Nursery propagation and seed biology of threatened flora for translocation.

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Kings Park Science has utilised an integrated conservation approach for many threatened species including:

- *Grevillea scapigera* (Proteaceae)
- *Symonanthus bancroftii* (Solanaceae)
- *Eremophila resinoso* (Scrophulariaceae)
- *Darwinia masonii* (Myrtaceae)
- *Lepidosperma gibsonii* (Cyperaceae)
- *Androcalva perlaria* (Malvaceae)
- *Ricinocarpos brevis* (Euphorbiaceae)
- *Tetratheca erubescens* (Elaeocarpaceae)

**Propagation & seed research integral components**
## Plant production for translocation
### Summary of main approaches

<table>
<thead>
<tr>
<th>Propagation method</th>
<th>Cost</th>
<th>Time frame for field ready plants</th>
<th>Equipment &amp; facility support needed</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seeds</td>
<td>Low</td>
<td>Short (4 - 8 m)</td>
<td>Low (basic accredited nursery facilities)</td>
<td>Greenstock with strong root systems</td>
<td>Only practical when seed is available &amp; seed biology understood i.e. seed quality, dormancy &amp; germination requirements</td>
<td><em>Acacia woodmaniorum</em></td>
</tr>
<tr>
<td>Cuttings</td>
<td>Low-medium</td>
<td>Short (4 - 12 m)</td>
<td>Low to medium</td>
<td>Overcomes seed bottlenecks Produces semi mature plants</td>
<td>Plants may not perform as well due to weaker root systems, not all plants strike from cuttings, slower than seeds.</td>
<td><em>Darwinia masonii</em></td>
</tr>
<tr>
<td>Division</td>
<td>Medium</td>
<td>Short - medium (6 -24 m)</td>
<td>Low to medium</td>
<td>Can work well with rhizomatous plants, overcomes seed bottlenecks</td>
<td>Slow to establish, takes up a large amount of space, only applicable to a niche group of plants</td>
<td><em>Lepidosperma gibsonii</em></td>
</tr>
<tr>
<td>Tissue culture</td>
<td>High</td>
<td>Medium-long (&gt;12 m)</td>
<td>High</td>
<td>Small amount of material required, overcomes seed &amp; other bottlenecks, large rates of multiplication</td>
<td>Many potential bottlenecks i.e multiplication, root induction, deflasking</td>
<td><em>Synaphea quartzitica</em></td>
</tr>
</tbody>
</table>
Plant production cont. (excluding tissue culture)

- Direct seeding *in situ* – potentially very cheap however emergence and persistence low
- Direct seeding into pots – useful when seeds are not limiting & germinate easily
- Prick out seedlings from Petri dishes – useful with fewer seeds & germination bottlenecks
- Cuttings – strike cuttings in punnets then remove & pot up (slower)
- Cuttings – strike cuttings directly into forestry pots (quicker)

However, seed derived plants commonly perform better than cutting derived plants

*Androcalva perlaria* *in situ* translocation – Wellstead region

<table>
<thead>
<tr>
<th>Propagation method</th>
<th>% survival - 2 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seedlings (n = 80)</td>
<td>78.8 ± 10.0</td>
</tr>
<tr>
<td>Cuttings (n = 80)</td>
<td>53.8 ± 13.4</td>
</tr>
</tbody>
</table>

Where possible seed is preferable though clearly not always an option…
Avenues for seed biology research

Germination requirements
(Temperature, light conditions, moisture, stimulants)

Enhancement & *in situ* germination
(priming, coating, pelleting, field trials)

Longevity
(rapid ageing, Probert analysis & ranking)

Storage
(temperature, duration, moisture content)

Seed-focused disciplines

Dormancy
(type, embryo attributes, treatments)

Seed attributes
(mass, shape, seed quality, appendages)

Dispersal
(cafeteria experiments, ant midden assessment)

Soil seedbank dynamics
(burial & retrieval, soil cores, persistence, dormancy cycling)
Seed dormancy within WA DRF

- ~429 DRF from ~45 families
- Based mainly on the characteristics of related species we find:
  - ~15 % likely to possess non-dormant seeds
  - ~19 % likely to have physical seed dormancy
  - ~44 % likely to have physiological seed dormancy
  - ~12 % likely to have underdeveloped embryos (MD or MPD)
- UP TO 75% MAY HAVE SOME FORM OF SEED DORMANCY
- May(?) confer seed persistence within the soil seed bank
Dormancy may interact with fire cues so what are the conditions for breaking dormancy & stimulating germination?

- ~44% (?) likely to respond to smoke (~20 families)
- ~20% (?) likely to be heat responsive (3 families)

Need to identify triggers for better management of DRF in situ and for ex situ conservation collections

Dormancy may interact with fire cues so what are the conditions for breaking dormancy & stimulating germination?

i.e. afterripening, wet/dry cycling, & stratification
Germination responses to incubation temperature and fire related cues

**Ricinocarpos brevis**

Smoke water

**Symonanthus bancroftii**

KAR$_1$

**Androcalva perlaria**

Heat
**In situ seed persistence**

*Symonanthus bancroftii* – Physiological seed dormancy

<table>
<thead>
<tr>
<th>Months of <em>in situ</em> burial</th>
<th>0 months</th>
<th>12 months</th>
<th>24 months</th>
<th>36 months</th>
<th>62 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Seed Fill</td>
<td>98.5 ± 1.0%</td>
<td>94.1 ± 2.5%</td>
<td>94.5 ± 5.5%</td>
<td>94.0 ± 2.0%</td>
<td>88.5 ± 6.2%</td>
</tr>
<tr>
<td>Lab germination</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>8.0 ± 2.3%</td>
<td>0.0 ± 0.0%</td>
<td>0.0 ± 0.0%</td>
<td>0.0 ± 0.0%</td>
<td>0.0 ± 0.0%</td>
</tr>
<tr>
<td>HW - 1 min</td>
<td>91.0 ± 4.4%</td>
<td>96.9 ± 3.1%</td>
<td>99.0 ± 1.0%</td>
<td>97.8 ± 1.3%</td>
<td>100.0 ± 0.0%</td>
</tr>
</tbody>
</table>

*Androcalva perlaria* – Physical seed dormancy

<table>
<thead>
<tr>
<th>0 months</th>
<th>5 months</th>
<th>12 months</th>
<th>17 months</th>
<th>24 months</th>
<th>28 months</th>
<th>36 months</th>
<th>76 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>Autumn</td>
<td>Spring</td>
<td>Autumn</td>
<td>Spring</td>
<td>Autumn</td>
<td>Spring</td>
<td>Autumn</td>
</tr>
<tr>
<td>% Seed fill</td>
<td>94.3 ± 2.5%</td>
<td>88.8 ± 2.1%</td>
<td>90.3 ± 1.4%</td>
<td>89.3 ± 2.8%</td>
<td>80.8 ± 3.0%</td>
<td>84.7 ± 2.2%</td>
<td>89.0 ± 2.0%</td>
</tr>
<tr>
<td>Lab germination</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>0.0 ± 0.0%</td>
<td>0.0 ± 0.0%</td>
<td>0.5 ± 0.5%</td>
<td>0.6 ± 0.6%</td>
<td>0.0 ± 0.0%</td>
<td>3.1 ± 1.7%</td>
<td>0.9 ± 0.9%</td>
</tr>
<tr>
<td>1 µm KAR</td>
<td>0.0 ± 0.0%</td>
<td>16.7 ± 7.6%</td>
<td>20.7 ± 11.3%</td>
<td>81.1 ± 14.4%</td>
<td>14.7 ± 2.7%</td>
<td>79.0 ± 7.7%</td>
<td>2.5 ± 0.8%</td>
</tr>
</tbody>
</table>

Dry season **Wet season** Dry season
Androcalva perlaria

Fire driving seedling recruitment

September 2014
Controlled fire

Max heat pulse (>60°C) ~4 mins – 2-3 cm below ground

Site were no plants seen for > 6 yrs

9 months later
>130 plants flowering & fruiting

December 2014
>100 seedling appeared ~ 3 months after the fire
Seed priming

- Used to improve various germination parameters
- *R. brevis* seeds exposed to priming treatments: 0 - 5 days
- Seeds were dried back before incubation
- Hydropriming enhanced total germination & rate

*Ricinocarpos brevis*

<table>
<thead>
<tr>
<th>Priming time</th>
<th>$T_{\text{initial germination}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>12 days</td>
</tr>
<tr>
<td>24 h</td>
<td>8 days</td>
</tr>
<tr>
<td>48 h</td>
<td>8 days</td>
</tr>
<tr>
<td>72 h</td>
<td>8 days</td>
</tr>
<tr>
<td>96 h</td>
<td>8 days</td>
</tr>
<tr>
<td>120 h</td>
<td>6 days</td>
</tr>
</tbody>
</table>

Hydropriming - water

Hydropriming - $\text{GA}_3 + \text{KAR}_1$
Priming improves water stress tolerance

- Germination in response to water stress assessed
- Different priming treatments
- Priming improved overall germination and water stress tolerance

*Ricinocarpos brevis*
Field emergence
-Seed based translocation-

Ricinocarpos brevis

<table>
<thead>
<tr>
<th>Shadecloth guard</th>
<th>Weekly irrigation</th>
<th>Seed Location</th>
<th>Seed pre-treatment</th>
<th>Average emergence (± SE) 9 weeks</th>
<th>Average emergence (± SE) 16 weeks</th>
<th>Average emergence (± SE) 18 weeks</th>
<th>Average emergence (± SE) 34 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>None</td>
<td>Surface</td>
<td>None</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>No</td>
<td>None</td>
<td>Surface</td>
<td>10% smoke water</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>No</td>
<td>None</td>
<td>Buried</td>
<td>None</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>No</td>
<td>None</td>
<td>Buried</td>
<td>10% smoke water</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Yes</td>
<td>None</td>
<td>Buried</td>
<td>Hydropriming with ( \text{GA}_3 ) and ( \text{Kar}_1 )</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>Yes</td>
<td>Buried</td>
<td>10% smoke water</td>
<td>0.0 ± 0.0</td>
<td>2.5 ± 1.3</td>
<td>4.0 ± 2.5</td>
<td>1.0 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>Buried</td>
<td>10% smoke water</td>
<td>0.0 ± 0.0</td>
<td>3.5 ± 3.5</td>
<td>5.0 ± 4.5</td>
<td>3.5 ± 3.5</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>Buried</td>
<td>Hydropriming with ( \text{GA}_3 ) and ( \text{Kar}_1 )</td>
<td>0.0 ± 0.0</td>
<td>7.5 ± 4.9</td>
<td>9.0 ± 4.7</td>
<td>4.5 ± 2.7</td>
<td></td>
</tr>
</tbody>
</table>

Encouraging improvement however more work to do!
Summary

• Kings Park Science has been involved in many different plant conservation projects
• An integrated conservation model is useful for good outcomes
• Most DRF are likely to possess seed dormancy
• Seed dormancy may enhance soil persistence
• Many DRF are likely to respond to fire related cues
• Understanding seed ecology improves germination under *ex situ* and *in situ* conditions
• Seed enhancement techniques can aid *in situ* conservation efforts
Thanks for listening

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