The University of Western Australia
CENTRE FOR LAND REHABILITATION

KINGS PARK
& BOTANIC GARDEN

About Native Seeds

5th December 2000

Presented by: Centre for Land Rehabilitation
University of Western Australia
NEDLANDS WA 6907

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About Native Seeds
5th December 2000, Perth, WA
Kings Park Theatrette, King's Park

8.30am- 9.00am	Registration
9.00am -9.30am	**Dr Siegy Krauss (BGPA)** Definition and delineation of Seed provenance
9.30am -10.00am	**Geoff Cockerton (Landcare Services)** Seed Collecting-protocols
10.00am -10.30am	**Anne Cochrane (CALM)** Seeds for the future:ex situ conservation in CALM
10.30am- 11.00am	**Morning tea**
11.00am-11.30am	**Dr Julie Plummer (UWA)** An ecological basis for seed dormancy
11.30am -12.30pm	**Bob Dixon(BGPA)** Seed treatments to enhance germination
12.30pm-1.30pm	**Lunch**
1.30pm -2.15 pm	Nursery tour
2.15pm - 3.15pm	**Luke Sweedman(BGPA)** Seed Storage
3.15pm -3.45pm	**Afternoon tea**
3.45pm-4.30pm	**General discussion Q & A**

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About Native Seeds
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Definition & delineation of Seed Provenance

Dr Siegy Krauss

Genetic principles for plant restoration

Siegy Krauss

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getting the right genetic mix

⇨ **Seed source (provenance)**

⇨ Seed quality (genetic)

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genetic provenance

"place of origin"
seeds used for native plant restoration should be sourced from "local" populations

⇨ Q. why?
⇨ Q. how local is local?

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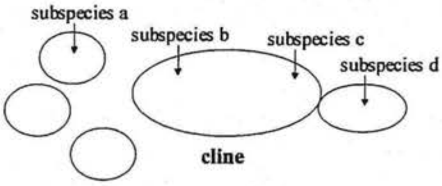
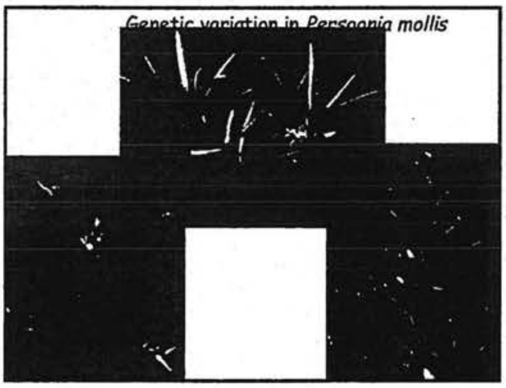
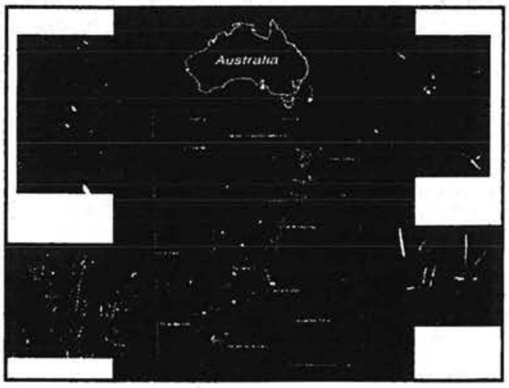
why genetic provenance?

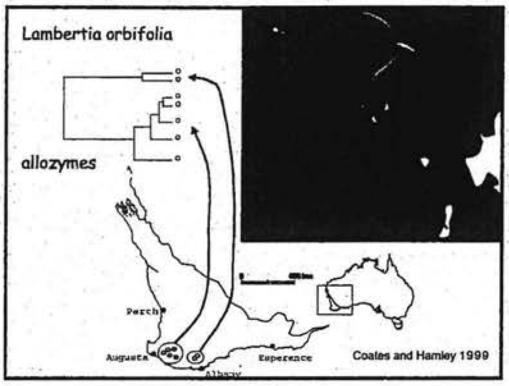
⇨because all species display significant levels of geographic variation



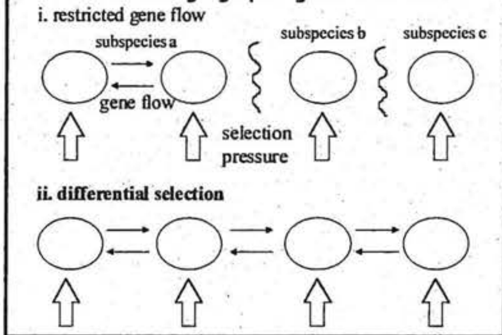
Genetic variation in Persoonia mollis





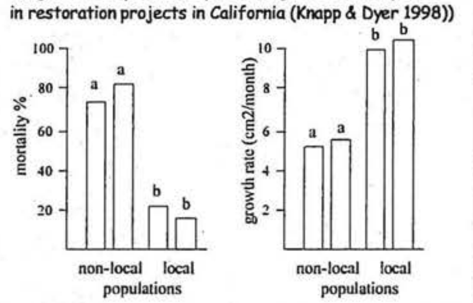


sources of geographic genetic variation



consequences of introducing "alien" seeds

(e.g. *Nassella pulchra*, a perennial grass commonly used in restoration projects in California (Knapp & Dyer 1998))

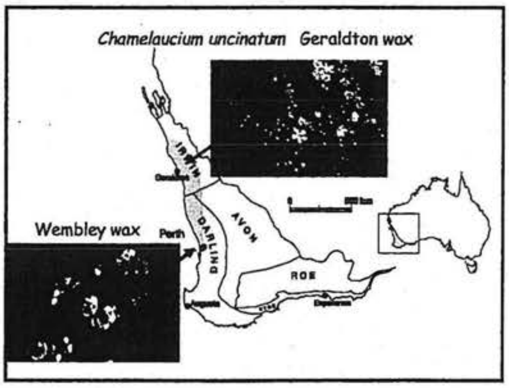


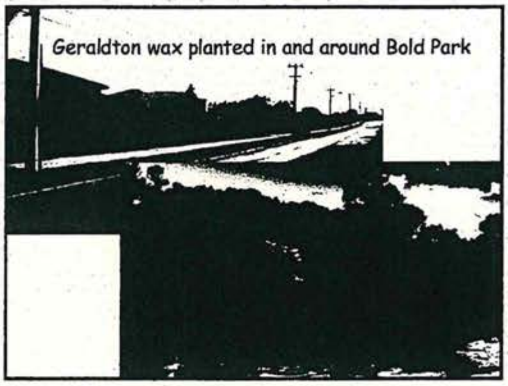
Consequences for non-local genotypes:

consequently, to promote self-sustaining rehabilitated populations, seeds must be sourced from local provenances

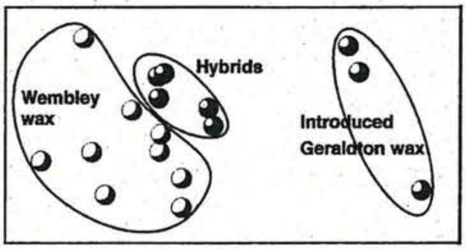
Alternatively, maintenance of local genetic integrity

Avoid introducing nonlocal genotypes which may swamp and extinguish a significant local variety





Population genetic variation in *C. uncinatum* in Bold Park



Provenance delineation is vital because...

(i) it is inefficient to use nonlocal genotypes

(ii) or the genetic integrity of a distinct local genotype may be threatened

How local is local?

(i) consider known taxonomic boundaries

(ii) consider distribution disjunctions

(iii) consider environmental variables (e.g. geomorphology, climate)

(iv) consider life history and breeding system

(v) direct analysis of genetic variation

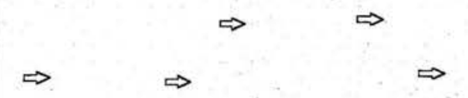
Wind ~~insect~~ pollinated > genetic provenance likely to have large provenance than insects mammal pollinated.

Genetic markers for the detection of geographic variation

	development	cost	power
allozymes	+	+	+(+)
RAPD	++	++	++
AFLP	++	+++	+++
microsatellites	+++	+++	+++
cDNA	++	++	++
RFLP	+++	++	++

Utility of AFLP to provenance delineation

-Requires no prior sequence knowledge
-Highly reproducible
-Require small amounts of tissue
-Large numbers of polymorphic loci
-Loci segregate as Mendelian markers
-Accurate resolution and scoring



presence/absence polymorphism

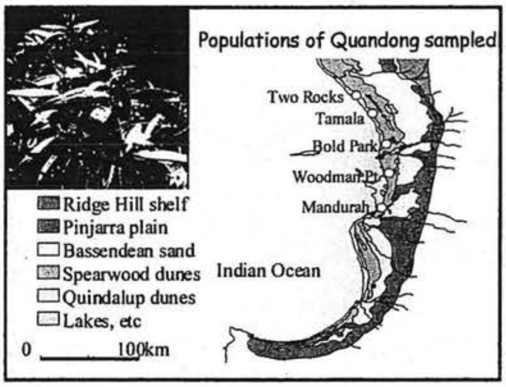
At Kings Park, AFLP applied to….

Persoonia mollis, Santalum acuminatum (Quandong) *Grevillea scapigera, Conostylis spp., Eucalyptus caesia, Mesomelaena, Leucopogon obtectus, Goodenia spp., Lechenaultia spp., Chamelaucium uncinatum*

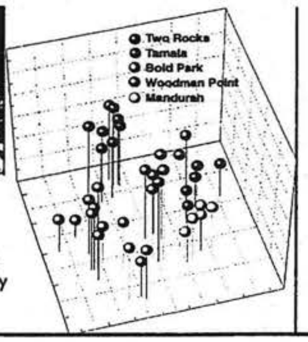
As well as

quokkas, ghost bats and dung beetles

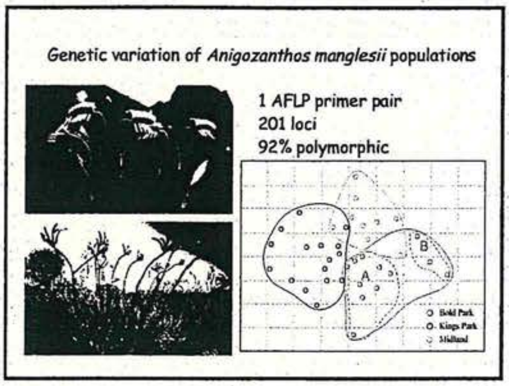
Populations of Quandong sampled

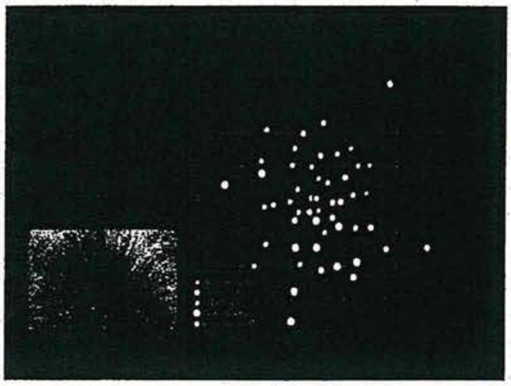


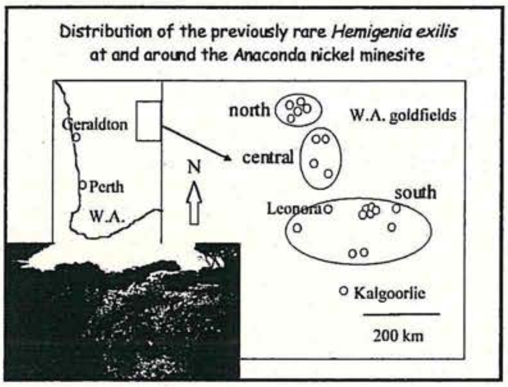
Population genetic variation in Quandong

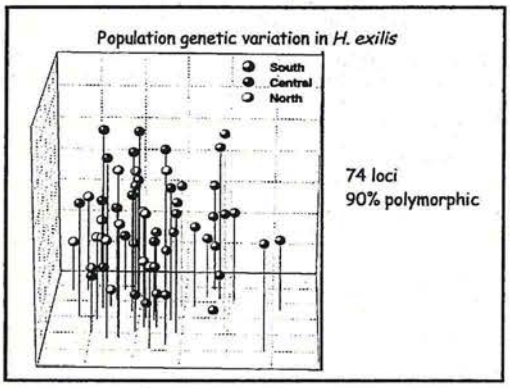


74 loci
60% polymorphic
extensive clonality

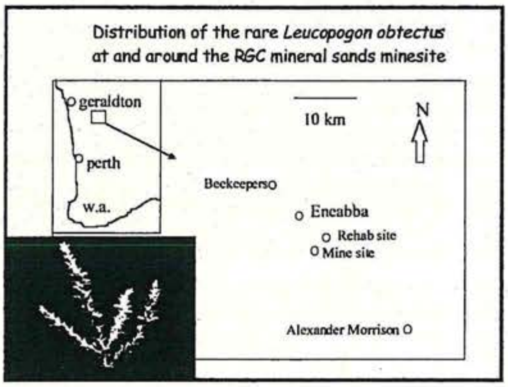


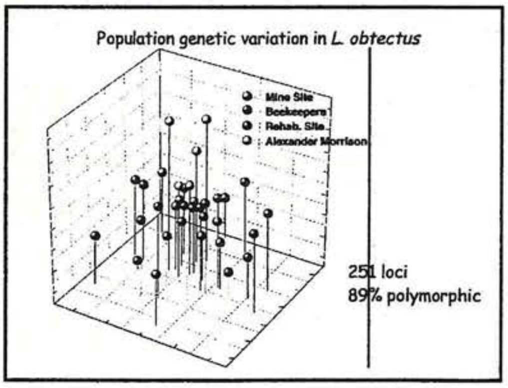






2 provenc
N+SR.





- All 4 popl^ns = same provenance.

getting the right genetic mix

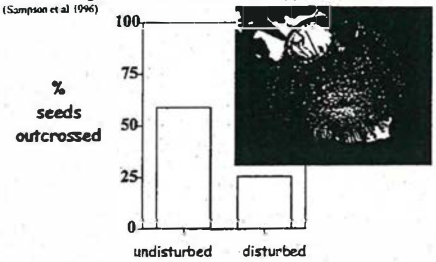
⇨ Seed source (provenance)

⇨ **Seed quality (genetic)**

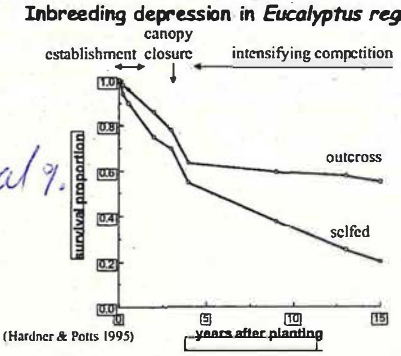
Genetic quality of seed

Outcrossing in the rose mallee *Eucalyptus rhodantha*

(Sampson et al 1996)



Inbreeding depression in *Eucalyptus regnans*



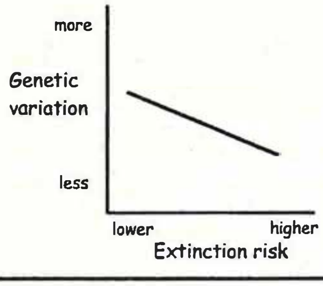
(Hardner & Potts 1995)

To maximise the genetic quality of seed

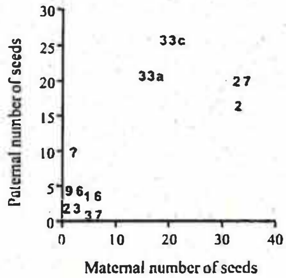
for outcrossing species -
(80% of all species)

⇨ target large undisturbed populations
⇨ and collect seed from many plants

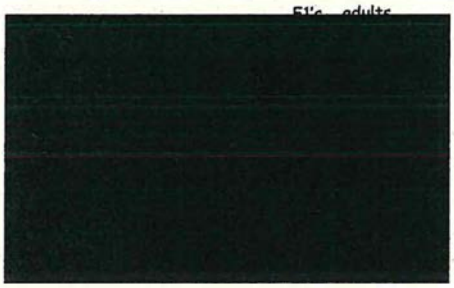
Maximise genetic variation in the restored population ensuring adequate genetic variation in restored populations as insurance against extinction in a changing world



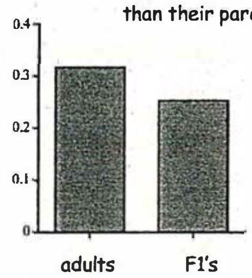
Contribution of each clone to the next generation



The F1's are more inbred than their parents!



The F1's have less genetic diversity than their parents!



Genetic principles for restoration of self sustaining, reproducing populations

(i) delineate local provenance
(ii) collect seed of high genetic quality
(iii) maximise genetic variation

Acknowledgments

Kings Park and Botanic Garden:

Peter Hood
Grace Zawko
Julia Mattner
Matt Barrett

The University of Western Australia

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Seed Collecting Protocols

Geoff Cockerton

Seed Collection Protocols

Geoff Cockerton B. Sc.
Landcare Services Pty Ltd

Abstract

The regulations governing the collection of native seeds from vacant crown land, pastoral leases, road reserves and other public areas are determined and administered by the Department of Conservation and Land Management's Wildlife Protection Branch. These regulations are in place in the absence of any industry standards and are designed to protect the native flora. They allow only a conservatively small portion (currently 20%) of seed to be taken from any population or individual plant by an individual at any one time. The "20% Rule" has been devised without substantial practical appreciation of native plant biology, reproductive strategies or seed longevity in the soil seed bank. In practice, some seed collectors do not adhere to the "20% rule" and are therefore regularly breaking the law. In many cases, however, the current practice of taking up to 100% of seed from a given plant or population can be justified on ecological and reproductive strategy grounds if the appropriate knowledge is available.

Background

The native seed industry in WA has been active and for over 20 years and seeds from as far as Augusta to Kununurra, from Exmouth to the South Australian border are produced on a regular basis. Even though the industry has been well established, there are still only relatively few individuals involved in seed production and in the revegetation industry. Reasons for this include the cost involved in establishing a seed supply business, the need for extensive specialist knowledge regarding plant identification, plant distribution, seeding biology, seed ripening and shedding, processing of the resulting crop and marketing.

End uses, in reducing order of magnitude, include revegetation by the mining industry, revegetation of near metropolitan and regional development areas, and the wheatbelt of WA. The latter has by far the greatest need for revegetation and yet the most limited availability of suitable seed sources.

Generalist seed collections have their place, however, local provenance seed collections are now the most common type of contract let. The definition of provenance has been bantered around for some time and the concept of regionalisation needs to be more widely accepted. Some clients place an arbitrary geographical boundary on acceptable provenance eg: 20 km radius of the revegetation site, that often makes the task of producing sufficient seed very difficult. A better method is to look at the biogeographic region and the specific habitat being revegetated and to harvest species that match those criteria.

Regulation

The process of seed collection is simple in theory; wait until the seeds are ripe and harvest them. However, the myriad of seeding strategies, life cycles and efficient collection methods of the 12,500 or so native species in this state means that on a regular basis, only around 2,500 varieties are collected and supplied.

The harvesting of native seeds falls under the Wildlife Conservation Act (1956) as amended and CALM regulations governing seed collecting are slowly evolving in a fashion that reflects practical harvesting methods. The recent acceptance of the biogeographic region / habitat hierarchy for determining provenance is one step in that direction. Licensing requirements are getting stricter but are also being modified to make compliance easier. Current regulations prevent the taking of more than 20% of seed from any one plant or population (in the case of annuals). Wildlife officers are charged with regulating this and some instances of prosecutions are known where these guidelines were exceeded. In reality, the damage done to some plant groups is minimal if greater than 20% of seed is taken.

Harvesting Protocols

One needs to understand the reproductive biology of the plant being harvested to determine the effect of seed harvesting. Some plant are short lived and rely on seed being stored in the soil seed bank for continuation of the population, Geosporous species. Others are long lived but still rely on seed stored in the soil. Others yet again; Bradysporous varieties, hold their seed on the plant for extended periods. All of these have different life cycles and should be tackled differently when seed is harvested.

Short lived plants without lignotuber relying on seed in the soil seed bank for survival of the population include *Acacia drummondii*, *A. pulchella*, *Gompholobium*, *Hovea elliptica*, *Boronia*, *Goodenia*, *Kennedia*, *Beyeria* and *Rulingia* species. These are obligate seeders and need seed to be produced in copious quantities to return to the soil seed bank so that the predation by insects and vertebrates does not destroy all seed. These are often referred to as disturbance opportunist species which establish rapidly after a fire or soil disturbance. These species are very sensitive to having too much seed removed from the system on a regular basis.

Some varieties that rely on the soil seed bank for survival of the population are also long lived. These include *Acacia aneura* (mulga) and the majority of inland *Acacia*, *Senna* and *Eremophila* species. These plants live for decades and have the opportunity to add seed to the soil seed bank over an extended period. The soil seed bank is therefore large. The harvesting of all seeds from a population in one year is not likely to affect the survival of the population. Due to seasonal conditions, it is also unlikely that seed is present in economically harvestable quantities every year. The taking of a large proportion of seed in the "boom years" is probably acceptable as long as the population is able to contribute some seed to the soil seed bank at other times.

Other species shed their seed each season and also have seed stored in the soil seed bank, however, plants have a lignotuber and can withstand repeated fires or removal of the above ground parts with no deleterious effect on the population. These include *Bossiaea ornata*, many *Grevillea*, *Adenanthos* species and *Acacia nervosa.* This group of plants do not rely on seed for survival of the population and the successful establishment of seedlings in the natural environment is very uncommon. They do produce viable seeds, however, if dispersal from the parent plant is not significant, the competition for resources (light, moisture) relegates the emerging seedling to a certain. The harvesting of all seeds from a population of these species does not in any way damage the parent plant or affect the populations chances of survival.

A fourth group of plants sets large amounts of seed which are held on the plant until a disturbance (fire, storm damage, flooding) triggers seed shedding. Parent plants are killed in the process. These species are obligate seeders and removal of all seed from the population has serious implications for survival of the population should a disturbance event occur. These include *Dryandra sessilis, D. formosa, Banksia prionotes, Eucalyptus salubris* and other Gimlets and Mallets, *Melaleuca uncinata* (some forms).

Another group includes the majority of *Eucalyptus*, *Melaleuca* and *Calothamnus* varieties which have a lignotuber or epicormic shoots, survive fires and other disturbance events, do not have seed stored in the soil seed bank and hold seed on the parent plant until triggered to shed. These species are not sensitive to having all seed taken as the survival of the individual and the population is not affected adversely. They resprout after having seeding branches removed and in many cases do so very rapidly. They usually flower two years after harvesting and produce large amounts of seed each year. This group of plants would warrant a higher percentage of acceptable seed harvesting.

Some progress towards the changing of the 20% rule has been made in that members of the Revegetation Industry have forwarded to CALM an outline of the above information. No formal changes have yet been approved.

Summary Table

Obligate Seeders	Short Lived Geospores	Need seed for continuation of the population	Should be harvested lightly
	Long Lived Geospores	Seed over many decades	Can be harvested heavily on an irregular basis
	Bradysporous species	Rely on seed fall for survival of the population	Should be harvested lightly
Resprouters	Geosporous species	Do not need seed for continuation of the population	Can be harvested more heavily
	Bradysporous species	Do not need seed for continuation of the population	Can be harvested more heavily

Suggestions:
Harvested lightly: 20 to 50% seed taken
Harvested heavily - upto 100% seed taken

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Seeds for the Future: *ex-situ* plant conservation in CALM

Anne Cochrane

Seeds for the Future:
ex-situ plant conservation in CALM

Anne Cochrane
Threatened Flora Seed Centre, CALM

Flora Licencing

All flora is protected in Western Australia under the *Wildlife Conservation Act 1950*. State laws regulate the picking or harvesting of native flora (flowers, seeds, whole plants) for commercial purposes and are administered by CALM. On Crown Land protected flora may only be taken by individuals who hold a licence issued by CALM. Special restrictions apply to taking flora from National Parks, Nature Reserves or Conservation Parks and taking flora for commercial purposes in these lands is prohibited. Flora may only be taken from private lands with the owner or occupiers permission. Flora taken from private property can only be sold under a Commercial Producer's Licence or a Nurseryman's Licence. The annual fee for these licences is $25. It is an offence to sell flora that has not been legally taken.

Scientific or *Other Prescribed Purposes*

People wanting to take flora for scientific study, education, hobby, propagation and other non-commercial purposes must obtain a *Scientific* or *Other Prescribed Purposes Licence*. Under this licence you are not permitted to sell any of the flora taken. The licence costs $10 annually.

Commercial Purposes

If the flora is being taken for commercial purposes a *Commercial Purposes Licence* must be obtained. You must also submit "returns" of the flora taken to CALM. The annual fee for this licence is $100.

Rare and Priority Flora

There are 334 species of plants listed as Declared Rare Flora. They are considered to be in danger of extinction, rare or otherwise in need of special protection. Rare flora cannot be taken without the specific written approval of the Minister for the Environment. This applies to both Crown and private lands. Many other species of flora are known from only a few collections or sites. They may be rare or threatened but require further survey prior to being considered for declaration as rare flora. These plants are listed as Priority Flora and cannot be harvested on Crown Lands under a commercial licence.

CALM's *ex-situ* conservation

Seed from rare and priority flora is collected for a number of reasons:

- Long term storage
  as a safe guard against extinction in the wild
- Recovery
  reintroduction and augmentation of threatened flora populations
- Genetic research
  differences between and within populations
- Seed biology research
  seed germination requirements
- Disease susceptibility research
  effect of disease on survival of threatened plants
- Botanic Gardens and Parks Authority
  for display and for stock plants for ex situ conservation and recovery

Overcoming Dormancy

Seed is of little use to CALM unless it can be germinated to produce a whole living plant whether for recovery work or for research. In many cases dormancy reduces our ability to germinate fresh seed and a range of cues are required to help stimulate germination. These may include heat shock to imitate a fire, the application of growth hormones to overcome after ripening requirements, leaching of inhibitors in the seedcoat or removal of a portion of the fruit wall or seed coat to simulate seed ageing. These cues are used over and above the standard requirements of moisture, adequate temperature and light.

Some examples of difficult to germinate genera within the Western Australian flora include *Adenanthos*, *Eremophila*, *Darwinia* and *Verticordia*. Many threatened species occur in these four genera and CALM's Threatened Flora Seed Centre has made a concerted effort to overcome dormancy in these groups.

1. Adenanthos

come up after fire well.

Adenanthos generally exhibit flowering and fruiting over long periods of time making cost-effective and efficient seed collection difficult. Seed traps constructed of fence droppers and fibreglass flyscreen wire have proven very successful for a number of species. The traps are not 100% effective for collection as some seed is wind blown away from the traps and other seed falls through the small aperture beneath the plant, but the traps collect much more seed with much less effort than was required formerly. The major problem experienced with this method of seed collection is the need to clear traps on a regular basis to prevent seed predators such as bush rats reducing the number of seed retrieved. Once collected, it is quite common for the fruits to be empty of the endosperm and embryo necessary for growth. Seed abortion may be attributed to lack of resources (for example nutrients, pollinators and/or pollen) or could perhaps be a predator avoidance strategy. Whatever the reason for the production of empty fruit, it is very difficult to determine whether the fruit is filled or not unless you cut the hard fruit wall or weigh the seed. Heavier seed contains endosperm and embryo. Despite success with collection and seed quality

→ can weigh fruit to see if viable seed occurs inside.

fly screen wire. in under plants.

7 are threatened

17 taxa on rare + priority list

determination, fresh seed is not readily germinable without some form of pre-treatment. Reasonable germination has been achieved using smoke, the complete seed coat removal in conjunction with applications of the growth hormone gibberellic acid.

2. Eremophila

Eremophila are an extremely versatile group of plants that may exhibit tolerance to fire, drought, frost, grazing and salinity and have the potential for minesite rehabilitation and rangeland revegetation programs. Seeds are notoriously difficult to germinate due to the hard fruit wall that is impenetrable to water and gas exchange, and to radicle emergence. In the laboratory, the fruit is sectioned to determine the presence of seed and to quantify seed set. Most species tested to date have been multi-loculed but few fruits contain the full compliment of seed and many are empty. Seed set (proportion of fruit with at least one locule containing seed) in 14 threatened species tested ranged from 1% to 91%. Fresh and one year old seed stored at –20°C can be readily germinated once extracted from fruits in the presence of the growth hormone gibberellic acid. Germination in 12 species ranged from 29% to 87% for fresh seed and 50% to 100% for stored seed. 18 DRF.

3. Verticordia

In *Verticordia* the seed forms in the hypanthium of the flower and the old flower becomes the fruit without a change in overall structure. Flowers fade and fall when seed is ripe. We have noted that there is considerable intra- and inter-specific variation in both seed production and germinability in the majority of threatened taxa tested. The seed to flower ratio, or "seed set" ranged from 0% to 68% (mean 21%) in 82 collections representing seed from 48 populations of 22 different taxa. Percentage germination ranged from 7% to 100% (mean 49%) for 68 collections. Germination is achieved by removing the fresh seed from the old flower and incubating it on the growth hormone gibberellic acid. Smoked water solution is often used to soften the hypanthium prior to extracting the seed. The frequently low annual reproductive capacity of some of the more restricted and critically endangered taxa may threaten their survival and unexpected disturbance events may result in population decline or even localised extinction. Many of these taxa will require recovery efforts to ensure survival over the long term. 8 are DRF. 76 taxa on rare/priority list.

cut from hypanthium

4. Darwinia

Like *Verticordia*, fresh seed of *Darwinia* species exhibit dormancy. Seed set appears to be much higher than for *Verticordia*, although this is perhaps due to the fact that it is easier to recognise filled fruits from those shrunken and aborted. A somewhat leathery seed coat prevents water and gas exchange and also radicle emergence. In the laboratory, seeds are extracted from the fruits and incubated on the growth hormone, gibberellic acid. Percent germination in 12 taxa representing 29 collections of rare and threatened *Darwinia* from the south-west of Western Australia ranged from 0% to 86%.

15 are DRF. 33 are rare priority

DEPARTMENT OF CONSERVATION AND LAND MANAGEMENT

DECLARED RARE AND PRIORITY FLORA LIST

for Western Australia

CONSERVATION CODES

R: Declared Rare Flora - Extant Taxa

Taxa which have been adequately searched for and are deemed to be in the wild either rare, in danger of extinction, or otherwise in need of special protection, and have been gazetted as such.

X: Declared Rare Flora - Presumed Extinct Taxa

Taxa which have not been collected, or otherwise verified, over the past 50 years despite thorough searching, or of which all known wild populations have been destroyed more recently, and have been gazetted as such.

1: Priority One - Poorly known Taxa

Taxa which are known from one or a few (generally <5) populations which are under threat, either due to small population size, or being on lands under immediate threat, e.g. road verges, urban areas, farmland, active mineral leases, etc., or the plants are under threat, e.g. from disease, grazing by feral animals, etc. May include taxa with threatened populations on protected lands. Such taxa are under consideration for declaration as 'rare flora', but are in urgent need of further survey.

2: Priority Two - Poorly Known Taxa

Taxa which are known from one or a few (generally <5) populations, at least some of which are not believed to be under immediate threat (i.e. not currently endangered). Such taxa are under consideration for declaration as 'rare flora', but are in urgent need of further survey.

3: Priority Three - Poorly Known Taxa

Taxa which are known from several populations, and the taxa are not believed to be under immediate threat (i.e. not currently endangered), either due to the number of known populations (generally >5), or known populations being large, and either widespread or protected. Such taxa are under consideration for declaration as 'rare flora' but are in need of further survey.

4: Priority Four - Rare Taxa

Taxa which are considered to have been adequately surveyed and which, whilst being rare (in Australia), are not currently threatened by any identifiable factors. These taxa require monitoring every 5-10 years.

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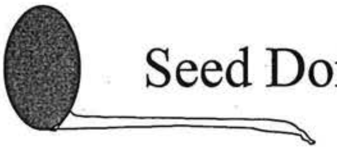
An Ecological basis for Seed Dormancy

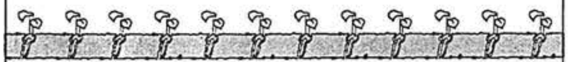
Dr Julie Plummer



Seed Dormancy

About Native Seeds
Dr Julie Plummer
UWA

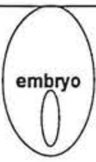
Why don't seeds germinate?



- **What is a seed?**
- **Is there any seed?**
- **Is the seed alive?**

embryo, endosperm + seedcoat = seed

- seed decays occur



Any living seed?

Viability Tests

- **Simple Tests**
  - Cut test
  - Flotation test

cut test - shows us embryo - white
endosperm - should be white + fleshy

flotation screens for living seed - seed will sink if viable - sometimes!
- seeds that have an embryo sink
but embryo may not be alive.
- float test doesn't always work - eg with Grevilleas. - float test not good

Viability Tests

- **Standard tests**
  - **Germination Tests**
    - Petri dishes
    - Paper Towelling
    - Flats in a greenhouse

  - **Chemical Tests - Tetrazolium Test**
    - 2,3,5-triphenyl tetrazolium chloride (TTC; 0.1-0.5%)
    - WARNING TOXIC!
  - **Excised Embryo Test**
    - Petri dish or sterile tube

Is it dormant?

i.e. is it alive but won't germinate?

Genome x Environment

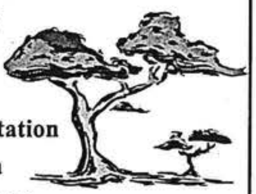
- **Why are seeds dormant?**
- **Where are the seeds from?**

- **Genome** - taxonomic relationships
- **Environment**
  - similar climate (water, temperature), soil, vegetation type

dormancy to avoid averse cond^ns

What are the major vegetation regions of WA?

- What are the regions?
- What environmental factors define them?
- What plants exist in each?
- What adaptations do they have to exist there?
- Seed dormancy is an adaptation
- Plants from the same area may have similar adaptations



Cues from the Environment

- Measuring time & temperature
- Measuring water & moisture
- Determining light, shade
- Fire
- Multiple factors

} can be combinations of these

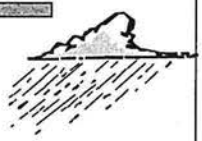
Bioclimates (Bagnolus & Gaussen, 1957)

1. DESERT (Eremean)	
bc Intermediate (summer & winter rain)	12 months dry
b Summer rain	
d Non-seasonal	
2. SEMI-DESERT (Sub-Eremean)	
a Mediterranean	9-11 dry months
b Tropical	
x Bixerix	
3. MEDITERRANEAN	
a Extra dry mediterranean	7-8
b Dry mediterranean	5-6 dry months
c Moderate mediterranean	3-4 (summer)
4. TROPICAL	7-8 dry months
a Dry hot tropical	(winter)

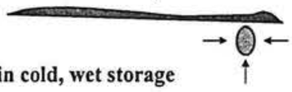
Measuring Time & Temperature

- After-ripening
  - Several months dry storage, warm temperature
    - Grasses: *Themeda australis, Stipa begeniculata* (11 months)
    - Arid-zone forbs: *Rhodanthe chlorocephala* (1m), *Schoenia filifolia* (8 m)
  - High temperatures reduce the time required
    - *Themeda australis* (20/60C)
    - *Schoenia filifolia* (80C for 11 days)
  - Hormone application - GA3
    - *Schoenia filifolia*

Measuring Time & Temperature

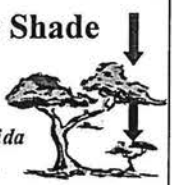


- Soil storage
  - physical or microbial breakdown of inhibitors



- Chilling
  - Several months in cold, wet storage
  - generally restricted to alpine species in SE Australia
  - Hormone application - GA3

Determining Light & Shade



- sunlight is high in RED
  - promotes in *Juncus pallidus, Aristida contorta, Erymophyllum ramosum, Craspedia* sp.
  - application of GA_3, KNO_3
- filtered through a canopy, high in FAR RED - generally inhibits
- some plants require darkness
  - *Spinifex hirsutus*
  - related to moisture in sand dunes

Fire

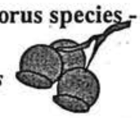
- many plants have evolved to cope with fire
- post-fire: reduced competition, increased light, nutrients
- heat- hard seed coats
  - legumes (eg *Acacia, Gompholobium, Chorizema, Daviesia)*
  - boiling, scarification, acid
- smoke - tents, solutions

Multiple factors

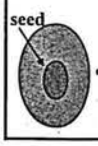
- dormancy may be complex requiring several factors sequentially
  - eg storage & smoke - *Orthrosanthus laxus*
  - eg heat, light & nitrate
  - eg dry storage & light - *Craspedia* sp

Which tissue is responsible?

- Fruit, seed coat, endosperm, embryo?
- Woody Fruit (eg Bradysporus species - held on plant)
  - *Banksia, Hakea, Eucalyptus*
    - heat to open fruit



- Succulent Fruit
  - (eg *Persoonia elliptica, Carpobrotus modestus*)
  - fruit has inhibitors, eaten - dispersal method



- Dry fruit
  - inhibitors: remove fruit, leach out

Germination Requirements

- Water
  - for metabolic processes
    - salinity: species vary but < 200 mM NaCl
    - diluted with rainfall
- Temperature
  - appropriate to rainfall period
- Oxygen
  - for respiration, active growth

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Seed treatments to enhance germination

Bob Dixon

The University of Western Australia
CENTRE FOR LAND REHABILITATION

KINGS PARK
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Seed Storage

Luke Sweedman

Seed treatments to enhance germination

Bob Dixon
Manager Biodiversity and Extensions
Kings Park and Botanic Garden

For seed to germinate they may require specific pretreatment before sowing, in some cases more than one treatment further enhances germination rates, and there is a great deal of variation even within plant species in the same genus. Therefore you cannot generalise on seed treatments. For germination to occur, the seed must not be in a state of dormancy and the environmental conditions for germination must be met (Ref 1).. Of course you also have to make sure the seed is viable before treatment/sowing.

In nature seed will germinate when the conditions are right and the seed are ready to germinate, that is, they have been exposed to the correct after ripening processes, seed germination inhibitors have disappeared and dormancy has been broken. You may also be aware in nature not all of the seed germinate at once, even after fire events there is always a residual seed bank in the soil. This also happens with direct seeding with germination rates higher in the second season after sowing (Ref 4).

So what we are trying to do is artificially enhance germination and expect to achieve 100% germination rates, this is rarely achieved even in controlled laboratory conditions.

There are numerous seed treatments available to enhance germination the main treatments used include-

- scarification ,physical removal of a portion of the seed coat, can be done by hand or machine . Generally used on hard coated seed eg *Acacia*, *Kennedia* (note seed can be stored for 1 to 2 years before sowing)
- hot water treatment , pouring boiling water over seed and allowing it to cool, generally sown straight after treatment. Generally used on hard coated seed eg *Acacia*, *Kennedia* (note as long as the seed has not imbibed and the germination process started, and if seed is dried correctly, it can be stored for several months before sowing)
- smoke treatment , the use of aerosol smoke or smoke water. Used on a wide range of genera eg *Anigozanthos*, *Grevillea*, *Thysanotus* (Ref 6), (note aerosol treated seed is easy to store, smoke water treated seed can be dried and stored as long as the seed has not imbibed and the germination process has started)
- gibberellic acid, usually used by researchers for small quantities of seed on difficult to germinate species note GA sometimes reduces germination rates (Ref 2).

Other specific seed treatments

Rapid ageing of seed is an important new method being used in seed treatment. *Anigozanthos manglesii*, *Stylidium affine* and *Loxocarya striata* respond well to temperatures of 100 degrees centigrade for 3 hours (Tieu pers comm). There may well be a number of other seed that also respond to this treatment, further research is required.

Baking seed in the sun has been used for a number of years and enhances the germination of a number of seed including *Verticordia grandis*, fresh seed sown straight after harvest and the pots left in the sun over summer, germination rates of 60-70% ; *Macropidia fuliginosa* responds to this treatment but will only germinate when the seed is at least 1 year old, 50-60% germination rate (Ref 3), indicating there are also other requirements for germination eg maturation of the embryo, note seed only germinates in falling temperatures; *Tripterococcus brunonis* also responds very well during this process the wings of the seed break down germination rates after this process are up to 90%.

Fruit removal and baking the seed in the sun again there are a number of species that respond to this process eg *Leucopogon verticllatus* the seed is covered in a hard almost woody seed coat and soft white fleshy fruit , remove the fruit by placing in a small quantity of water for about 2 weeks to ferment then wash off the fruit and sow, this gives a 65% germination rate; *Lomandra ordii* using this process gave a 51% germination rate (note you must use fresh seed); *Persoonia comata* (also applies to *P saccata*) ferment the fruit and pinch off the remainder with dry newspaper though this only gave 8% germination rate, the addition of leaching the seed in running water for 3 weeks increased germination rates to 46%, scarification without the running water gave 40% germination rates (Ref 3).

Bimodal dormancy. The temperature for many species for after ripening etc is very important even with some common species. One of our most difficult species is *Conostylis aculeata* as with most metropolitan areas of bushland we can never get enough seed and have to use it immediately after collection and therefore have to get over the temperature problem. It has peak germination 60% in autumn March to May, winter, spring and summer dormant 5-10% germination rate, the following autumn is okay. Smoke and GA only raises or elevates germination to this level (Tieu and Meney pers comm). Some *Grevillea* species are best enhanced with smoke plus heat treatment (Ref 5).

Scarification is usually an easy process , however some seed require specific scarification treatment eg *Grevillea bracteosa* 2 year old seed gave 71% germination rate when the straight edge of the seed was scarified, broken off with the blade of a knife (Ref 3) note scarfiying in the usual manner produced very poor results; *Grevillea scapigera* using fresh seed responds in the same way , however the germination period is over a longer period of time (in the field older seed respond to smoke treatment but fresh seed do not respond to smoke treatment alone).

Splitting or cutting woody fruit . This is regularly used on *Santalum* species using a bandsaw or vice ot allow the seed to swell and germinate. A band saw is also used on the native walnut to cut straight through the woody fruit and expose the seed, this should only be done in summer as wet and cold conditions rot the seed. *Eremophila* species have a natural split in the woody seed capsule, use a hooked knife to split it open, again it must be done in the spring/summer.

References

1. Bell, D. T. (1999) Turner Review No. 1. Australian Journal Botany 47, 475-517.
2. Bell, D.T., Rokich, D.P., McChesney,C.J. and Plummer, J.A. (1995). Effects of temperature, light and gibberellic acid on the germination of seeds of 43 species native to Western Australia. Journal of Vegetation Science 6: 797-806.
3. Lamont, B and Watkins, P. (Eds)(1985). Propagation by seed. Horticulture of Australian Plants. Western Australian Dept. Agriculture, South Perth W.A.
4. Meney, K. (1999). Mt Eliza escarpment restoration plan. Kings Park and Botanic Garden, West Perth, Western Australia 6005.
5. Morris, E. C. (2000) Germination response of seven east Australian Grevillea species (Proteaceae) to smoke, heat exposure and scarification. Australian Journal of Botany 48: 179-189.
6. Dixon, K.W., Roche, S. and Pate J.S. (1995) The promotive effect of smoke derived from burnt native vegetation on seed germination of Western Australian plants. Oecologia 101:185-192.

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Further reading

Floradata, native seed database (in press). More information from Michael Lloyd at acmer.project@mailbox.uq.edu.au.

SEED STORAGE DISCUSSION

General Seed Physiology and Longevity

- Seeds excavated from beneath monasteries and roads in Sweden and Norway have been grown and dated at 1700 years old (Spergula arvensis)
- Seed found at depths of 120cm in iron age digs in Denmark from 100BC
- Egyptian tombs, wheat at 2000 to 3000 years old.
- Arctic lupin 10,000 years old found in 1954 in the Yukon were found in Lemming burrows deeply buried in permanently frozen silt of Pleistocene age in central Yukon. Six seeds germinated within 48 hours after returning to room temperature over 12 years in a dry place.
- However there are well documented numbers of common species well authenticated for over 100 years

(1986 Crop Science Society of America, Physiology of Seed Deterioration.CSSA Spec,Pub no 11)

Precepts of successful Seed Storage

Lower temperatures reduce seed metabolism and therefore slow seed ageing

Low moisture contents impedes seed aging reactions and increases storage life

Seed longevity in storage still governed by factors including genotype, maturity at collection and storage environment ie temperature and humidity.
General rule is that for each 1% decrease in moisture content or 5degrees Celsius reduction in temperature, a seeds storage life is doubled (Harrington 1972 Physiol of Seed Deterioration)

Types of Seeds (Roberts 1973)

Orthodox

Can be dried to a low moisture, generally considered to be around or down to 5% net weight basis. Seed tends to be small and are the most common species especially from arid and temperate climates and generally have long periods of viability most Australian Seed

Recalcitrant

Large and fleshy and are killed below a moisture content of 20%. Cannot be kept for more than a few months under any conditions. Eg Chestnuts Coffee seed,

Recommendations for seed storage of native species is based almost entirely on Research from northern hemisphere agricultural species.

Issues contributing to successful seed storage

1. Quality of material collected.

This is the key factor in determining a successful outcome for storage. Before all other issues, seed needs to be of a high standard and collected at the right time of maturity

2. Cleaning

This should be done efficiently and in as least damaging a way as possible to the seed coats. Any insect infestation should be checked and dealt with

3. Test for viability and germinability
Done by cut test and by growing samples
4. Processing
Ensuring correct records are kept giving all collection details especially locations, soil type etc
5. Storage
Use appropriate storage techniques, if long term consider moisture levels. Humidity levels (eg 15% and 15degrees poss ideal)

Storage Needs

Environmental conditions under which seed is stored depends on the purpose the collection is to serve

- Evaluate what you are using the seed for eg revegetation or other
- Consider the time that you need to store the seed
- Budget

The Successfully Stored Seedlot

1. Collected from the field ripe and seeds falling from the fruit eg Acacias
2. Collecting bags placed dry, low humidity, warm and well ventilated area from the field. Not exposed to high temperatures (15-20%relative humidity if possible)
3. Checked for insect infestation and cleaned
4. Seed separated for viability and or germination testing
5. Bulk sample placed in appropriate storage facility
6. Monitored every 12 months or so.

Containers for storage

Moisture is the overriding factor in seed longevity and seeds should be protected from the ambient atmosphere.

Containers need to be airtight
- Glass jars
- Aluminium containers
- Plastic containers
- Foil packets

Recommendation
Foil packets hermetically sealed using CO2

It is important where practical to remove oxygen from the storage container and replaced with inert CO2. This helps to prevent oxidative aging reactions within the seeds and also prevents predation by microorganisms or insects, which can contribute substantially to seed decay during storage

Types of Storage Conditions

Short term
Seed containers stored at or below ambient temperatures in a dry dark place. Seed stored when clean at ambient humidity.
Expected longevity
One to five years but varies by species. Eg *Asteraceae* and *Mimosaceae* Acacias, Eucalyps

Medium Term
Some type of cooling with CO2 used in packets. Seed placed in cool store at or near freezing.
Expected Longevity
Five to 20 years but varies by species. Asteraceae.

Long Term
Seed of low moisture contents. Sealed in CO2 foil packets. Stored at -20c
Expected Longevity
25 to 100 years

Ultra long term
Seeds frozen at critical moisture contents to prevent damage. Stored in liquid nitrogen at below -130c
Expected Longevity
Indefinite? rare flora.

Luke Sweedman
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THE CENTRE FOR LAND REHABILITATION was established at The University of Western Australia in 1995. The centre, led by Dr David Jasper, is based in the Soil Science and Plant Nutrition Group within the Faculty of Agriculture. With strong links to other research centres at the University, it is a multi-disciplinary centre applying soil science, geomechanics, hydrology, soil biology, plant nutrition, plant biology, ecology and resource economics to the management of disturbed lands.

The Centre for Land Rehabilitation has four major objectives:

- To increase understanding of **processes contributing to stable landforms and sustainable ecosystems** in mine rehabilitation
- To contribute to the development of **management strategies to restore and maintain physical, chemical, and biological fertility** in degraded agricultural soils
- To increase understanding of the **plant and soil resour ces of rangelands** and develop strategies for their sustainable management
- Through application of appropriate science disciplines, contribute to **rehabilitation and management of other disturbed land** s, including urban land and wetlands

The objectives of the Centre are being achieved through the major activities of research, education and training. Applied and basic research is being conducted to develop cost-effective solutions for rehabilitation of disturbed lands and disposal of wastes. The practice of land rehabilitation is also being advanced through practically-oriented short courses directed to both managers and operators.

The University of Western Australia

CENTRE FOR LAND REHABILITATION

Centre of Excellence

The Centre is supported by the State Government under its Centres of Excellence Programme. These funds support core research and training of staff in the Centre. The aim of the funds is to provide a catalyst for further growth of the Centre.

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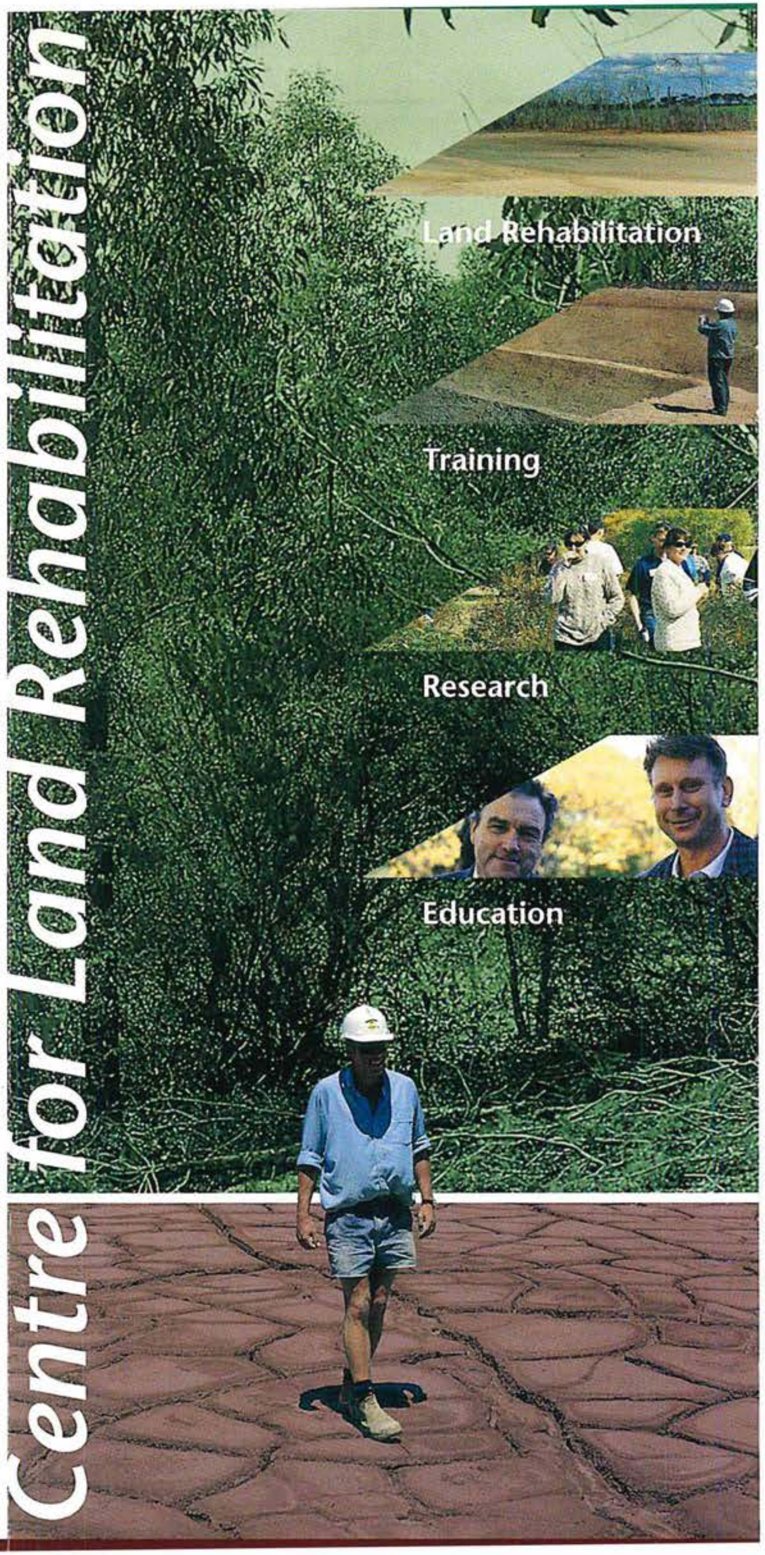
To receive our newsletter which highlights current research and workshops, please contact Sandra Maynard. Email: sandra.maynard@uwa.edu.au.
Check our website for current training schedules.

The Centre for Land Rehabilitation wishes to acknowledge the support of the following organisations:

THE UNIVERSITY OF WESTERN AUSTRALIA

BHP

COMMERCE AND TRADE
GOVERNMENT OF WESTERN AUSTRALIA



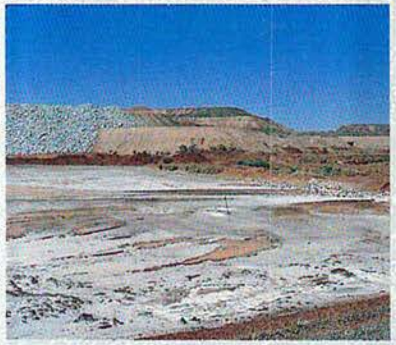
TRAINING

The Centre for Land Rehabilitation offers a wide range of courses covering management strategies for sustainable ecosystems. Including:

Restoration with native plants

Understanding environmental processes and maximising successful revegetation with native plants.

Minewaste management



Understanding the mineralogy, geochemistry and weathering of mine materials to optimise rehabilitation.

Environmental monitoring

Two days of practical methods for all mining monitoring methods, including case studies from industry.

RESEARCH

Applied and basic research of a high quality is conducted in co-operation with industry and Government, to develop cost-effective solutions for rehabilitation of disturbed lands and disposal of wastes that meet the needs of both industry and the community .

These new research projects range from research in mine tailings rehabilitation, including a collaborative project in the People's Republic of China, through to developing soil biological indicators for sustainable grain production in WA.

Gold residue rehabilitation research

Ecophysiology of stress tolerance in Halosarcia

Inoculating VA mycorrhizal fungi into mine soils

Water & nutrient uptake by pastures growing on bauxite residue

To register to receive information about specific courses or research please complete the Registration form on the panel oppostie.

Mailing list registration

Contact details

Title: ....

First Name: ....

Surname ....

Organisation: ....

Position: ....

Address: ....

.... Postcode: ....

Tel: ....

Fax: ....

Email: ....

Areas of special interest:

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Potential courses you would attend:

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Preferred location of courses:

Tick a box ☐ Metropolitan
☐ Country location ....

....

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