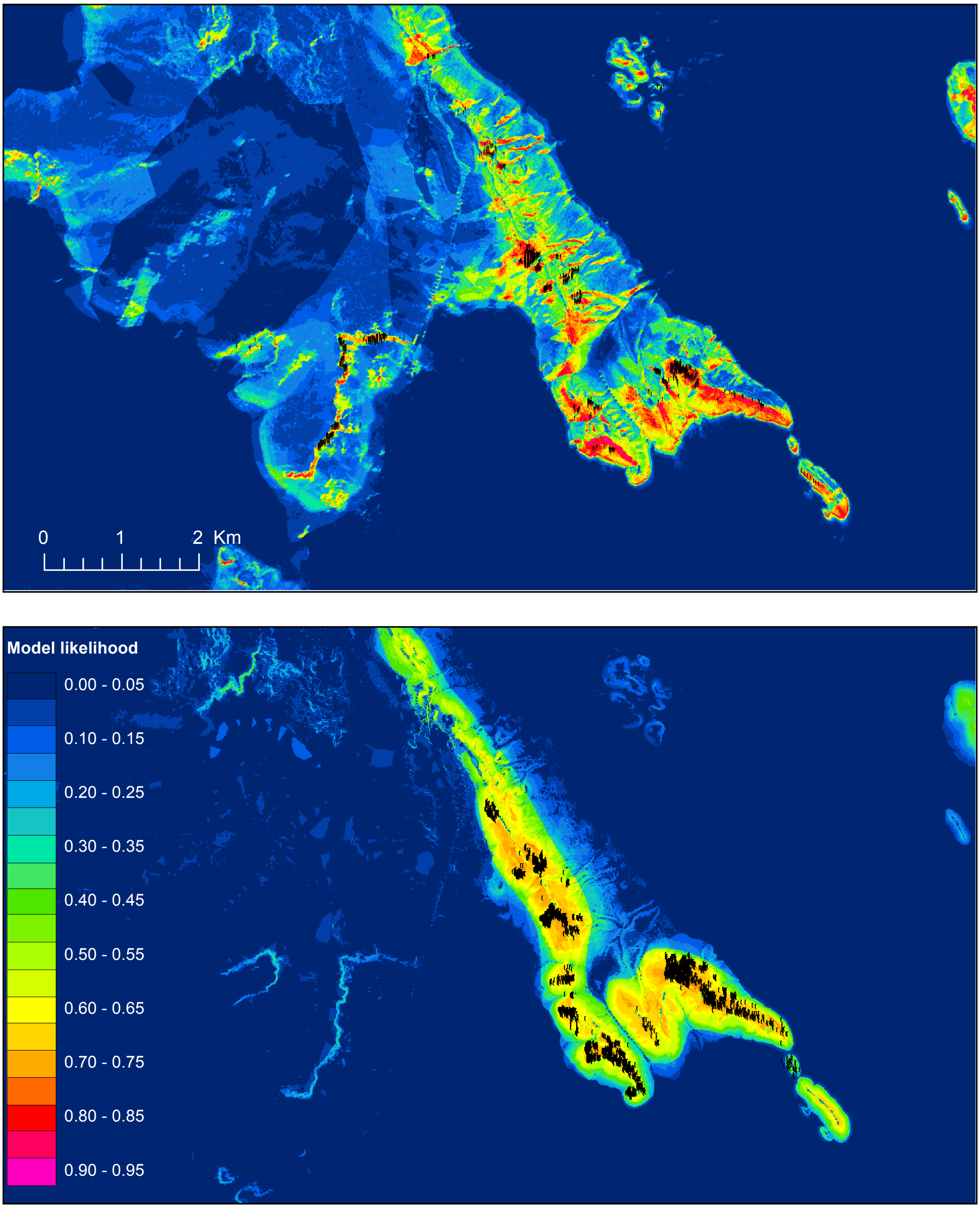

Figure 35 Maxent model outputs showing probabilities of presence (blue = low, red = high) for *Lepidosperma gibsonii* (top) and *Darwinia masonii*. Black dots indicate known localities.

A breakdown of parameters contributing to model predictions shows that, for *L. gibsonii*, winter 2 pm solar radiation contributed to 40% of the predictive model, with elevation and slope contributing most of the remainder (Table 29). For *Darwinia masonii*, slope alone makes an 80% relative contribution to model predictions, with elevation, geology and summer midday SR making up most of the rest. Fire history since 1969 did not contribute to model predictions.

Models for both species predicted their respective distributions well (AUC = 0.988 for both *D. masonii* and 0.98 for *L. gibsonii* species: the maximum possible value = 1). Models predicted a detailed pattern of presence probabilities for *L. gibsonii*, with many small areas identified with a >90% likelihood of presence, but a broader pattern of high (60-75%) probability of occurrence for most of the Mt Gibson range for *D. masonii* (Figure 35). Both models predicted few localities outside of the known range, with the exception of Yandanhoo Hill in the far east.

Table 29 Estimate of relative contributions of the environmental variables to Maxent models for *Lepidosperma gibsonii* and *Darwinia masonii*.

Lepidosperma gibsonii	
variable	% contribution
Winter SR 2pm	40.3
elevation	29.5
slope	13.6
geology	4.6
aspect	3.3
fire	2.2

Darwinia masonii	
variable	% contribution
slope	79.5
elevation	15.4
geology	1.7
summer SR noon	1.4
fire	1.0

The response curves from the model output indicate that the association of *L. gibsonii* distribution with SR is a negative one (Figure 36), with probabilities of *L. gibsonii* presence of 80% predicted for areas with <0.4 w.m^{-2}.hr^{-1} and a rapid fall in likelihoods at around 0.7 w.m^{-2}.hr^{-1}.

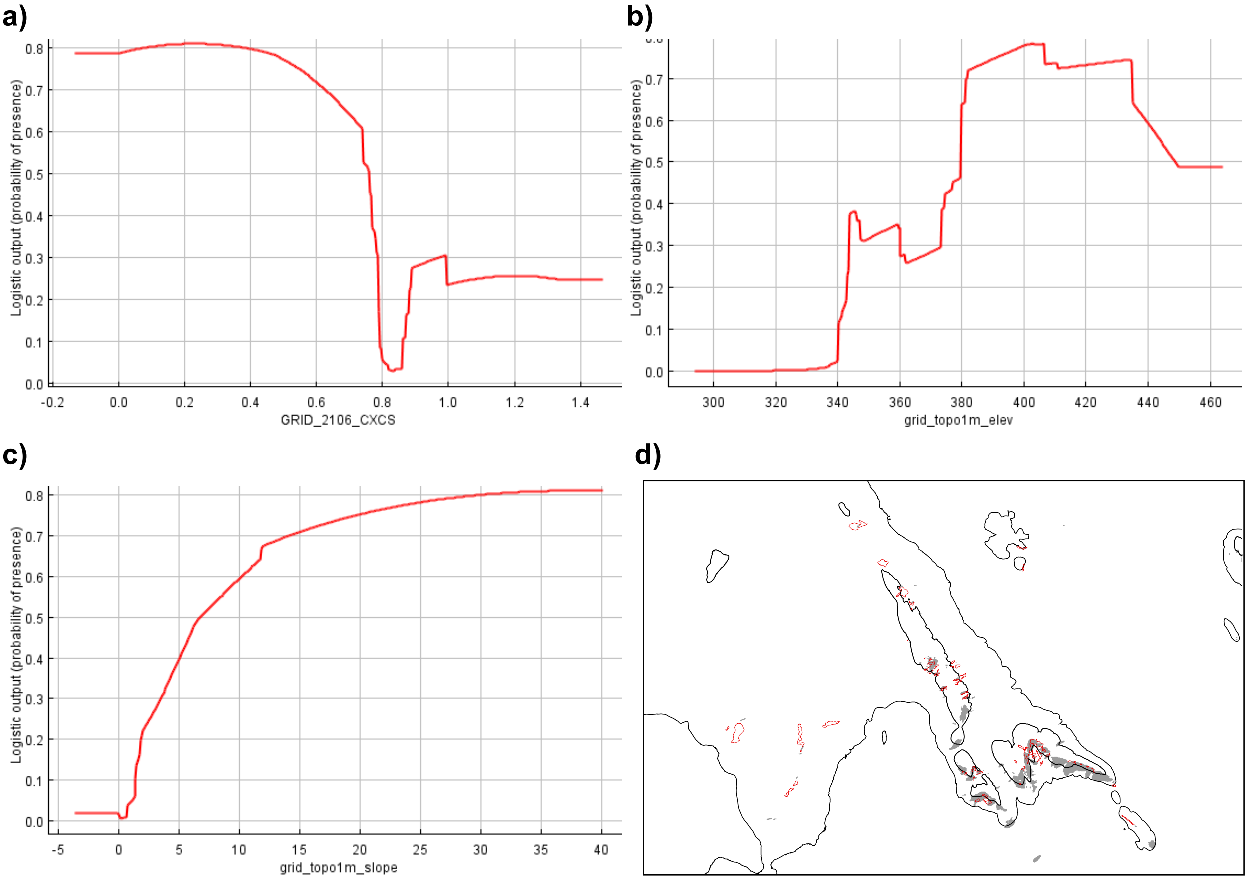

Figure 36 Response curves of the key variables influencing Maxent model predictions of *Lepidosperma gibsonii* showing presence probability estimates if the shown parameter is used alone as a model input. **a)** Winter 2 pm solar radiation receipt (w.m^{-2}.hr^{-1}), **b)** elevation (m) and **c)** slope (degrees). **d)** Distribution of *L. gibsonii* (red outline) in relation to winter 2 pm solar radiation <0.6 w.m^{-2}.hr^{-1} (grey) and elevation (340 and 380 m contours)

The response curves of the principal environmental parameters predicting *Darwinia masonii* distribution – elevation, slope and geology – suggest that their contribution to the model is solely to select the Mt Gibson range (Figure 37). Slopes over 7-8°, elevation over 380 m and all geology types except for 14 and 99 have an associated probability of 50-60% if considered alone. Geology unit 99 represents areas not covered by the mapping. As mapping focussed on the ridges and slopes of the range, a negative association with this unit effectively identifies the range and foothills. Unit 14 is “White Rock (unclassified, including granite & its group, acidic dyke rocks, feldspar porphyry & meta-sediments phyllitic rock)” this captures the footslopes of the ranges and the saddles between major hills. Thus elevation, slope and geology parameters combine to indicate simply that *D. masonii* is associated with the slopes and ridges of the BIF range.

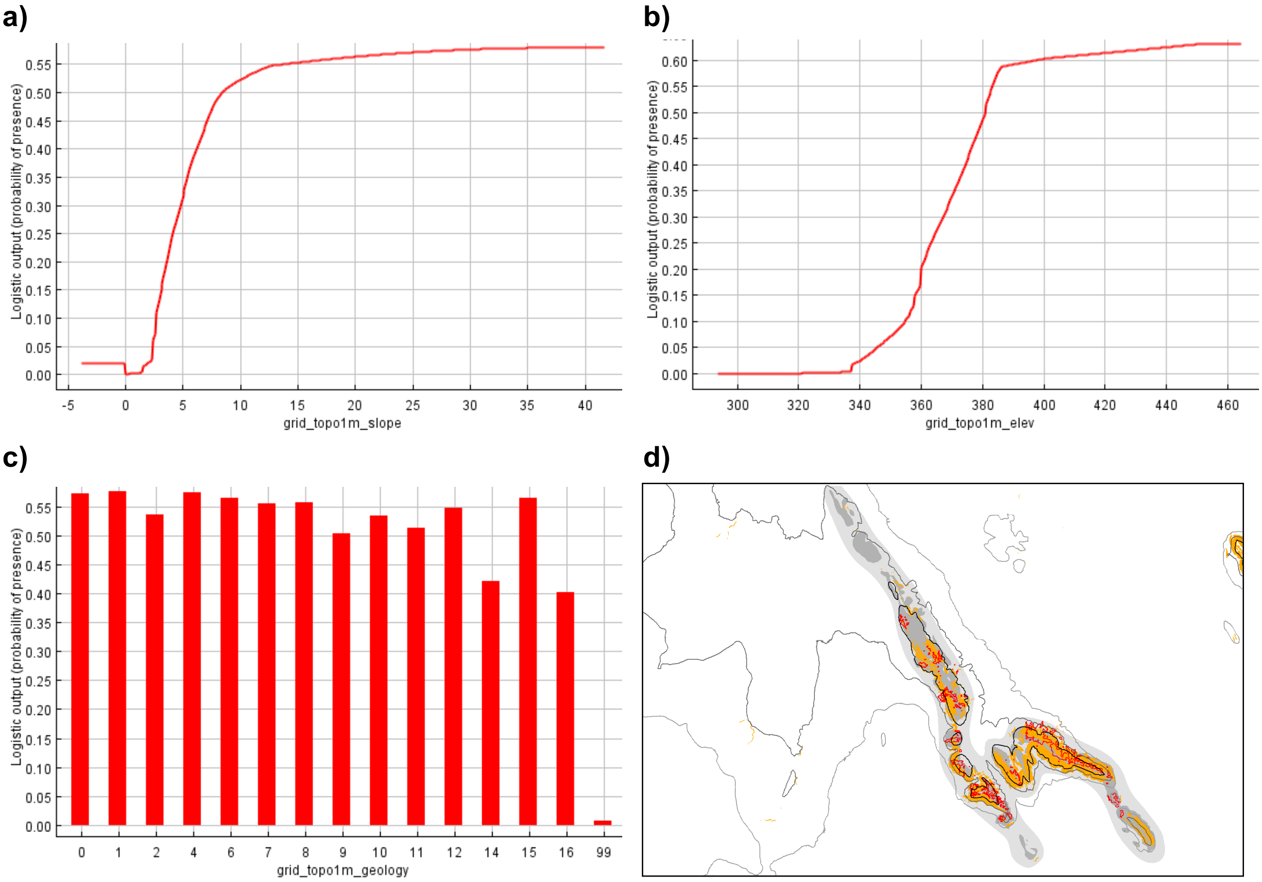

Figure 37 Response curves of the key variables influencing Maxent model predictions of *Darwinia masonii* showing presence probability estimates if the shown parameter is used alone as a model input. **a)** slope (degrees), **b)** elevation (m) and **c)** geology – see text for codes. **d**) distribution of *D. masonii* (red outline) in relation to slope >12° (orange), elevation (340, 360 and 385 m shown) and geology (white = 99, light grey = 14, all others, dark grey).

Implications

The maxent models for *D. masonii* and *L. gibsonii* identify the species' respective habitats with differing degrees of detail. The *L. gibsonii* model identifies locations which are the coolest part of the landscape. Low solar radiation means relatively low receipt of light and heat, which have differing implications for plant behaviour and plant environment. Plants require light for photosynthesise but, particularly if deficient in water or nutrients, too much light can damage plant tissues: during summer droughts, damage to plant photosystems from excess sunlight can lead to long-term damage. Heat loads associated with solar radiation receipt can also influence plant physiology, but more importantly, by promoting evaporation, soil moisture availability. Field-based manipulative experiments examining the interactions of heat, soil moisture and photoinhibition and photosystem damage would be required in order to disentangle the physiology reflected in the association of *L. gibsonii* with low radiation areas. However the strength of this association is clear. The habitat of *D. masonii*, on the other hand, is not so circumscribed and appears to be simply gravelly / rocky iron-rich loams.

Lepidosperma gibsonii is already known to occupy many of the areas that it is most strongly predicted to occupy. It is suspected that most of the remaining highly predicted areas will also contain populations if they were to be surveyed. In contrast, *D. masonii* is predicted to occur broadly across the ranges with a high likelihood but not predicted to occur anywhere with a very high likelihood. Many areas where populations are most highly predicted are known to not support *D. masonii* individuals. These results suggest three things. Firstly, that while the factors limiting the distribution of *L. gibsonii* are described by the environmental variables modelled, the distribution *D. masonii* may be limited by factors not included in the model inputs. Unincorporated factors might include attributes of the regolith – soil depth, underlying rock structure, etc – and longer-term fire-regime. Fire history since 1968 was included as a model input but did not contribute to model predictive capacity. On the other hand, it is likely that longer term fire history patterns not captured by the limited temporal coverage of the data may be important. It seems likely that some areas of the Mt Gibson range are more fire-prone than others – rocky open sites may be less able to carry fire and therefore burn less frequently, for instance.

Secondly, potential restoration and translocation areas for *L. gibsonii* need to be low SR and able to maintain higher soil moisture levels than the landscape average, but all such existing locations are likely to be already occupied. For *D. masonii*, models suggest that iron rich gravelly loams seem likely to be all that is required of a suitable translocation or restoration site, and there are many such areas available which are currently unoccupied if translocation was required. However further details of the substrate may still be important to ensure restoration success – for instance it is not known whether the key attributes of such substrates for *D. masonii* survival will be recreated in restoration.

The final implication, therefore, is that restoration trials for each species should include treatments addressing these uncertainties – i.e. shade and moisture for *L. gibsonii* and attributes of soil depth and rockiness for *D. masonii*.

Summary

- Species distribution models were constructed for *D. masonii* and *L. gibsonii* using the Maxent technique to assess the association of both species with key environmental variables.

- Models were able to provide good descriptions of the species' respective distributions, and identified different habitat attributes for each, consistent with different observed micro-site distributions of the two species.

- Results have implications – for survey for new populations, selection of potential translocation sites, construction of restoration areas and research into plant interactions with their environments – which are particular each species.

Recommendations

- Restoration areas for *L. gibsonii* should be shaped as slopes or gullies with lower radiation receipt.

- Restoration trials for *L. gibsonii* should include treatments varying shade and moisture.

- Mapping of soil or regolith data for the region to refine distribution models to improve understanding and predictions of the habitat and restoration requirements for *D. masonii*

- Restoration areas for *D. masonii* may not require particular topographies, but attention to soil requirements may be important.

- Restoration trials for *D. masonii* should include treatments varying degrees of soil depth and rockiness.

4.5b Translocation study of environmental boundaries

A pilot translocation trial was installed in May 2005 on a ripped drill pad on Iron Hill East. In this trial, 206 *Darwinia masonii* cuttings were planted in a grid connected to a drip feed irrigation system which supplied water for an hour at a time, twice a month over the first two summers. The irrigated plants were fenced to exclude herbivores. Twenty additional plants were planted outside of the fenced and irrigated area. Ten percent (2) of these unwatered plants survive to 2010, while 89% of the watered plants survive. The surviving irrigated plants have grown rapidly to a large size and (tripling in size in their first 18 months) and have flowered extensively and precociously (with half flowering in the first year).

A second restoration trial was established in the winter of 2009. This experiment included planting nursery stock of *D. masonii* and *L. gibsonii* – established respectively from cuttings and separated clumps, together with seedlings of *Acacia cerastes* (a local species with P1 conservation status).

Plantings occurred in four sites with differing field soil substrates which included 1) deep red loam/clay plains east of Extension Hill – a material potentially available in abundance for restoration as it underlies most of the planned waste rock dump, 2) white-yellow sands of sandplains west of Extension Hill and 3) gravelly and 4) rocky loams of the north Extension Hill slope and ridge (Table 30).

Three 10m x 10m replicate plots per site were cleared and fenced before planting (Table 30). A total of 780 plants of each species were planted in July/August 2009, with 65 per plot. Monitoring of these plants started after one month and is ongoing at 3 month intervals. Monitoring has included demographic (survival, health, height, fecundity) and ecophysiological parameters (leaf gas exchange and plant water status – measured via a Li-6400 gas analyser; LI-COR Inc, Lincoln, Nebraska, USA and a Scholander-type pressure chamber; Wescor Inc., Logan, UH, USA).

Table 30 Location of Translocation Sites on the Mt. Gibson Range.

Site	Substrate	Replicate	Locality	
1	BIF Rock	1	S29° 34’ 03.9”	E117° 09’ 21.9”
2	BIF Rock	2	S29° 34’ 03.1”	E117° 09’ 21.1”
3	BIF Rock	3	S29° 34’ 04.1”	E117° 09’ 21.1”
4	BIF Gravel	1	S29° 34’ 04.2”	E117° 09’ 20.5”
5	BIF Gravel	2	S29° 34’ 03.5”	E117° 09’ 19.8”
6	BIF Gravel	3	S29° 34’ 04.3”	E117° 09’ 19.6”
7	Sand	1	S29° 34’ 04.8”	E117° 09’ 17.8”
8	Sand	2	S29° 34’ 04.2”	E117° 09’ 17.3”
9	Sand	3	S29° 34’ 04.7”	E117° 09’ 16.6”
10	Clay	1	S29° 34’ 19.1”	E117° 10’ 29.4”
11	Clay	2	S29° 34’ 18.3”	E117° 10’ 30.4”
12	Clay	3	S29° 34’ 17.2”	E117° 10’ 30.3”

By April 2010 (9 months after planting), results showed clear differences among substrates. *Acacia cerastes* established on all of the four substrates, but least successfully at the Clay site (Figure 38). *Darwinia masonii* and *L. gibsonii* demonstrated a greater specificity for substrate type with survival successful only in the BIF Rock and BIF Gravel substrate. While occasional *L. gibsonii* plants persist on the other sites they show very limited vigour and survival prospects. In terms of mean plant health and height, plants of all three species performed better growing on BIF rock and BIF gravel loams than on the clay and sandy substrates. Measurement of the physiological attributes of these plants – rates of photosynthesis, transpiration and water potential – confirm the poor performance of *D. masonii* and *L. gibsonii* on sand and clay substrates, and indicate relatively poorer performance of *Acacia cerastes* on clay.

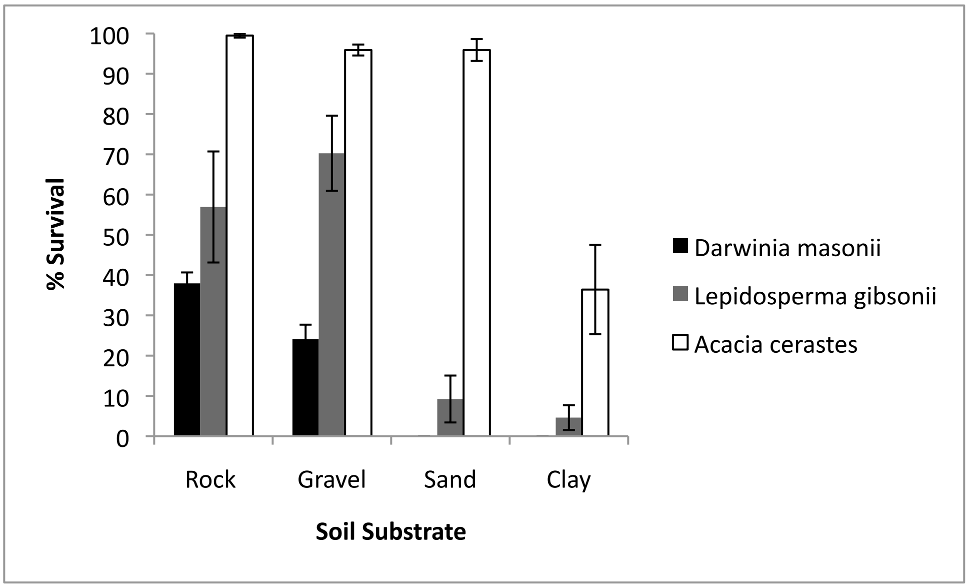

Figure 38 Survival rates of *D. masonii* and *L. gibsonii* planted together with *Acacia cerastes* on four differing soil substrates – nine months after planting.

Table 31. Chemical properties of translocation substrate soils. Significance (ANOVA) indicates *p<0.05, ***p<0.001

	units	rock	gravel	sand	clay	sig
EC	mS/m	7.00 ± 2.00	5.67 ± 2.08	5.33 ± 2.08	2.00 ± 0.00	*
pH	($CaCl_2$)	5.07 ± 0.15	4.5 ± 0.35	5.33 ± 0.38	4.33 ± 0.58	*
Organic C	%	1.91 ± 0.21	1.54 ± 0.32	0.98 ± 0.04	0.53 ± 0.12	***
Cu	mg/kg	0.77 ± 0.12	0.53 ± 0.25	0.23 ± 0.06	1.13 ± 0.15	***
Ca	mg/kg	610 ± 61	393 ± 250	393 ± 93	223 ± 170	
Fe	mg/kg	76.3 ± 8.7	74.0 ± 8.2	75.0 ± 2.6	27.7 ± 2.5	***
K	mg/kg	90.7 ± 8.5	74.3 ± 22.0	43.0 ± 4.4	76.7 ± 46.2	
Mg	mg/kg	66.3 ± 11.1	41.0 ± 21.5	36.0 ± 16.8	60.0 ± 52.0	
P	mL/g	48.3 ± 12.7	64.3 ± 28.9	11.0 ± 1.0	66.0 ± 33.8	
Mn	mg/kg	24.7 ± 3.8	13.3 ± 7.2	16.3 ± 1.2	39.3 ± 25.5	
Na	mg/kg	15.7 ± 6.1	14.0 ± 7.5	14.0 ± 4.6	7.7 ± 4.7	
S	mg/kg	17.0 ± 3.5	20.3 ± 8.0	12.7 ± 3.2	10.0 ± 3.5	
N total	%	0.10 ± 0.01	0.07 ± 0.02	0.05 ± 0.00	0.04 ± 0.01	***
B	mg/kg	1.00 ± 0.44	0.80 ± 0.52	0.57 ± 0.12	0.40 ± 0.17	
Co	mg/kg	0.08 ± 0.02	0.04 ± 0.01	0.08 ± 0.02	0.56 ± 0.35	*
Ni	mg/kg	0.17 ± 0.06	0.13 ± 0.12	0.17 ± 0.06	0.30 ± 0.10	
P	mg/kg	5.67 ± 2.08	3.67 ± 1.53	4.00 ± 0.00	5.33 ± 0.58	
Zn	mg/kg	1.00 ± 0.36	0.67 ± 0.23	0.5 ± 0.17	0.83 ± 0.23	
Pb	mg/kg	1.00 ± 0.00	0.87 ± 0.15	0.43 ± 0.06	0.87 ± 0.12	***

The properties of translocation site soils were assessed via chemical analysis (WA Chem Centre, one bulked sample of 5 subsamples of 0-10cm depth per plot) and soil moisture probes. Three moisture probes were installed in one plot of each substrate type: these were set to record every 10 minutes (Hobo microstation S-SMC-M003 ECH2O soil moisture probes: Onset Computer

Company). Soil chemistry differed several respects between sites, most significantly in C content, Fe, Cu, Pb and total N. Electrical conductivity, pH and Co also varied significantly (Table 31). pH was highest at the Sand site and lowest at Clay – although all were acid, and EC was much lower at the Clay site than any other (associated with lower Na and Ca). Organic C content and % total N were highest at the Gravel and Rocky loam sites. Fe was lowest, and Cu and Co highest at the Clay site. Of these differences, N is likely the most important for plant growth and survival.

Higher % organic C in Gravel and Rock site soils may be indicative of a simple, but significant, role of gravel and rock in these substrates. By excluding water and other inputs., the presence of gravel and rocks in soil means that identical rainfall inputs over a unit area of surface is concentrated into a smaller volume of soil so that in a uniform rainfall event, rocky soils receive and hold a greater volume of water than non-rocky soils. The same process occurs with other soil inputs sourced from above-ground, such as organic C.

In seasonal and low rainfall regions, soil water availability is usually more limiting to plant growth than soil nutrition. Soil probe outputs show that while soils attain similar maximum and minimum soil moisture concentrations, the Sand, and Clay site soils appear to dry out more rapidly than those at the Rock and Gravel sites (Figure 39). Clay site soils appear to wet more quickly, or more responsively to smaller rain events, but the Gravel and Rock site soils also reached slightly higher maximum water contents than the other sites (27-28% v 24-25%). Soils of different composition and texture bind water with different potentials, so that water in soils of differing texture but similar water content may, from a plant's point of view, differ in the availability of water and the ease with which it can be extracted. Also important for plant survival is the period for which plants experience water availability levels above or below key thresholds (e.g. wilting point). While observed soil dry-down rates may not appear to differ markedly, the slightly slower drying curves of the Gravel and Rock sites may lead to plants experiencing significantly longer periods of favourable water availability in these sites.

The pattern of soil moisture with depth in the soil profile is also unknown and may differ between sites. Rock and gravel cover effectively reduce the area of exposed soil surface and thereby act to limit surface evaporative losses on a volume basis.

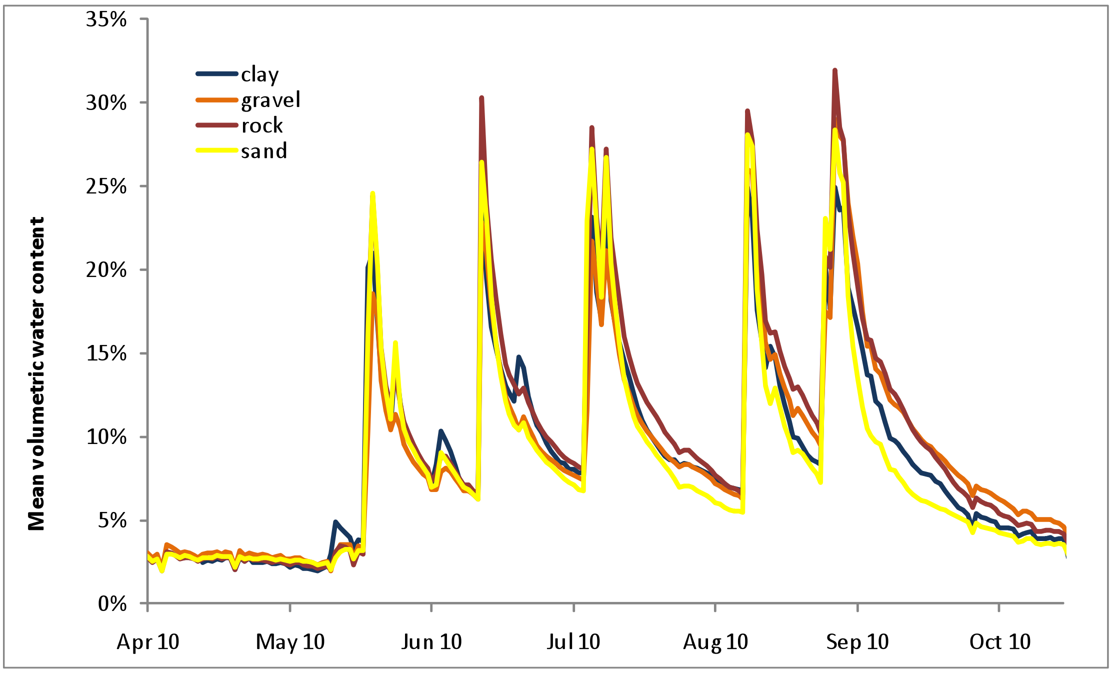

Figure 39 Mean daily maximum soil moisture content at 5cm depth for translocation trial sites on differing substrates (average of three Hobo microstation S-SMC-M003 ECH2O soil moisture probes, Onset Computer Company). Winter 2010

Summary

- Both *D. masonii* and *L. gibsonii* have the ability to be planted and survive in restoration sites

- Survival and establishment of translocated *D. masonii* and *L. gibsonii* individuals was effectively limited to BIF rock and BIF gravel sites

- Survival to 9 months of transplanted *D. masonii* greenstock averaged under 40% at its best performing site (BIF rock)

- Transplanted *L. gibsonii* survival (to 9 months) was greatest on BIF gravel sites (~70%) but was also high (>50%) on BIF rock sites

- Translocation sites differed in several soil properties, of which patterns of moisture content and total Nitrogen may be the most critical.

Recommendations

- Sand and clay substrates may not be effective restoration materials for *D. masonii* and *L. gibsonii*, although mixing clays with quantities of rock and/or gravel may be worth trialling.

- Final restored structure surface should incorporate a large proportion of BIF rock or gravel for successful restoration of both *Darwinia masonii* and *Lepidosperma gibsonii*.
- Field studies in which *D. masonii* and *L. gibsonii* are translocated into restoration substrates designed with varying amounts of rock and gravels, and with rocks at varying depths are recommended to ensure optimal restoration success.

4.5c Drought study

Many of the studies in this and the next section are comparative, with the target species studied in comparison with a range of other taxa. Comparator species included 2-4 of the most closely related taxa (*Darwinia acerosa*, *D. purpurea*, *Lepidosperma* sp. 'costale' Wanara, *L.* sp. 'costale' Beanthiny, *L.* sp. Wubin biconvex, *L.* sp Wubin scabrid) as well as species which are less closely related but co-occur with *D. masonii* and *L. gibsonii* at Mt Gibson but are also common / widespread across a range of habitat types – *Gahnia drummondii* (Cyperaceae) and *Amphipogon caricinus* var. *caricinus* (Poaceae) and *Aluta aspera* subsp. *aspera* (Myrtaceae). The purpose of comparisons with related species is to identify if observed traits are adaptations specific to the target species or shared among close relatives, and therefore pre-dating the evolution of the modern species and excluding specific adaptation to their modern habitats. Comparisons with widespread species indicate the extent to which observed traits are shared with other species in the same habitat.

Drought studies were performed under controlled glasshouse conditions and involved comparisons with several closely related *Lepidosperma* and *Darwinia* taxa. These experiments took two forms, one investigating root growth and biomass accumulation under drought conditions and the other measuring physiological response to declining water availability.

In the root growth and biomass accumulation study 80 individuals from each species were transplanted into free draining custom-made PVC tubes (1 m deep and 0.1 m diameter). Soils were white sands with addition of 59 mL of diluted nutrient solution (200μM $Ca(NO_3)_2$, 100 μM K_2SO_4, 4 μM KH_2PO_4, 54 μM $MgSO_4$, 0.24 μM $MnSO_4$, 0.10 μM $ZnSO_4$, 0.018 μM $CuSO_4$, 2.4 μM H_3BO_3, 0.030 μM Na_2MoO_4, 40 μM Fe-EDTA – following Poot and Lambers 2008). Tubes were held upright in a metal frame and watered twice daily from overhead sprinklers during an establishment period (2 months) prior to drought treatment. Plants were subsequently exposed to drought (no watering) and control (250 mL of water twice a week) treatments over a period of four months.

After 72 days, droughted plants had significantly higher root growth and lower stem growth (ANOVA $p<0.05$) than non-droughted plants in both species (Figure 40). Examination of the distribution of dry mass down the profile in the tube-pots showed that this extra root growth occurred at all depths.

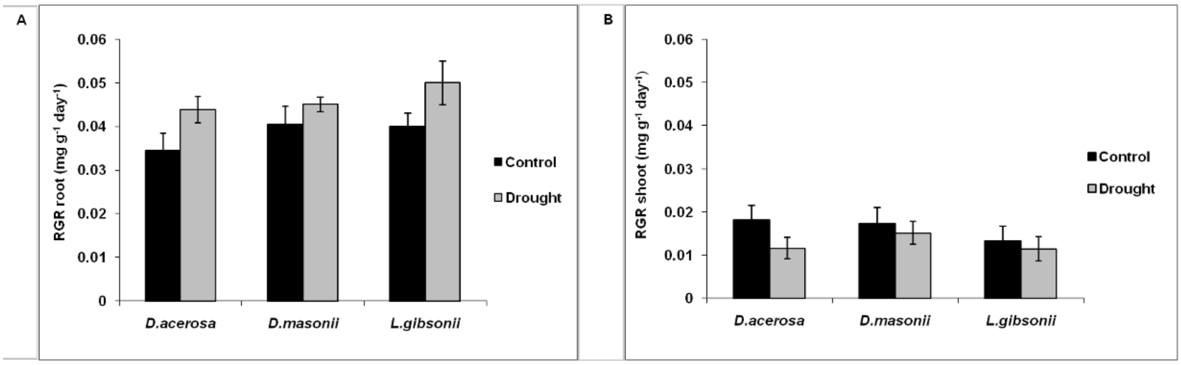

Figure 40. Relative growth rates (RGR) of shoot dry mass (A) and root dry mass (B) over a 72 day period. Comparison of control (black bars) and drought plants (grey bars) *Darwinia acerosa, D. masonii* and *Lepidosperma gibsonii* respectively. Bars represent means ± SE (n=8 per harvest).

In this experiment, *D. masonii* plants had greater total leaf surface area than did *L. gibsonii* (likely simply a result of plant selection), but *L. gibsonii* had a significantly larger difference in total leaf area between control and droughted plants (drought plants had 50% of the area of watered plants) than did *D. masonii* (10% difference).

Treated plants in this study were also examined for their plant water status and photosynthetic activity. Water status was assessed via measurement of both pre-dawn and midday xylem pressure potential using a Scholander-type pressure chamber (Wescor Inc., Logan, UH, USA). Midday plant water potential reflects both the level of soil water potential and the transpiration activity of the plant during the day. The absence of photosynthetic activity during the night means that pre-dawn plant water potential are generally in equilibrium with the soil water potential. Photosynthesis, C-assimilation and transpiration rates were measured for each plant in the study using a portable infrared gas analyser (Li-6400, LI-COR Inc, Lincoln, Nebraska, USA).

The difference between midday and pre-dawn water potential of drought plants was greater for drought plants than controls in *L. gibsonii* but less so for *D. masonii.* There was little difference between species in their rates of photosynthesis and transpiration, although *L. gibsonii* did have slightly lower rates than *D. masonii* at 72 days.

In a second study, 15 plants of each of *D. masonii* and *L. gibsonii* were grown, together with closely relative taxa *D. purpurea*, *D. acerosa*, *L.* sp Wubin bi-convex and *L.* sp Wubin scabrid, in free draining 25 cm diameter × 40 cm deep pots. This study was designed in order to control soil moisture conditions as closely as possible and to ensure that samples of each species were exposed to identical soil moisture conditions. Pots were lined with a fine nylon fabric prevent soil loss and filled with a known weight of oven dried, commercial loamy sand. All materials were weighed in initial dry conditions and then watered to field capacity. Pots were subsequently weighed at intervals to determine soil gravimetric water content. Pots were then watered in controlled volumes to maintain or manipulate water content at desired levels for testing over a period of 85 days. Plant physiology was measured using the Li-6400 as described above and plant drought response curves based on soil water potential were assembled for each species. The relationship between gravimetric water content and soil water potential was established for the experimental soil using an inverse van Genuchten Equation (van Genuchten 1980). This equation was constructed from measurements of the two parameters made using the pressure plate method (Wild 1988) (n = 3) at -0.01, -0.10, -0.3, -1 and -1.5 MPa as well as using the vacuum desiccator method (Bulut 1996) at -39, -98, and -316 MPa.

Water potential is expressed in units of pressure required to remove water from the target material. Its units are negative as the process requires a vacuum to extract water. Values of water potential close to 0 indicate water can be extracted with little effort, while larger negative values indicate dryer conditions.

Plants were exposed to soil water potentials between -0.00052 to -1.3 MPa. Establishing the point at which plant function ceased is a delicate matter as a small change in soil water content corresponded to a large change in soil water potential in very dry soils so that the critical soil water potential when the gas exchange of the plants ceased occurred over a narrow range (a few hours to a few days). Results identify the lowest soil water potential under which plants were measured to be functioning and the next lowest measurement when they were not. *Lepidosperma* sp Wubin scabrid appeared to cease gas exchange at a higher water potential (between -0.6 and -0.7 MPa) than the other *Lepidosperma* species (-1.0 and -1.1 MPa for both *L. gibsonii* and *L.* sp Wubin bi-convex). Differences between the *Darwinia* species were less clear (all between -0.8 and -1.3 MPa) but encompass the range of the *Lepidosperma* species (Figure 41).

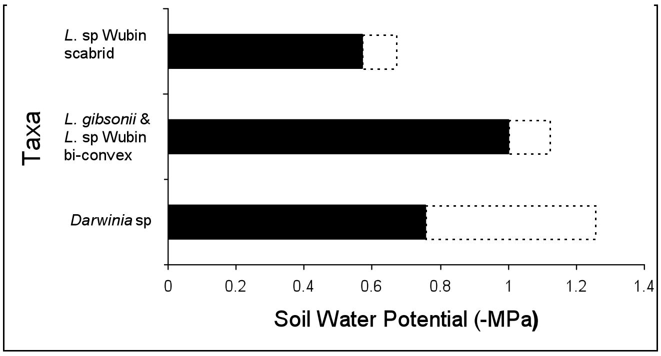

Figure 41 The soil water potential at which measured photosynthesis rate (A) became zero or negative. Black bars are based on last time of measurement when A was still positive, dotted ranges are based on the next time of measurement when A was zero or negative.

Drought response curves differed between genera as *Lepidosperma* species had lower rates of photosynthesis at maximum water potentials than the *Darwinia* species did. *Darwinia masonii* recorded among the highest rates of photosynthetic activity in the study, and *Lepidosperma gibsonii* recorded the lowest even when well watered (Figure 42).

Water use efficiency (WUE) was calculated for examined plants – on the basis of ratio of Carbon assimilated per water lost – across the range of soil water potentials (Figure 43). *Darwinia* species were able to maintain their WUE with declining water availability, while *Lepidosperma* species were able to increase their efficiency. Both *D. masonii* and *L. gibsonii* had relatively low and broadly similar WUE. *Darwinia acerosa*, a DRF species from granite areas closer to Perth, had significantly higher WUE than other *Darwinia* (and *Lepidosperma*) species (t_{78} = 3.64, P <0.001). This habitat of this species has higher mean rainfall than that of any other tested.

It is important to note that the soil water potentials that were able to be imposed in these experiments – and which were ultimately lethal at their lowest levels – were all considerably wetter than the levels that are frequently recorded in the field (e.g. Figure 44). This illustrates the difficulty of transferring glasshouse experimental results to field conditions, but also identifies the key strategy employed by most of the experimental species, including *D. masonii* and *L. gibsonii*, which is to avoid drought. Drought avoidance, as opposed to drought tolerance is a strategy whereby, plants do not function under drought conditions, but instead cease photosynthetic function and try to conserve water and survive through to a time when soils become moist again. The implication of this strategy is that plant tissues must have the capacity to desiccate to a very high degree, remain alive under such conditions and then regain metabolic function when soils rewet.

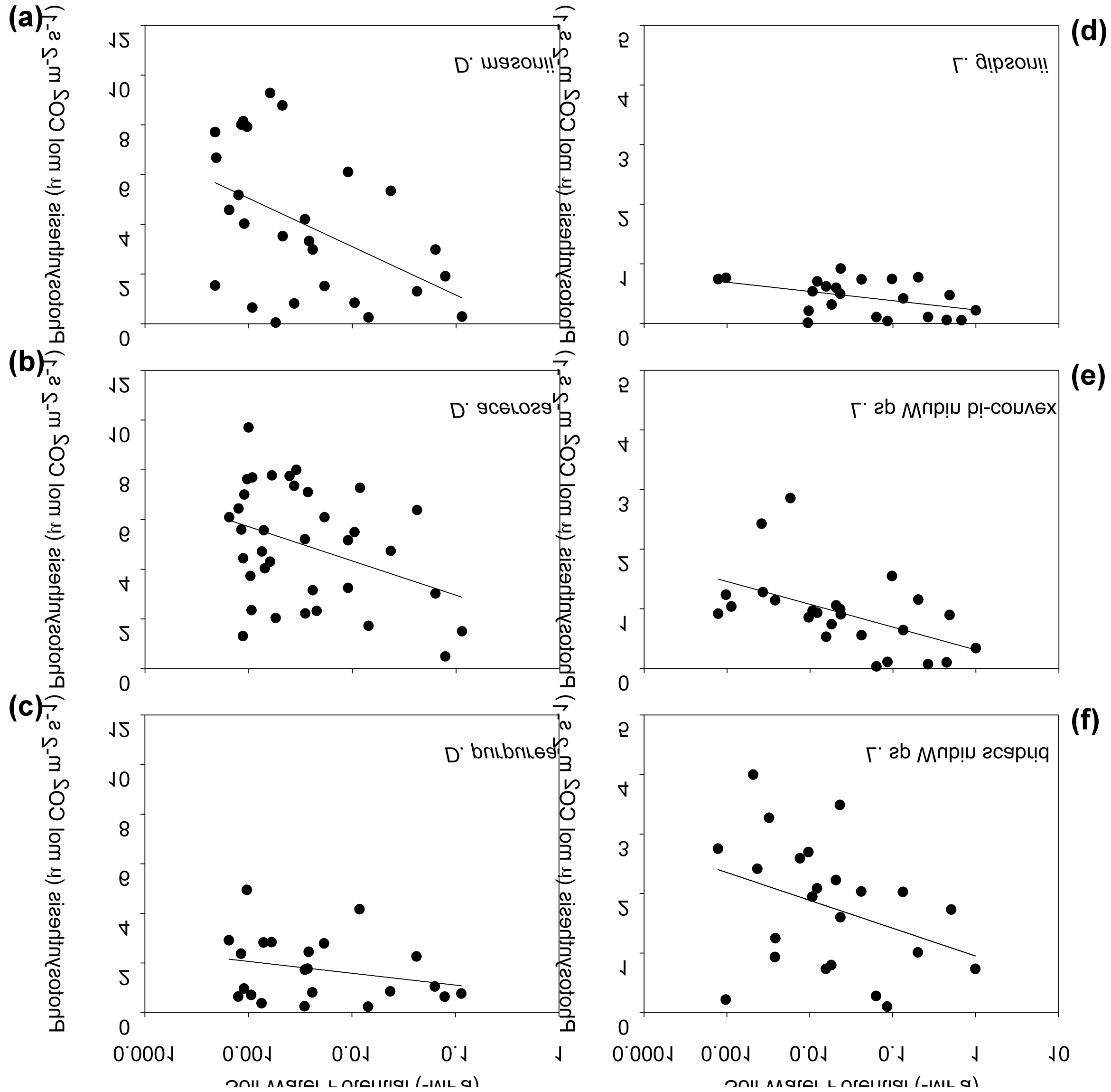

Figure 42. The response of photosynthesis to varying soil water potential for the target species (a) & (d) and their respective congeners. Points are multiple measurements of individual plants taken at various points through time. Note different Y-axes for each genus and log X-axis scale. Linear regressions fitted to the log transformed soil water potential data are significant for *Darwinia masonii*, *D. acerosa*, *Lepidosperma gibsonii* and *L*. sp Wubin bi-convex ($p<0.05$)

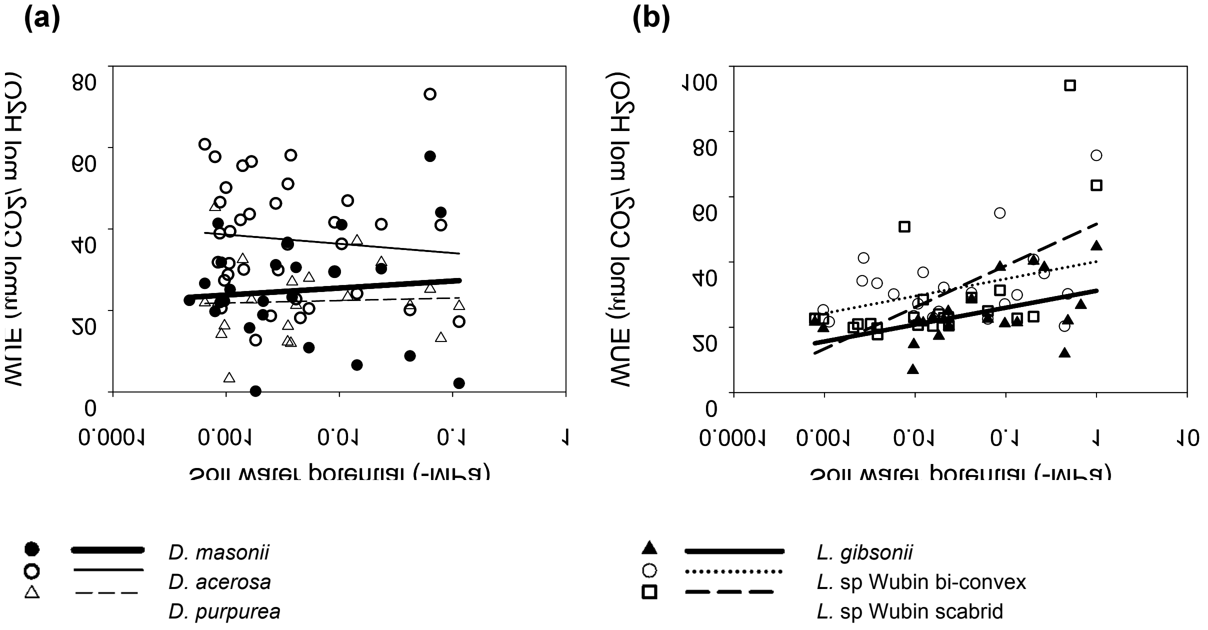

Figure 43. Intrinsic water use efficiency (WUE) of (a) the *Darwinia* species and (b) the *Lepidosperma* species with varying soil water potential. Intrinsic water use efficiency is photosynthesis/ stomatal conductance.

Both species have relatively low water use efficiency when they are functioning, and respond to lower soil mater availability by increasing root growth at the expense of reduced shoot growth. *Lepidosperma gibsonii* has low maximum photosynthetic rates, lower WUE, a greater root growth response to dryer conditions. *Darwinia masonii* on the other hand has high maximum rates of photosynthesis

Summary:

- Comparative studies of *D. masonii* and *L. gibsonii* and related species of other environments demonstrate that the target taxa do not possess unique capacity to function or use water at lower levels of water availability.

- Both species do respond to declining soil moisture levels by increasing root growth at the expense of investment in leaves and shoots.

- *D. masonii* and *L. gibsonii* appear to persist over the arid summer period by closing down plant function and maintaining a dormant state through to next winter.

4.5d Plant response to environmental variation

The environment experienced by *D. masonii* and *L. gibsonii* has been investigated via assessment of site factors, soil and surface properties and modelling of distributions in respect of environmental variables (**4.5a Abiotic associations**). The role of fire in the mortality and recruitment of plants has been examined in section 4.2 Population Demography, and the role of climatic variation – as much as was possible within the study period (section **4.2** and **4.3b Seed production**). The response of *D. masonii* and *L. gibsonii* to experimentally induced drought conditions is described in section **4.5c** above. This section presents addition studies of the physiological behaviour of *D. masonii* and *L. gibsonii* under field conditions.

Seasonal monitoring of plant ecophysiology has been ongoing at Mt. Gibson since 2008. This survey (of 145 plants of 5 species) characterises ecophysiological strategies in terms of seasonal variation in leaf gas exchange and plant water status, comparing *D. masonii*, *L. gibsonii* and *A. cerastes* with common/widespread relatives (*Aluta aspera* subsp. *aspera* and *Amphipogon caricinus* var. *caricinus*. 8-11 plants are monitored every three months at each site.

Table 32. Location of sites used in ecophysiological monitoring.

Site	Species	Location	Age Class	Locality	n
1	*D. m.*	Mt Gibson	Adult	S29 35 37.4 E117 11 03.7	11
2	*D. m.*	Iron Hill	Seedling	S29 36 25.4 E117 10 46.4	8
3	*D. m.*	Mt Gibson South	Adult	S29 36 12.9 E117 12 00.6	11
4	*D. m.*	Extension Hill South	Adult	S29 35 04.4 E117 10 00.7	11
5	*L. g.*	Iron Hill	Adult	S29 36 16.8 E117 10 23.4	11
6	*L. g.*	Iron Hill North	Adult	S29 35 59.3 E117 10 14.6	10
7	*L. g.*	Extension Hill South	Adult	S29 35 04.9 E117 10 00.0	11
8	*L. g.*	Mt Gibson	Adult	S29 35 39.1 E117 11 03.7	11
9	*L. g.*	Mt Gibson	Seedling	S29 35 39.1 E117 11 03.6	11

Ecophysiological monitoring indicates that there are no differences in mean water potential or leaf gas exchange attributes (photosynthesis and stomatal conductance) between *D. masonii* and *L. gibsonii* and non BIF-endemic species from all sites (e.g. Figure 44 – gas exchange parameters, and other sites show similar patterns). All species examined show the same diurnal and seasonal patterns, with both gas exchange and water potential parameters indicating active growth and C-uptake during winter, but an almost complete cessation of growth over summer and autumn. These results do not support the theory that *D. masonii* and *L. gibsonii* have root foraging (or other) strategies which enable their access to water that other species are not – but which may cause them to be limited to the BIF substrate. They are, however, very drought tolerant through the simple drought avoidance strategy of shutting down function completely over summer and autumn. The process of this shut down is illustrated in Figure 45 and Figure 46.

These physiological measurements (e.g. Figure 44) additionally illustrate the effect of winter drought, with pre-dawn water potentials in winter of 2010 much higher than the levels measured exactly 12 months previously, and approaching those observed in the summer of 2008/09.

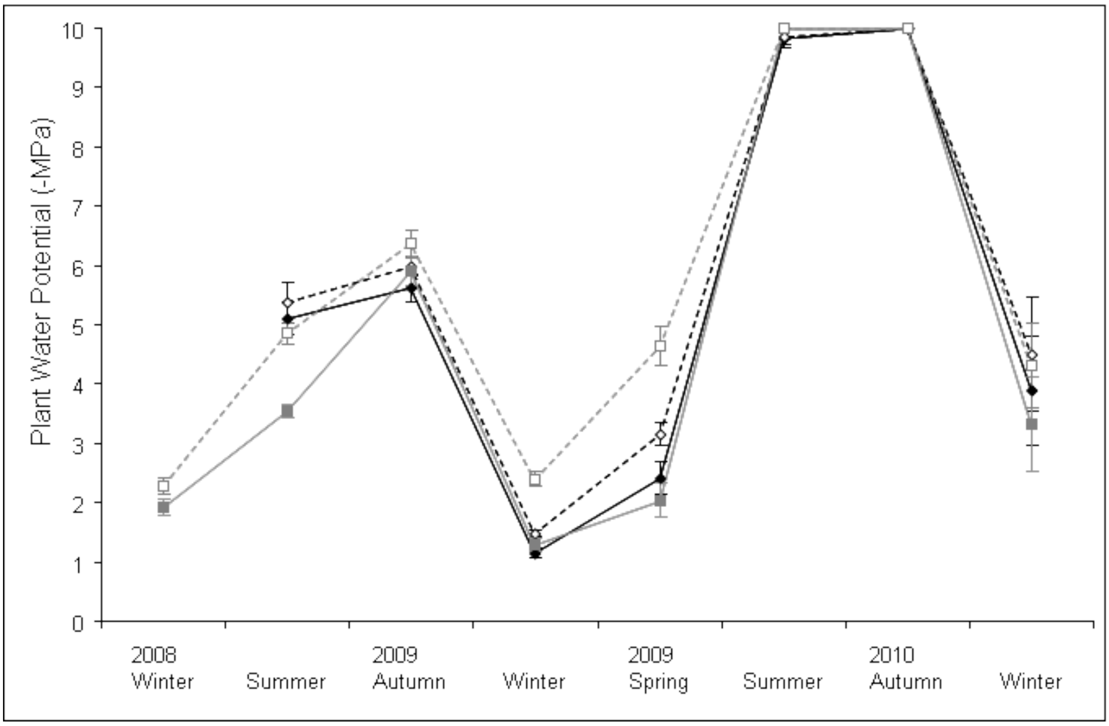

Figure 44. Mean (± SE) Water potential of Mt Gibson *Darwinia masonii* (grey) and Iron Hill *Lepidosperma gibsonii* (black) populations from mid 2008 to mid 2010. Water potential readings observed pre-dawn (dashed: 0400-0600hrs) and PM (solid: 1200-1400hrs).

4.5e Plant health

Plant health scores have been discussed previously under **Population demography** (**4.2**), interactions with predators and parasites are described under **Biotic Interactions** (**4.5g**, below) and plant physiological behaviour is discussed above (**4.5c, 4.5d**).

Figure 45. *Darwinia masonii* foliage passing through seasonal changes. Leaves and stem are produced in autumn and winter and function through to spring. They enter a dormant state over summer, and regain their green colour and photosynthetic function with the onset of rains. Most leaves survive through one or two summers and function through two or three winters. Left (July): Previous season's leaves re-greened and new season growth commenced. Centre left (December): last season's growth lost, showing much bare stem. Centre right (January) and Right (April): last season's growth yellow-grey in drought mode.

Figure 46. *Lepidosperma gibsonii* leaf growth commences with onset of rains and continues through winter (left). Growth ceases in late spring and leaves change colour through December (centre left) and January (right).

A quantitative health score based on comparing foliage colour with colour tables was trialled for both species, with the aims of reducing the subjectivity of scoring, and of deriving further information on plant vigour. However, foliar colour is observed to change seasonally in both species, with even the healthiest plants attaining a colour in early autumn comparable with that of the least healthy plants in early spring. This coloration is likely an adaptive mechanism which protects leaf photo-systems from damage due to excess light and heat during the period when they are unable to repair themselves, or make use of available light, due to the lack of water. Photosynthesis ceases as soils dry during over summer and leaves remain yellow or orange through until rains return, whereupon leaves re-green and start photosynthesis and growth again. Leaves of both species appear capable of surviving through one or two summers, although, in more sheltered positions they may survive longer. Leaves therefore function through 2-3 winters. Any quantitative colour score would need to be finely calibrated for season and soil moisture content.

4.5f Below ground adaptations

The root growth and foraging abilities of both species were examined in experimental studies investigating root vertical and horizontal growth capacities, and in their ability to penetrate small pore spaces and fissures. Excavation of the root systems of *D. masonii* seedlings and *L. gibsonii* clumps was also undertaken in the field. The response of root mass growth of both species to experimentally imposed drought has been described previously (section 4.5c).

Vertical root growth was relatively rapid in glasshouse experiments. Root extension was determined by comparing root depths among plants of each species grown from cuttings (*D. masonii*) and clump separation (*L. gibsonii*) in white sand in 1 m deep and 0.1 m diameter PVC tubes (as detailed in 4.5c) when harvested at 28 days versus 72 days after planting. Eight individuals of each species were harvested at each period. Over this 44 day period, roots of well watered plants of both species when harvested at 72 days, were on average 24 and 25 cm deeper than those of plants harvested at 28 days. These measurements suggest a rate of vertical growth of 5.5 and 5.7 mm.day^{-1} for *D. masonii* and *L. gibsonii* respectively. Under drought conditions, *D. masonii* root growth was similar (6.1 mm.day-1) but *L. gibsonii* root extension declined (to 3.0 mm.day^{-1}). As the experimental plants of *L. gibsonii* were initially larger than those of *Darwinia masonii*, and plant growth often increases with size, it may be more appropriate to compare the ratio of root growth rate relative to initial size (relative growth rate), which would then be 0.4 and 0.9 over this 44 day period. By the end of the study program, the root systems of many individuals of both species had reached the bottom of the 1 m pots.

A second glasshouse study examined rates of horizontal root extension by growing cuttings transplanted into custom made containers (1.8 m long, 0.2 m wide and 0.15 m deep) filled with course white sand to a depth of 0.1 m. These long pots had transparent polycarbonate bases covered externally with black plastic to maintain humidity and shield roots from light. Plants were flushed once a fortnight with approximately 500 ml of the standard nutrient solution and grown in well-watered conditions. Root growth was periodically examined by observing the transparent base of each pot, and all pots were harvested and root distributions assessed after five months when the first roots were observed to have reached the end of the 1.8 m long pots. In this study five individuals of each of six species (*D. masonii* and *L. gibsonii* and two closely related taxa) were compared. In this study horizontal root growth was measured at around 1.5 mm.day-1 for *D. masonii*, slower than its close relative, the sandplain species *D. purpurea*, but similar to another shallow soil endemic species (*D. acerosa*, which occurs on granites). *Lepidosperma gibsonii* horizontal root extension rates averaged 3.5 mm.day^{-1}.

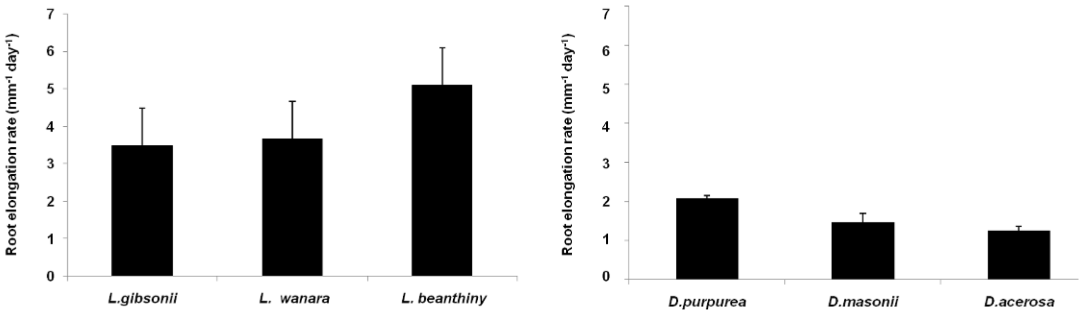

Figure 47. Horizontal root extension over the bottom of 1.8 m-long containers over a period of 44 days of three *Darwinia*, and three *Lepidosperma* taxa, once the roots reached the bottom. Error bars indicate standard errors (n=5)

A final study examined the capacity of roots of target species, and of two near relatives per genus, to explore soil areas by entering into small apertures such as pores and cracks in rocks. This study utilised plants grown in climate controlled glasshouse in 550mm x 90mm diameter PVC tubes with soils and nutrient additions (as above) and watered daily. Five replicate control plants of each species were grown in 55cm tubes without any restrictions, and another five replicates were grown in a sealed stack of six 5cm high sections, each of which had a sheet of stainless steel woven wire mesh (Metalmesh) siliconed to its base. A 15 cm top segment with a mesh bottom was attached upon the top of the stack to accommodate the planting of seedlings. Mesh sizes were based on the measured diameters of roots for each species and ranged from 34 to 530 µm. Plants were harvested when roots from control plants were observed to have reached the bottom of their pots (at 5 months). Sections were carefully separated on harvesting with root dry mass and root length measured and the number of root mesh penetrations counted. Controls were also cut into sections corresponding to the sections of the treatments, in order to provide comparison with an unobstructed root profile. Each section of roots (both treatments and controls) was scanned at high resolution, using the WinRhizo Pro 2007d software package (Regent Instruments, Quebec, Canada) and an Epson Perfection 4990 photo scanner.

Sections of fine roots taken from free growing plants were also examined under a microscope to identify elements of their anatomic structure (Figure 48). Of key significance is the diameter of the root itself, and of the root stele. Previous work (Zwieniecki and Newton 1995) had identified the root stele diameter as the major factor limiting pore entry of roots of shallow soil species. The stele occupies the centre of the root section and consists of the bundled vascular cells which transport water, nutrients and sugars around the plant. Stele diameters did not vary within genera but larger among *Lepidosperma* species (averaging 223 µm, relative to the average of 102 µm for *Darwinia* species). Root diameters were more variable, averaging 3-5 times that of the stele.

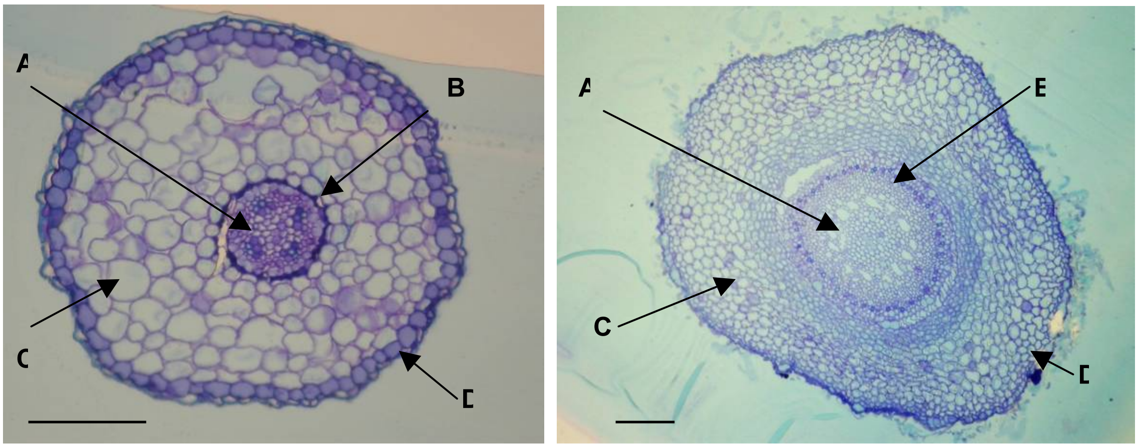

Figure 48. Root sections of *Darwinia masonii* (left) and *Lepidosperma gibsonii* (right). Root samples were imbedded in resin using biowave technology, sectioned, and stained with toluidine blue. Letters indicate the root stele (a), endodermis (b), cortex (c) and epidermis (d). Bars represent 100 µm.

Examination of number of root penetrations, root mass and total root length below mesh barriers identified differences between species within genera, as well as between genera. *Darwinia* root penetration was severely limited at the 100 µm mesh barrier and effectively prevented at a 63 µm mesh. Root penetration by *Lepidosperma* species was reduced at the 260 µm mesh, and no *Lepidosperma* species penetrated the 150 µm mesh.

Root excavations were performed in the field using brushes and a compressed air jet. Two *D. masonii* seedlings and one *L. gibsonii* clump were excavated from within the mining footprint on Extension Hill. In both cases, root systems were successfully traced up to the point when roots passed into, or beneath larger, immovable rocks. Unfortunately, in each case this occurred within 10-15 cm and roots passed into rock and regolith which could not be excavated with the tools available.

In the case of *D. Masonii*, plants were 5 months old and 5-6cm tall. They were each found to be emerging from seeds (still attached) buried at 3-4 depth, and with root systems traced a further 6 cm, to a total depth of ~10 cm (Figure 49). In the case of *L. gibsonii*, the excavated plant was a small sized mature individual likely dating from the 1969 fire. It had a well developed root system, with ~30-40 individual roots emerging from the clump base. These spread diagonally downwards, but could be traced for only 10-15cm before entering crack and pore spaces in rock-like regolith material (Figure 50).

Figure 49. Excavated *Darwinia masonii* seedling from within the mining footprint on Extension Hill; October 2009, following May 2009 experimental fire. Seedling height 5 cm, seed depth 3 cm, roots excavated to 10 cm depth – and continuing into rock cracks. Arrows show seed attached to stem (below) and ground level (above).

Figure 50. Site from which the root system of a *Lepidosperma gibsonii* plant was excavated from within the mining footprint on Extension Hill. The roots of the excavated plant (basal diameter 80 mm) extended to 15 cm depth before terminating at, or entering hard regolith surfaces (several indicated with arrows).

Examination of seven mining drill cores from Extension Hill, including from localities close to individuals of *D. masonii* and *L. gibsonii* revealed extensive weathering of underlying regolith with abundant solution channels, large cracks and pores. Many of these cracks, including to depths below the surface much exceeding the height of the vegetation above ground, contained visible root material (Figure 51). While one core (with sand overlaying weathered rock) had no visible roots, and one had roots only to 1.1m others had roots extending to over to 10 m, one to 13 m (Figure 52). While it was not possible to identify the plant species, and there are a number of candidate species, it is feasible that either study species could possess root systems with the capacity to exploit weathered regolith to this depth.

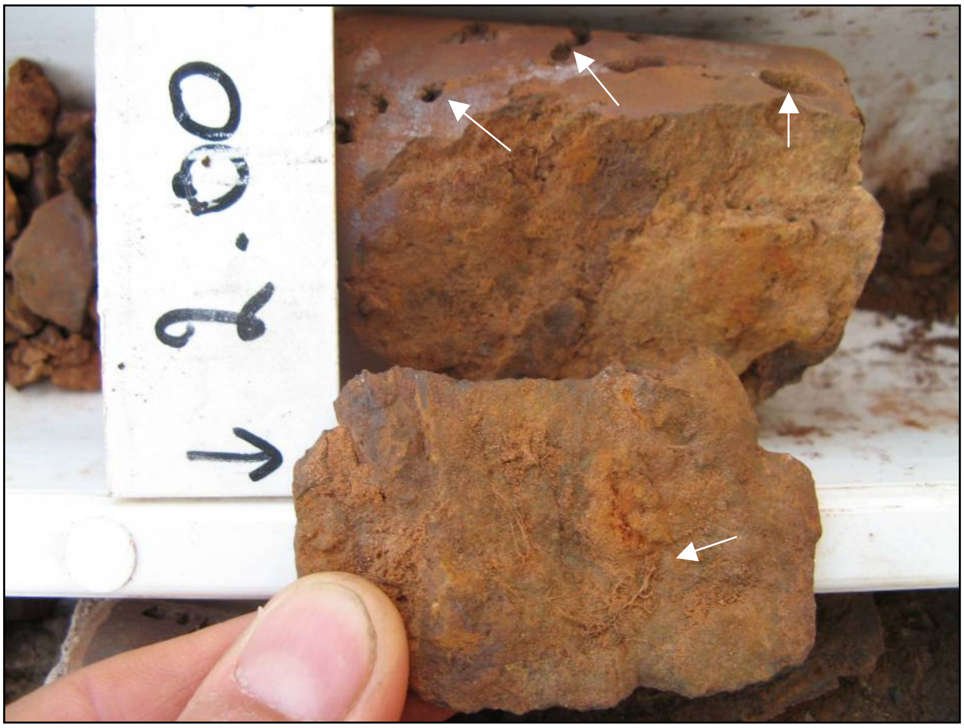

Figure 51. Example of roots (lower white arrow) observed in a natural break in a rock core form Extension Hill, here at 2 m depth. Note also solution channels in rock above (upper white arrows)

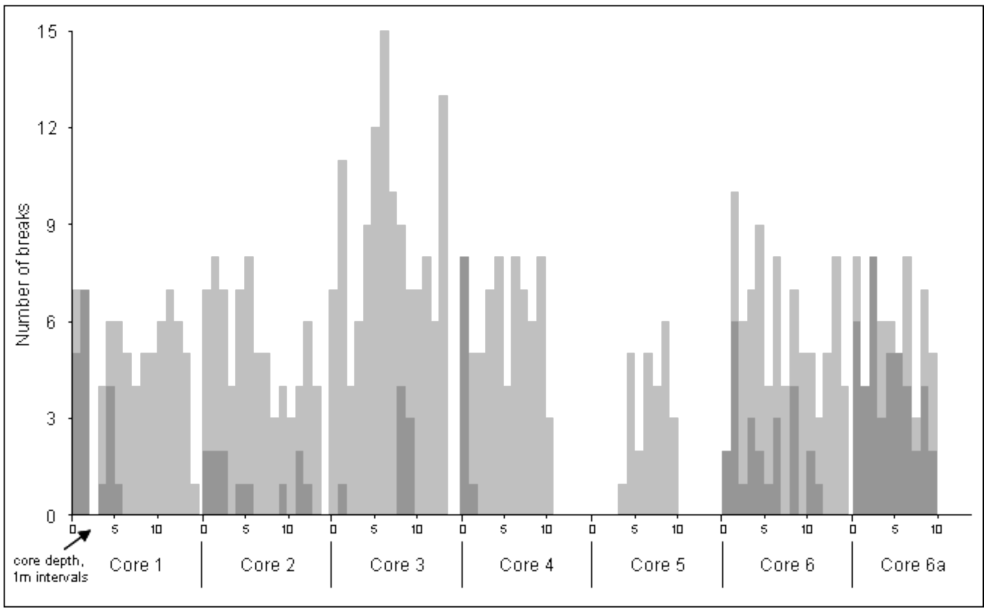

Figure 52. Frequency of root observations (dark grey) among breaks (fissures and cracks, light grey) in 1m sections of seven 50mm drill cores from Extension Hill.

Planned excavations of whole root systems with the aid of mining technology were not undertaken as mining development had not reached this stage during the period of the study.

Summary

- Results of these research programs show a capacity of both *D. masonii* and *L. gibsonii* for rapid horizontal and vertical root growth under good conditions and root growth is relatively increased in drying soils.

- Measured rates of root growth were faster for both species in vertical orientations than horizontally. Both species had similar rates of vertical growth (5-6 mm.day^{-1}), but *L. gibsonii* grew faster horizontally (3.5 v 1.5 mm.day^{-1}).

- Roots of both species grew to 1m depth and a similar horizontal extent over a period of a few months in glasshouse conditions.

- *Lepidosperma gibsonii* roots are generally larger than those of *D. masonii*, and are more restricted in the size of soil and root spaces that they can enter.

- Neither BIF species showed root growth adaptations that were significantly different from close relatives from non-BIF habitats.

- Excavations indicate a capacity of roots to enter large cracks, pores and fissures in regolith, and examination of drill cores show that the regolith is highly porous and that some plant species do achieve root depths exceeding 10m in the BIF system.

Recommendations

- Proposed examination of root systems in mine pit faces should proceed as mining commences through populations of *D. masonii* and *L. gibsonii*, but are likely to prove challenging as roots pass through solution channels and fissures in rock. These studies may help to determine rooting depth of *D. masonii* and *L. gibsonii* and their use of surficial versus deeper layers in the soil profile. Results would inform requirements for restoration substrates.

- Mapping of regolith may indicate a relationship with the distribution of *Darwinia masonii* around the range which may reveal an important but as yet unknown association of the species with particular fracture patterns or density.

4.5g Biotic interactions

Darwinia masonii

Vertebrate grazing impacts have been considered a possible threat for *Darwinia masonii* populations. However, mortality among seedlings protected from herbivory by a coarse (chicken wire) steel mesh was similar to that observed among uncaged seedlings (see **mortality/survival** under **4.2 Population Demography** above). Further, no evidence of mammalian herbivory was observed on any plant, seedling or adult in the demographic survey plots or outside of these (with the exception of a single cutting damaged by a presumed rabbit-bite, but not actually grazed, and therefore likely to be an isolated instance), and it is not considered to be a current threat. Four tagged flowering plants were observed to have had branch tips cut off (2007 only). Further evidence of this impact was observed elsewhere in 2007, and was presumed to result from the actions of parrots eating developing seeds or flowers.

Invertebrate herbivory. Galls were observed on a very small number of *Darwinia masonii* individuals (Figure 53), but other evidence for foliar loss or damage from insect attack was not noted.

Figure 53. *Darwinia gibsonii* leaf gall.

Termites were observed on stems of 15 of 357 tagged *Darwinia masonii* individuals. One of these individuals was from a site last burnt in 1969, the remaining 14 were from older sites: respectively this represents 0.7% and 15% of individuals in these site ages. Termites were observed with their trails ascending the stems of *D. masonii* plants, and in one case had hollowed out a dead stem, their impact never appeared significant, and the mean of growth rates and health scores of affected individuals did not vary greatly from the mean of termite-free individuals in the same areas.

Pollination experiments describing the role of birds (white cheeked honeyeaters) and insects in pollen transfer are described under section 4.3e. Extensive s**eed predation** by moth larvae and **seed dispersal** by various ant species are described under sections 4.3b and 4.3f respectively.

Mycorrhizal associations: Examined field-collected root-systems of *D. masonii* clearly showed Vesicular-Arbuscular mycorrhizal (VAM) colonisation, but no obvious Ectomycorrhizal (ECM) formation. VAM associations are ubiquitous in Myrtaceae, and ECM associations common in Myrtaceae. Few shrubby Myrtaceae have been investigated for ECM associations, but an ECM partnership between *Chamelaucium uncinatum* (a sister-genus of Darwinia) and *Pisolithus* sp. has been documented at Kalbarri. No evidence of fruiting of ECM fungi was observed in the vicinity of *Darwinia masonii* plants during this project; however, some ECM-forming fungi were observed fruiting elsewhere in the range (*Amanita* spp., *Torrendia inculta*, *Pisolithus* sp., *Entoloma* sp., which were probably associated with *Eucalyptus* and/or *Acacia* spp.). Mycorrhizal formation is potentially important for restoration success for *D. masonii* (e.g. for seeding survival and growth rate, as has been shown for a variety of VAM and ECM-forming species), and should be further investigated in comparative restoration trials, in particular whether restoration-planted seedling are spontaneously able to form mycorrhizal associations in restoration surfaces, or whether inoculation is required or beneficial.

Fungal infections: Only a single unidentified bracket fungus has been observed fruiting on *D. masonii* trunks during this study. Wood of dead or burnt *D. masonii* plants persists for many years after death, with no obvious indication of fungal rotting. In contrast, living *Melaleuca nematophylla* was frequently observed colonised by *Fulvifomes* sp., and numerous plant species had dead wood colonised by *Pycnoporus coccineus* and numerous corticioid wood-rotting species. It is likely that wood rots have a very minor impact on *D. masonii*.

Evidence for **competition or facilitation** (i.e. negative or positive interactions with neighbouring plants) was also not observed, however such interactions are best determined by manipulative experiments under field conditions. Such experiments are suggested for future restoration research.

Lepidosperma gibsonii

Vertebrate grazing Mortality among seedlings protected from herbivory by a coarse (chicken wire) steel mesh was identical to that observed among uncaged seedlings (see ***L. gibsonii*** **mortality/survival** under **4.2 Population**

Demography above). Nonetheless, significant herbivory was observed in a number of surveyed plots. Herbivory was recorded on 100% of individuals in three surveyed plots: one each at Iron Hill (IH1), Iron Hill North (IHN1) and the Emu Fence. Herbivory was assessed as estimated proportion of leaves or scapes which had been grazed (usually to close to the ground). In these plots, herbivory of individual *L. gibsonii* clumps represented 15 to 100% of foliar loss (Figure 54). Complete foliar loss (100% herbivory) was observed in 35 surveyed plants. One fifth of all surveyed *L. gibsonii* adults had >20% herbivory, 16% were at least half eaten. Herbivory among seedlings was not observed as frequently: 6% of tagged seedlings had 20% or more foliar loss.

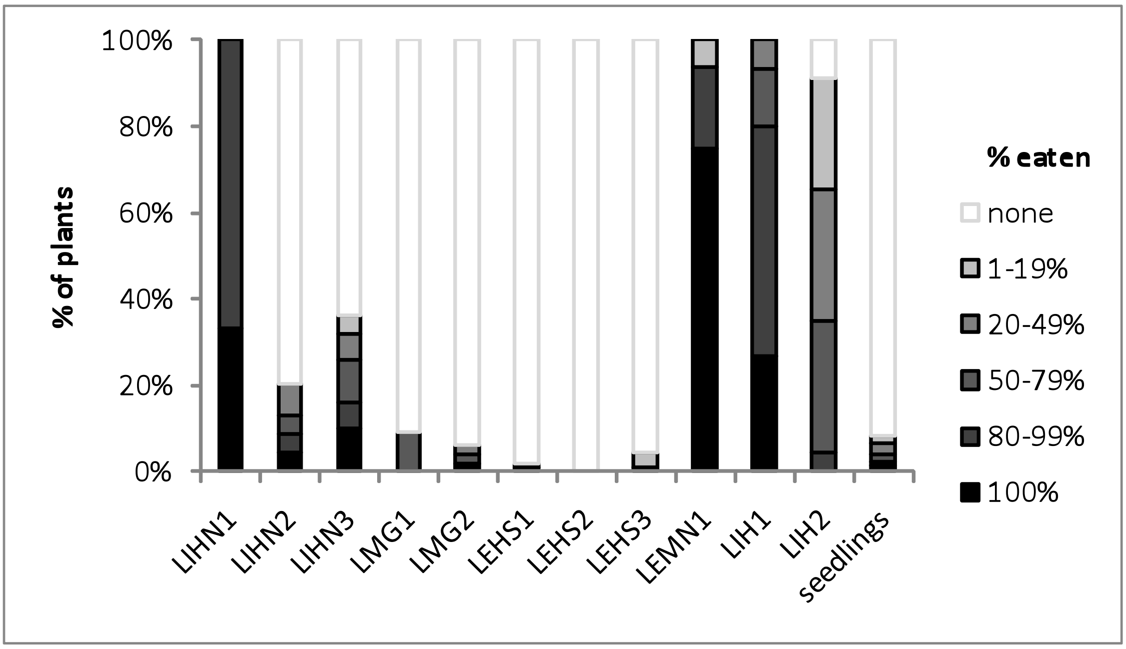

Figure 54. Frequency (number of plants) and intensity of maximum observed herbivory (% of foliage eaten) in mature *L. gibsonii* in each plot and in seedlings pooled across plots.

Sites with significant herbivory are located in the south and the north of the survey area, but are also lower on slopes (e.g. IH1, IHN1) or not on slopes (e.g. Emu Fence). Judging by the presence and abundance of nearby faecal scats, this grazing may be attributable to rabbits and / or goats. Macropods may also play a role.

Plants previously observed to have experienced herbivory often showed significant recovery on subsequent surveys, but sites with extensive herbivory seem to also experience frequent herbivory. While evidence for an impact of herbivory on survival rates is unclear, the evidence for an impact on reproductive output is clear. Grazed plants have their reproductive capacity reduced to exactly the same extent as they are eaten as leaves and culms are impacted to the same extent. That is to say, plants in the most heavily grazed sites may have their reproductive output reduced to close to zero through grazing.

Invertebrate herbivory An unidentified scale insect (Hemiptera: Coocoidea) was observed to be abundant on *L. gibsonii* culms. In some populations (e.g. near the Emu-proof fence) they covered a large areas of the available photosynthetic surface, and in such cases are likely to significantly affect the growth rate and reproductive potential due to the parasitic, sap-sucking lifestyle of the insect.

Fungal infections An unidentified rust fungus has been observed on the culms of *L. gibsonii* plants from most populations, albeit usually in small numbers (1-5 culms per clump). Infected culms have been observed successfully fruiting in 'good' years. Since culms only last 2-3 years, these rusts are potentially minor parasites of *L. gibsonii*, but could potentially have a stronger effect in poor seasons, or under increased infection rates. Five species of smut fungi (four *Moreaua*, one *Heterotolyposporium*) are known to infect inflorescences of *Lepidosperma*, and have been observed in populations of *L. costale sens.lat*. in the Midwest, but not yet on *L. gibsonii*. When abundant, *Lepidosperma* smuts can sometimes have a significant impact on reproductive output, as they are systemic and destroy all florets within an inflorescence.

Summary:

- Interactions with other organisms play an important role in the life-cycle of both *D. masonii* and *L. gibsonii*.
- Herbivores had a negligible impact on *Darwinia masonii* plants. Seed predation (by larvae of an unidentified moth species) can be significant, although is spatially and annually variable. Positive or neutral interactions with pollinators (chiefly white cheeked honeyeaters) and seed dispersers (a number of ant species) also play a major role in *Darwinia masonii* life history.
- In *L. gibsonii*, grazing – presumed to be by goats and rabbits – can have a significant impact on growth and reproduction but was observed in only a handful of localities. Other leaf parasites, notably a rust pathogen and a scale insect also occur infrequently (and often at these same sites).
- Both the seed-eating moth, and a gall forming insect observed on *Darwinia masonii* individuals are unidentified, likely unrecognised

species and possibly specialist on *Darwinia masonii* and therefore also potentially rare and threatened species. The bract feeding beetle observed in collected *D. masonii* seed is less likely to be a specialist species.

Recommendations:

- Manage populations of goats and rabbits across the distribution of *L. gibsonii* and in restoration sites in particular, and monitor herbivory impacts of macropods on *L. gibsonii*.
- Ensure habitat requirements for key *D. masonii* pollinators are retained.
- Monitor and take into account the dynamics of moth impacts when collecting *Darwinia masonii* seed for restoration.
- Identify the seed-eating moth species and survey for its occurrence in co-occurring species and related *Darwinia* species.

4.6 RESTORATION AND TRANSLOCATION

4.6a Storage of propagation material for translocation

Cuttings were collected from 300 genotypes of *Darwinia masonii* and 250 genotypes of *Lepidosperma gibsonii*. All material was taken from within the mine footprint on Extension Hill and under the DRF collection permits of EHPL and BGPA. Collections were made at several times but established best when soils were moist and plant tissues were fresh and growing (i.e. mid-winter), material can then be stored and transported in cool and moist conditions. Collected material was delivered to Nuts About Natives (NAN), a specialist native plant nursery in October 2008. They reported that 385 *D. masonii* cuttings (12% of total cuttings) from 150 different genotypes (50% of genotypes) had successfully initiated after 3 months. At the same time, 815 (44%) of the 1846 pots of *L. gibsonii* divisions showed new root and shoot growth and could be considered successfully initiated. These represent 187 genotypes (75% of collected genotypes) and could be considered successfully initiated.

Both DRF species are able to be successfully initiated into cultivation, and are now stored in cultivation at NAN’s nursery. These plants are maintained at NAN as an off-site genotype stock with the intention that they will be multiplied up (by taking further *D. masonii* cuttings and separating *L. gibsonii* clumps) to provide an off-site collection and perhaps ultimately to contribute to stock for population restoration. The nursery plants are maintained on unshaded external benches, regularly monitored, and watered at moderate intervals.

BGPA has made a number of additional collections, using the same techniques and keeps a small number of genotypes of both species in glasshouses at Kings Park for experimental purposes. Here, *L. gibsonii* grows vigorously, and flowers, under watered glasshouse conditions, and is easily split and repotted to create larger number of individuals (clones). *Darwinia masonii* cuttings survive and flower but have not shown the same degree of vigour in their growth, *Lepidosperma gibsonii* appears to be susceptible to a moth whose subterranean caterpillars (likely a native species and possibly present at Mt Gibson) consume dead and older live, leaf material. *Darwinia masonii* cuttings appear to prefer lower humidity levels than may often occur in glasshouse conditions. Seedlings established from *D. masonii* seeds derived from experimental studies are also potted up and growing in Kings Park glasshouses. These appear to grow with slightly greater vigour than cuttings.

Glasshouse plants of both species experimentally exposed to drought conditions do not appear to be able to enter or recover from a dormant, drought-mode state equivalent to that observed in field plants through late summer and autumn (see **4.5c Drought study**). Propagated plants planted in field translocation trials in winter did appear to achieve and recover from this dormant state in the following summer and winter periods.

While the use of nursery stock from cuttings or clump separation is a proven and suitable technique for population restoration, its drawbacks imply that investigation into the use of seed as a restoration resource for *D. masonii* and *L. gibsonii* are worth continuing. The drawbacks of greenstock include its infrastructure, resource and time demands (pots, potting media, glasshouse bench space, irrigation, pest management, time and expertise to establish cuttings, plant out, etc) as well as implications for genetic selection and diversity. Selection in propagation – i.e. with survival of plants better suited to glasshouse, but not necessarily field, conditions – can lead to a loss of genetic diversity and capacity. In terms of numbers, collection and establishment of genotypes numbering in the (low) hundreds is feasible and demonstrated, larger numbers may be possible but with diminishing returns in terms of required effort. Finally, the translocation of large and/or unbalanced numbers of genetic clones means that some genotypes may become grossly over-represented in restoration, which should ideally aim to replicate source levels of genetic diversity. The most effective way to ensure reinstallation of genetic diversity without creating artificial imbalances in genotype representation is to use seeds as a restoration source, either from collected seed or soil seedbanks.

In this area recommendations differ for *D. masonii* and *L. gibsonii*. Seed of *L. gibsonii* has proven difficult to collect and germinate and is likely to continue as such, however *D. masonii* seed is produced in larger numbers and more easily collected. As germination of treated fresh or standard stored seed of *D. masonii* is also low, this immediate approach is not recommended for restoration. However, germination of buried seed retrieved after 9 months of burial in field soils and then treated with smoke water reached 90% (see **4.3d Seed bank demography**). This suggests three possibilities: 1) It is technically feasible to explicitly follow this approach, exhuming bagged seeds after a period of burial in field soils, for germination in pots for planting as seedling greenstock; 2) Trialling a process of broadcasting of *D. masonii* seeds into field restoration areas, followed by application of smoke or smoke chemicals after some period, and which does seem likely to lead to the emergence of seedlings after some time; 3) Continued research into cues responsible for the pattern identified in burial trials may enable replication of these in seeds under lab conditions (e.g. by storing seeds under alternating warm/ hot temperatures and following this with a period of cool and moist conditions and the application of smoke) and cued seed could then be applied to restoration areas ready to germinate – at higher rates.

Summary:

- Techniques for the successful collection, establishment, maintenance and propagation of both *D. masonii* and *L. gibsonii* have been proven at both BGPA and an independent specialist nursery.
- Proven propagation techniques involve greenstock production from cuttings (*D. masonii*) or separated clumps (*L. gibsonii*).
- Both species can persist as tube-stock when stored outside in hygienic nursery conditions and watered through dry periods. *L. gibsonii* may show improved growth under more humid glasshouse conditions.

Recommendations:

- *ex situ* collections of live plant and seed material and multiple (>100 for live plants) genotypes should be monitored and maintained and supplemented as required.
- Seed collections made for restoration purposes should be viability checked and maintained in standard, pest-free, temperature and humidity controlled seed banks.

- Propagation of live plant material from wild collections and nursery stock likely pose the most cost effective approach for the short-medium term storage and production of plants for restoration purposes.

- Collection and storage of seed for restoration purposes may yet prove effective and is relatively cheap, however allowance must be made for the likely ultimate rate of seed germination, difficulty of collection (for *L. gibsonii*) and the potential cost (in time and money) of developing seedlings from seed.

- Uncertain and low seed production rates, and poor return in terms of demonstrated germination rates (to date) means that this approach is not yet recommended.

- For *D. masonii*, further research into seedling production under lab, glasshouse or field conditions appears promising and may provide a preferable approach to providing a genetically diverse and numerous source of restoration plants.

4.6b Translocation methods

Plants from the BGPA collection, supplemented by material previously cultivated at NAN (from BGPA collections) were used in translocation trials at Extension Hill with acceptable survival rates (as described in **4.5b Translocation study**). Evidence from the *D. masonii* pilot watering trial (also see 4.5b) indicates that *D. masonii* survival may be approximately doubled by irrigation in the initial years, although the two trials commenced in years differing markedly in their total rainfall receipt.

The trials performed demonstrate successful establishment and survival of both *D. masonii* and *L. gibsonii* in the rocky/gravelly loams of the Extension Hill range. While it is yet possible that some substrate attribute essential to growth and survival of these species may be missed in a regular replacement of gravel/rocky loam over waste rock, and these should be confirmed in restoration trials, the indications that this would succeed are good.

Potential localities for translocation (as opposed to restoration) of populations have been identified and mapped in section **4.5a** (**Abiotic associations**).

Plans for studies of plants planted into reconstructed substrates and designed to identify optimal soil and subsurface features for the growth and survival of *D. masonii* and *L. gibsonii* were contingent upon the commencement of mining and the provision of trial areas with trial substrate materials derived from the mining process. These are still to take place.

Summary:

- Trial translocation of propagated cuttings of *D. masonii* and split clumps of *L. gibsonii*, planted into field substrates in early winter and watered only at planting have proven successful.
- Attributes and localities of potential translocation sites within the Mt Gibson-Extension Hill range have been identified.

Recommendations:

- Experiments manipulating restoration substrates using mine waste components or other available and appropriate materials are recommended as mining construction commences.

4.7 *EX SITU* CONSERVATION

4.7a Seed storage

Batches of 1000 filled seeds of each of *Darwinia masonii* and *L. gibsonii* have been deposited at each of the WA Seed Technology Centre in the Botanic Gardens and Parks Authority at Kings Park, the DEC-operated Western Australian Threatened Flora Seed Centre, and Kew Garden's Millennium Seedbank at Wakehurst Place in the UK. These three facilities are premier global and national seed storage facilities.

Viability of long-term stored *L. gibsonii* seeds has not been tested due to its recent collection date, however *D. masonii* seed collected from 2004 and stored for three years appears to retain equivalent levels of viability when compared to seed collected (and simultaneously tested) in 2007. The soil seedbank strategy of both species strongly suggests that seed viability is likely to persist though storage under standard seed storage conditions for many years.

Summary:

- Batches of 1000 filled seeds of each of *Darwinia masonii* and *L. gibsonii* have been deposited at each of three, Australian and international, conservation seed storage facilities.

4.7b Germplasm storage

Germplasm storage techniques were investigated using standard approaches from excised seed embryos for both *D. masonii* and *L. gibsonii*, and shoot cuttings for *D. masonii*. The medium used for in vitro propagation was basal medium as per Bunn (2005) supplemented with BAP 0.15 µM, pH 6 and 6 gL-1 agar.

For *D. masonii*, shoot cutting material was trialled from 12 individuals representing 5 populations in a total of 35 separate 120 ml culture tubes. After a period of three months, all 35 tubes had initiated root and stem tissue and were growing well and multiplying (Figure 55). Culture lines are being maintained successfully in culture at room temperature with alternating cool storage (short to medium term storage).

Figure 55. *Darwinia masonii* in tissue culture (bar = 20 mm)

For *L. gibsonii*, tissue culture was attempted via embryo extraction in an experiment combining investigation into the role of heat shock on *L. gibsonii* seeds. Fruits were soaked in water for 24 hours to soften up the endosperm, then surface sterilised in 1% bleach for 20 minutes and rinsed in sterile water three times for approximately 3-5 minutes each. Embryos were then extracted under a binocular microscope in a laminar flow cabinet and plated on a special media of ½ MS + GA3 + Zeatin (6g per litre Agar & 20g per litre Sucrose). Prior to imbibing water, fruits were split into control and multiple heat treatment samples, with the latter exposed in a temperature controlled oven to 100°C for a period of 10 to 90 minutes for the heat shock treatment. Each of the seven treatments included a total of 16 embryos. Plated embryos were incubated @ 15°C in dark conditions examined for growth. Embryos with growing root and shoot systems were transferred to culture tubes and maintained under the same conditions as per *D. masonii* (above).

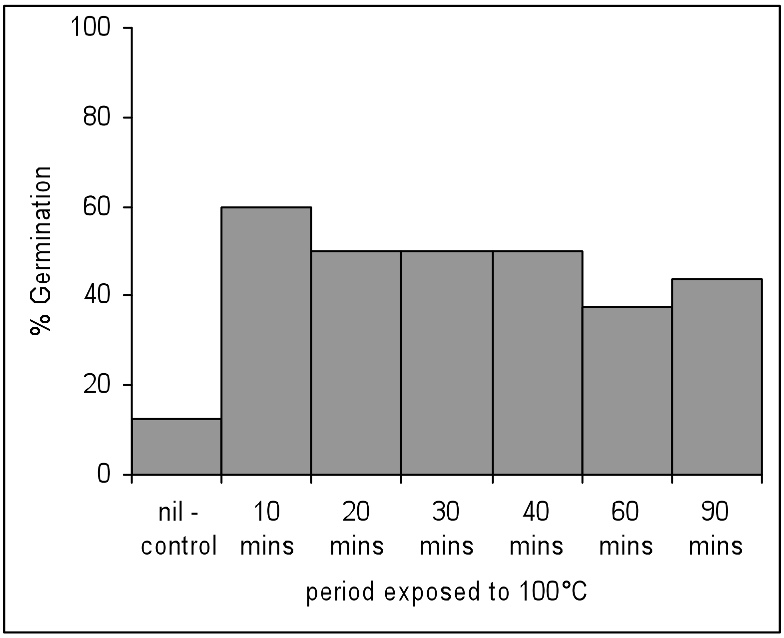

Figure 56. Germination rates (including to establishment of roots and leaf shoots) for seeds of *Lepidosperma gibsonii* exposed to varying periods of heat treatment.

An average of 49% of excised *L. gibsonii* embryos exposed to temps of 100°C produced roots and shoots and survived to establish successfully in tissue culture (Figure 56). Less than a quarter of this proportion of seeds not exposed to high temperatures germinated and established, and there is some limited indication that exposure to 100°C for longer periods may lead to lower establishment.

Summary:

- *in vitro* culture, with root initiation has been achieved with multiple genotypes of both *Darwinia masonii* and *Lepidosperma gibsonii*, using stem tissue and embryo extraction respectively.
- Culture lines can be maintained in culture with alternating room temperature / cool storage (for short to medium term storage).
- Cryostorage is an option for long-term storage of key clonal germplasm if required.
- Micropropagation is feasible should it be required as a propagation option for both species.
- Genotypes of both species are stored as live plants at two locations off-site (including a significant collection of Extension hill genotypes of both species)

- Genotypes of both species are additionally stored as seed at three secure locations off-site, representing a total of 3000 seeds of each species.

Recommendations:

- *ex situ* collections of live plant and seed material and multiple (>100 for live plants) genotypes should be monitored and maintained.
- The effectiveness of *ex situ* storage of germplasm in a variety of forms is demonstrated for both species, but live plant collections likely pose the most cost effective approach for the short-medium term.
- Collection and storage of seed as an *ex situ* conservation measure may also be effective, and is relatively cheap, however allowance must be made for the likely ultimate rate of seed germination, difficulty of collection (for *L. gibsonii*) and the potential cost (in time and money) of developing seedlings from seed.

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