Coorong, Lower Lakes and Murray Mouth Recovery Project – Vegetation Program Seed Germination and Propagation Research Project

Final Report


May 2016

SOUTH AUSTRALIAN SEED CONSERVATION CENTRE

Government of South Australia
Department of Environment, Water and Natural Resources

Botanic Gardens of ADELAIDE
ADELAIDE MOUNT LOFTY WITTUNGA

Australian Government

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Summary

This report is from the South Australian Seed Conservation Centre for the project to undertake seed germination and propagation research for plant species to be utilised in the Vegetation Program, a revegetation and habitat restoration program funded as part of the Coorong, Lower Lakes and Murray Mouth Recovery Project by the Commonwealth and South Australian governments. The time frame for this project is from February 2013 to May 2016. The project aim was to find suitable propagation methods for at least 24 plant species that were identified as priority species for the region.

Collections of viable seed were obtained from 27 plant species. The majority of collections had high viability and 22 out of 27 were found to be greater than 50% viable. Germination experiments were conducted on all the species. Maximum germination rates were between 38% and 100% and were above 50% for 21 species.

Data presented in this report has also been incorporated in the Seeds of South Australia website (www.saseedbank.com.au), which is freely accessible to the public. To facilitate the propagation of these plants by future practitioners, a page has been loaded for each species with photographic images of plants, flowers and seeds as well as information on seed collection and germination.

Background

The purpose of this project was to investigate the germination and/or propagation requirements of key species identified by the Coorong Lower Lakes and Murray Mouth (CLLMM) Recovery Project by the Commonwealth and South Australian Governments. The priority species for this project were selected because they were reported to be difficult to propagate by restoration practitioners. Research into the germination requirements and propagation methods for these species is required in order to increase the number of species available for revegetation programs. This will expand the diversity of species able to be used in revegetation, and increase the likelihood of reconstructing ecologically functional habitats.

The South Australian Seed Conservation Centre (SASCC) is well equipped to identify plant species, collect seeds and investigate their germination requirements. Seeds are routinely tested for viability and germination as part of the regimen of the seed bank curation. The SASCC has equipment for incubating seeds under a variety of temperatures and moisture levels, designed to mimic the conditions seeds would experience in their natural habitat. A range of chemical and physical treatments are also used that can increase germination in difficult seeds. These include plant hormones, heat and smoke treatments that simulate fire cues and stratification or after ripening treatments that can be used to alleviate dormancy and enhance germination levels. In addition to this equipment, staff at the SASCC have experience with germinating seed from a wide range of habitats and across many genera. Information for designing germination experiments is drawn from scientific articles and text books (Langkamp 1987; Baskin and Baskin 1998; Bonney 2003), data from the Tasmanian Seed Conservation Centre (http://gardens.rtbg.tas.gov.au/tscc-germination-database/) and the Millennium Seed Bank seed information database (http://data.kew.org/sid/). Observation, practical experience and knowledge of the local growing conditions also provide clues to environmental cues for germination, for example, disturbance, fires, flooding, arid environments etc.

The SASCC also works closely with the staff at the Mount Lofty Botanic Gardens Nursery when setting up propagation experiments to investigate methods for obtaining rooted cuttings and growing plants from seed in tube stocks ready for planting.

The project aim was to find suitable propagation methods for at least 24 plant species that were identified as priority species for the region. In this report we present data for 27 species that were tested for seed viability and germination. In the interests of sharing information with the wider community, data from this project has been loaded onto the Seeds of South Australia website, which is freely accessible to the public.

Methods

Seed Collection, Cleaning and Quantification.

Plant populations with adequate seed set were located and mature seed was collected for 27 species. The seeds were left to dry and cleaned using a combination of sieves and aspiration to remove other plant material. Species with seeds encased in fleshy fruits (*Astroloma conostephioides, Astroloma humifusum, Leucopogon parviflorus, Nitraria billardierei*) were treated with a solution of pectinase (1% (w/v)) for approximately 24 hours, then the flesh of the fruit was washed away by rubbing through a sieve. Dry seeds were stored in a controlled environment room maintained at 15˚C and 15% relative humidity before germination experiments were commenced. Loss of seed viability is minimised when seeds are kept in cool, dry conditions.

The seeds were quantified by weighing out 100 seeds (5 replicates of 20 seeds), and then calculating the average weight of one seed. The number of seeds in the seed lot was estimated by dividing the weight of the seed lot (g) by the weight of one seed (g). The number of seeds per gram was determined by the formula:

1 ÷ weight of 1 seed (g) = number of seeds/g.

Two species were added to the original target list:

Spyridium fontis woodii is endemic to South Australia and currently known from a single extant roadside population near Woods Well. It grows in shallow sands over calcrete with *Eucalyptus diversifolia* and coastal heath plants.

Hibbertia riparia is a common plant species growing throughout the CLLMM area and more research is required into the germination of this and other *Hibbertia* species.

Seed Viability Testing

Seed viability was estimated by x-raying 50 seeds or cut testing 20 seeds to determine the percent of seeds that are filled. Seeds were also dissected to examine the condition of the embryo and endosperm. Seed dissection is a readily available tool for estimating seed quality and correlates to the expected germination percentage in the sample. This can be done in the field or in a laboratory using small snips or other similar tools. Cut seeds can be viewed with a hand lens or dissecting microscope. Images of viable cut seeds were taken to assist seed collectors to examine the quality of seeds in the field before collection.

Germination Experiments

Seed germination was tested using a variety of methods depending on the plant species. The protocols for the germination tests vary depending on the predicted requirements for each species. The specific methods were derived from several sources including previous experiments within the seed bank, background information from textbooks or practitioners and published articles.

Table 1 lists the treatments that were applied to seeds and the conditions of the incubation periods when germination was scored. For each species a number of different treatments were tested. Each treatment was applied to 50 seeds which were then plated into 90mm glass petri dishes on a supporting media such as agar (1% (w/v)) or moist sand. Germination was recorded on a weekly basis when the radicle had emerged from the seed and had grown at least half the width of the seed. The rationale for each treatment is listed in Table 1. A combination of treatments were used in several experiments and these are described in sequential order in Appendix 1.

Table 1 List of treatments used for germination experiments and the rationale behind each treatment.

Treatment	Method	Rationale
Aerosol smoke	Seeds were placed in a tent connected to a metal drum via a pipe. Smoke from burning clean straw passed through the pipe into the tent for 15 min.	Chemicals present in smoke have been shown to trigger germination in some species that are fire responsive.
After Ripening (AR)	Seeds were placed in Petri dishes with dry washed sterile sand and incubated in ovens or incubators set to specified temperatures for a period of time.	Storing seeds at different temperatures in a dry environment is known as after ripening. This treatment has been shown to alleviate dormancy in some species.
Constant temperature incubator	Incubator set at constant temperature with a 12 h photoperiod	Used as an alternative to diurnal cycling, embryos may grow faster at one optimal temperature.
Control	No treatment.	The control shows the germination response of untreated seeds.
Dry Heat	Dry seeds were placed in a temperature controlled oven for a set period of time.	Used to mimic extreme hot conditions that is required by some species to germinate eg, baking sand or bushfire.
Fruit removal	The fruit portion was removed from *Exocarpos* seeds in some experiments.	Fruit removal reduces the chance of fungal contamination of seeds and mimics part of the process of animal ingestion.
Gibberellic Acid (GA)	Seeds soaked in a solution of GA dissolved in water. Concentration and duration of soaking may vary for different species. Continuous application of GA was delivered by adding GA to agar before pouring into plates.	GA is a plant hormone and is available as a powder or liquid solution. GA is used to alleviate physiological dormancy and promote germination in seeds.
Hydrogen Peroxide (H_2O_2)	Seeds soaked in hydrogen peroxide 30% (v/v) for 15 mins with gentle agitation, then rinsed 3 times with water.	Hydrogen peroxide is a strong oxidizer and is often used as a bleach or cleaning agent to sterilize the seed coat of any fungus or bacterial agents. The treatment may also breakdown chemicals in the seed coat that inhibit germination.
Leaching	Seeds were placed into a solution of water with gentle agitation to continuously mix the solution. Water was refreshed daily.	Leaching is used to mimic conditions where seeds are soaked by flooding or heavy rains. This process may leach out inhibitors present within the seed or seed coat which prevent or delay germination.

Treatment	Method	Rationale
Nicked seed coat	The outer layer of the seed coat was carefully nicked using a sharp scalpel to disrupt the water impermeable layers surrounding seeds with physical dormancy.	This process alleviates physical dormancy by allowing water to enter the seed. Another method to break physical dormancy is to pour boiling water onto seeds and let it cool. This is easier for bulk treating seeds.
Pectinase treatment	Seeds were soaked in a solution of Pectinase (1%) for several hours then fruit tissue was cleaned away by gentle rubbing through a sieve.	Pectinase is an enzyme which breaks down the cell walls in fruits and is used to soften the fruit tissue so it can be washed off the seeds. This process removes material that may promote fungal growth on the seeds and also mimics the seed cleaning that occurs through animal ingestion.
Potassium nitrate (KNO_3)	Seeds were soaked in a solution of Potassium Nitrate prepared by dissolving in water to a concentration of 100 mg/L. Soaking times may vary for different species. Continuous application of KNO_3 was delivered by adding KNO_3 (100 mg/L) to agar plates.	Potassium Nitrate is used to stimulate germination of seeds with physiological dormancy. The increased amount of nitrate signals a lack of plant competition for soil nutrients e.g., conditions after a fire. This mechanism of 'gap sensing' can indicate a positive germination response to other fire cues such as heat and smoke treatments.
Smoke water	Smoke water was prepared by connecting a container of water to a metal drum via a pipe. The smoke from burning clean straw was piped through water for 15-30 mins. This concentrated smoke water was stored at -20 ˚C until use and was diluted before treating seeds.	Chemicals present in smoke have been shown to trigger germination in some species that are fire responsive. Smoke-water is available commercially.
Spring/autumn incubator	Incubator set to 10°C for 12 h followed by 22°C for 12 h with 12 h photoperiod	Used to mimic temperature and day light hours of an average South Australian spring/autumn.
Stratification (STRAT)	Seeds were incubated in moist conditions in incubators set to specified temperatures for a period of time.	Storing seeds at different temperatures in a moist environment is known as stratification. This treatment has been shown to alleviate dormancy in some species.
Summer incubator	Incubator set to 15°C for 10 h followed by 30°C for 14 h with a 14 h photoperiod	Used to mimic temperature and day light hours of an average South Australian summer.
Wet heat	Seeds were placed in a tea strainer and exposed to hot water. Temperatures and times may vary for each species.	Hot water treatments may alleviate physical dormancy by disrupting the water impermeable outer layers of the seed coat.

Treatment	Method	Rationale
Wet/Dry cycling	Seeds were placed in Petri dishes with sterile sand at a specified temperature. During incubation seeds were wet on a weekly basis for 6 hours then allowed to dry out.	Wetting and drying simulates the soil seed bank environment as episodes of rainfall will cause the seeds to undergo several wetting and drying cycles before germination.
Winter incubator	Incubator set to 5°C for 4 h followed by 15°C for 20 h with a 10 h photoperiod	Used to mimic temperature and day light hours of an average South Australian winter.

Vegetative Propagation

Vegetative propagation methods were tested for the *Astroloma* species as these were the most difficult to germinate and it was likely that alternative methods to germination would be needed to propagate these plants.

An initial experiment was set up at the Mount Lofty Botanic Gardens Nursery on 15th April 2014. Fresh cuttings were taken from Naracoorte Caves National Park on 14th April 2014. A total of 100 cuttings from each species were dipped into purple Clonex and placed into a media of Perlite : cocopeat at a ratio of 90:10. Cuttings were kept in a misting tent with bottom heat applied for approximately 7 months before they were examined for root growth. Another experiment was set up at the Adelaide Botanic Gardens Nursery in a shade house. The cuttings were dipped into Clonex purple and placed into the same media and the pots were put into foam boxes (with drainage holes) and covered with white shade cloth. Cuttings were misted daily but no bottom heat was applied.

Information Sharing

The data gathered for each species has been summarised in a species sheet and posted on the Seeds of South Australia website (www.saseedbank.com.au). The data compiled on this website is freely available to the public. The website provides the following information for each species:

- Seed collection time
- Regions where the species has been recorded
- Latin name derivation
- Distribution and habitat
- Status
- Plant description
- Fruit type
- Seed type
- Embryo type
- Seed collecting notes
- Seed cleaning notes
- Germination results

Results

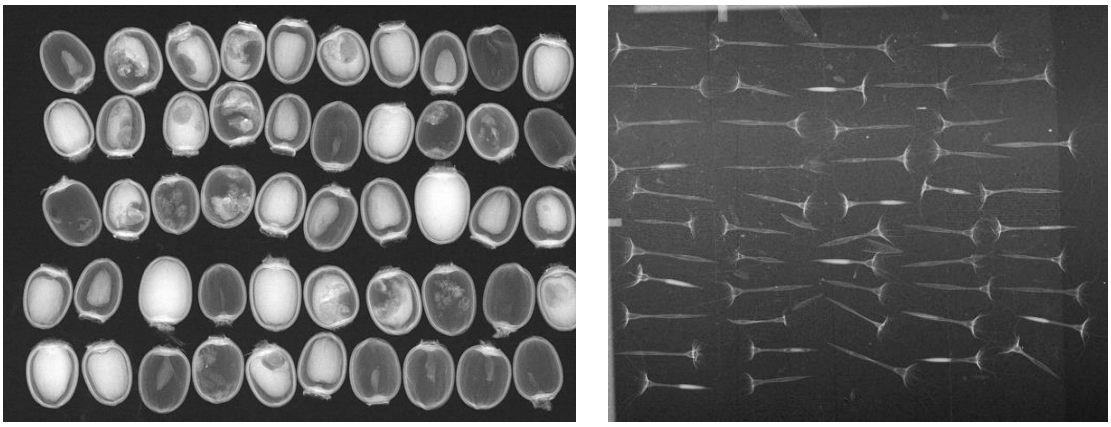
Seed Collections and Seed Viability

Sufficient quantities of viable seed for germination testing were collected from 27 species (Table 1). Seed viability was tested for all of the species collected and ranged from 14% to 100%. However, the majority of collections had high viability and 22 out of 27 species were found to have greater than 50% viability.

Seeds collected from *Leucopogon parviflorus* had low viability (30%) mostly due to poor development and seed fill. Seeds collected from *Calytrix tetragona* also had low viability (18%) as the majority of the seeds were predated which could be detected by small holes in the fruit. The seed collected from Exocarpos sparteus was also low (14%) with most of the seeds predated or poorly developed. X-ray images of these species are shown in Figure 1.

The number of seeds per gram is included in this table as an indication of seed size and also to use as a guide when using seed weight to estimate the number of seeds required for revegetation.

Images showing microscopic detail of viable seeds for the 27 species are shown in Figure 2. An image of the whole seed or fruit is shown as well as a viable cut seed. These images can be used as a guide during seed collection as a visual reference for viable seed. It is recommended that seeds are cut and examined with a hand lens prior to collection to ensure that viable seed is collected. The seed inside should contain a healthy endosperm and embryo that fills the seed with a creamy or white starchy matrix. Nonviable seeds are often shrivelled or discoloured inside or may have been predated which leaves holes in the seeds. These seeds are not viable and cannot germinate.



A B

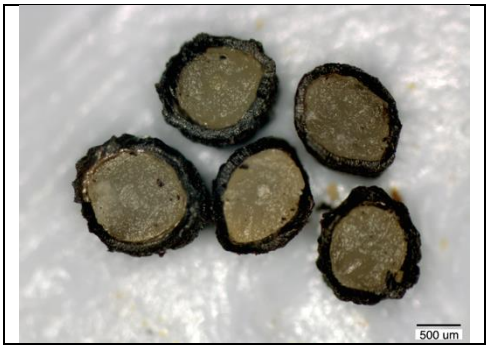
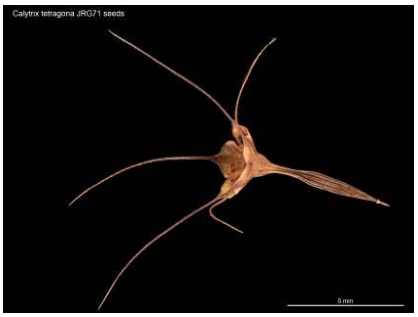
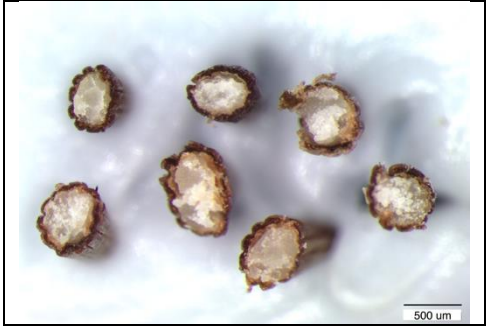
Figure 1. X-ray image of A) *Exocarpos sparteus* seeds 14% viable; B) *Calytrix tetragona* seeds 18% viable

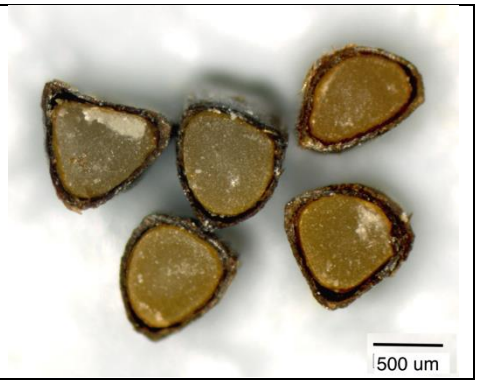
Table 2. Seed collection data and viability testing results.

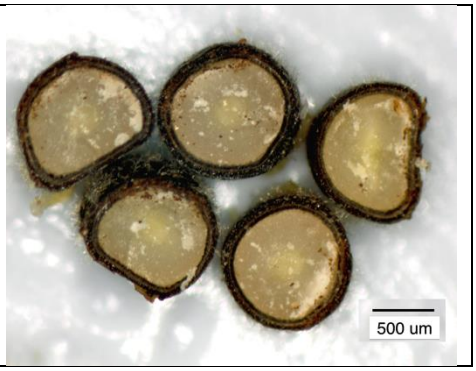
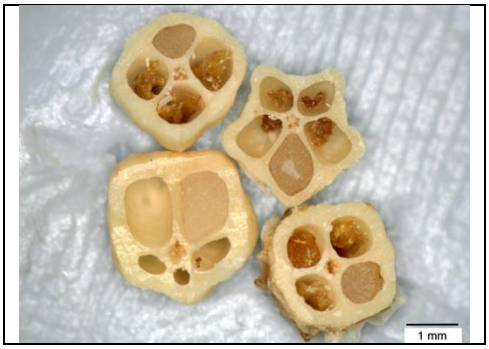
No	Family	Genus	Species	Date seed collected	Location	Number of Seeds collected	Number seeds/g	Viability
1	Euphorbiaceae	*Adriana*	*quadripartita*	30-Jan-13	Woods Well	860	40	78%
2	Epacridaceae	*Astroloma*	*conostephioides*	4-Oct-12	Naracoorte Caves NP	1912	20	95%
3	Epacridaceae	*Astroloma*	*humifusum*	7-Nov-13	Frahns Scrub	2960	15	92%
4	Rutaceae	*Boronia*	*coerulescens* ssp. *coerulescens*	10-Oct-14	Cox Scrub	1040	340	62%
5	Myrtaceae	*Calytrix*	*tetragona*	20-Nov-13	Monarto CP	5660	600	18%
6	Santalaceae	*Exocarpos*	*sparteus*	5-Feb-14	Ngarkat CP	1470	45	14%
7	Santalaceae	*Exocarpos*	*syrticola*	6-Feb-14	Salt Creek	944	40	83%
8	Frankeniaceae	*Frankenia*	*pauciflora* var. *gunnii*	21-Feb-13	Salt Creek	66000	7690	92%
9	Cyperaceae	*Gahnia*	*filum*	15-Nov-13	Currency Creek	1500	725	80%
10	Dilleniaceae	*Hibbertia*	*riparia*	27-Oct-14	Scott CP	1200	755	86%
11	Dilleniaceae	*Hibbertia*	*sericea*	20- Oct-14	Bonney Reserve	1040	400	56%
12	Sterculiaceae	*Lasiopetalum*	*baueri*	7-Nov-13	Ferries Macdonald CP	1300	690	52%

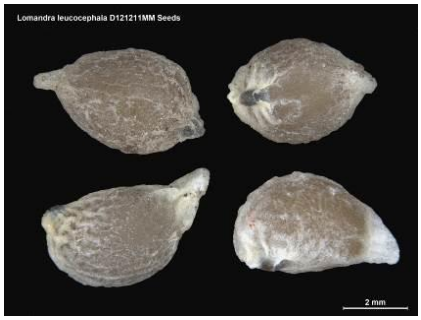
No	Family	Genus	Species	Date seed collected	Location	Number of Seeds collected	Number seeds/g	Viability
13	Epacridaceae	*Leucopogon*	*parviflorus*	30-Jan-13	Princess Hwy, Coorong	2876	50	30%
14	Liliaceae	*Lomandra*	*densiflora*	20-Nov-13	Frahns Scrub	1500	75	100%
15	Liliaceae	*Lomandra*	*effusa*	22-Nov-13	Frahns Scrub	2425	40	100%
16	Liliaceae	*Lomandra*	*juncea*	20-Nov-13	Monarto CP	1220	80	100%
17	Liliaceae	*Lomandra*	*leucocephala* ssp. *robusta*	12-Dec-11	Langhorne Creek	5000	50	100%
18	Liliaceae	*Lomandra*	*multiflora* ssp. *dura*	8-Jan-14	Finniss Oval	2964	60	100%
19	Polygonaceae	*Muehlenbeckia*	*adpressa*	16-Nov-15	Kangaroo Island	3100	220	85%
20	Polygonaceae	*Muehlenbeckia*	*gunnii*	23-Oct-13	Finniss - Milang Road	1140	70	100%
21	Zygophyllaceae	*Nitraria*	*billardierei*	28-Feb-13	Langhorne Ck - Wellington Rd	6200	16	96%
22	Thymelaeaceae	*Pimelea*	*glauca*	3-Nov-15	Rail Reserve, Winery Rd	3800	470	75%
23	Rhamnaceae	*Pomaderris*	*paniculosa* ssp. *paniculosa*	10-Dec-14	Tailem Bend – Karoonda Rd	5800	900	95%
24	Rhamnaceae	*Spyridium*	*fontis-woodii*	30-Jan-13	Woods Well	750	2230	74%
25	Rhamnaceae	*Spyridium*	*subochreatum*	7-Nov-13	Monarto CP	4100	2780	70%
26	Chenopodiaceae	*Tecticornia*	*indica* ssp. *leiostachya*	21-Feb-13	Salt Creek	4700	910	49%
27	Sterculiaceae	*Thomasia*	*petalocalyx*	11-Nov-13	Naracoorte Caves NP	900	720	16%

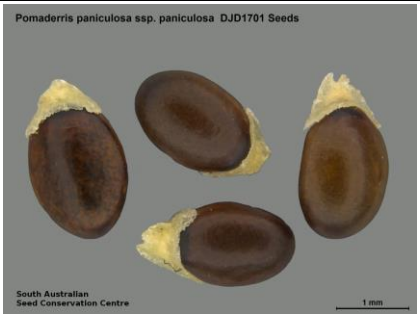
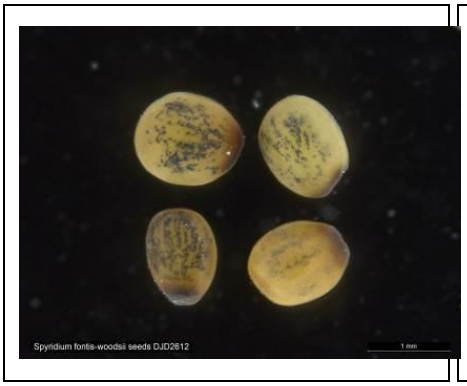
Figure 2. Images of seeds and fruits taken through a microscope and viable seeds cut open to show the inside of a healthy, ripe seed.

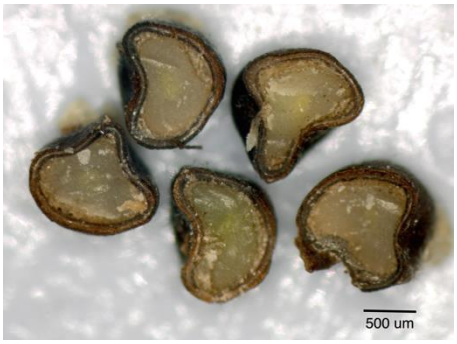
Species	Seed Image	Viable Cut seed
Adriana quadripartita (Seeds)		
Astroloma conostephioides (Woody drupe)		
Astroloma humifusum (Woody drupe)		
Boronia coerulescens ssp. *coerulescens* (Seeds)		
Calytrix tetragona (Fruit with awns)		

Species	Seed Image	Viable Cut seed
Exocarpos sparteus (Nut)		
Exocarpos syrticola (Nut)		
Frankenia pauciflora var. *gunnii* (Seeds)		
Gahnia filum (Seeds)		
Hibbertia riparia (Seeds)		

Species	Seed Image	Viable Cut seed
Hibbertia sericea (Seeds)		
Lasiopetalum baueri (Seeds)		
Leucopogon parviflorus (Woody drupe)		
Lomandra densiflora (Seeds)		
Lomandra effusa (Seeds)		

Species	Seed Image	Viable Cut seed
Lomandra juncea (Seeds)		
Lomandra leucocephala ssp. *robusta* (Seeds)		
Lomandra multiflora ssp. *dura* (Seeds)		
Muehlenbeckia adpressa (Seeds)		
Muehlenbeckia gunnii (Seeds)		

Species	Seed Image	Viable Cut seed
Nitraria billardierei (Woody drupe)		
Pimelea glauca (Seeds)		
Pomaderris paniculosa ssp. *paniculosa* (Seeds)		
Spyridium fontis-woodii (Seeds)		
Spyridium subochreatum (Seeds)		

Species	Seed Image	Viable Cut seed
Tecticornia indica ssp. leiostachya (Seeds)		
Thomasia petalocalyx (Seeds)		

Germination Experiments

The results from germination experiments for 27 species have been summarised in Table 3 and compiled in Appendix 1. Several methods were tested for some species and the reasons behind different treatment strategies are outlined in Table 1. The germination levels for 21 species were greater than 50% for at least one treatment. Overall, seed germination was greater than 35% which showed that successful propagation methods through seed could be developed for all species following the methods described.

Methods for successful germination have not been previously reported for most of the species tested in this project. A range of different methods were found to enhance germination across the species tested. The germination response of the species tested were grouped into the following categories.

Non-dormant

Three species were found to be non-dormant as they germinated within 30 days without treatment (Baskin and Baskin, 2004). These were *Frankenia pauciflora* var. *gunnii*, *Gahnia filum*, and *Tecticornia indica* ssp. *leiostachya*. *Muehlenbeckia gunnii* also had high germination without any treatment although not all seeds germinated within 30 days.

Responsive to Gibberellic Acid

Gibberellic acid is a naturally occurring plant growth regulator, which has an important role in initiating seed germination and can be used to overcome physiological dormancy in many species. Other treatments may also alleviate physiological dormancy such as stratification at different temperatures, temperature cycling or dry after ripening. In this study, germination in several plant species increased after treatment with gibberellic acid and further work is needed to find the environmental conditions that would also trigger this response. Some seeds are classified as having deep physiological dormancy when there is no germination response to exogenously applied gibberellic acid.

Seeds did not germinate in the control treatment for *Adriana quadripartita* but germination increased in treatments that had gibberellic acid and warmer temperatures. The highest germination (60%) was observed after treating with gibberellic acid (1000 mg/L) and incubating using photo and thermo-periods simulating spring/autumn and summer conditions. *Exocarpos sparteus* and *Exocarpos syrticola* both had increased germination after application of gibberellic acid and incubation in winter conditions. *Lomandra effusa* had the highest germination response after treatment with gibberellic acid. *Nitraria billardierei* and *Lomandra leucocephala* ssp. *robusta* had the highest germination in response to treatment with gibberellic acid and dry heat treatment. Germination in *Lomandra juncea* was increased after treatment with aerated water followed by gibberellic acid.

Responsive to Fire Cues

Seedlings from many plant species have been observed to emerge post fire. The effects of heat, smoke and increased available nitrogen in the soil can act as a germination stimulant. Germination of *Lomandra densiflora*, *Lomandra multiflora* ssp*. dura* seeds had a positive response to smoke water.

Boronia coerulescens ssp. *coerulescens*, *Hibbertia riparia* and *Hibbertia sericea* had the highest germination after treatment with a combination of gibberellic acid and smoke water. *Leucopogon parviflorus* had the highest germination after treatment with a combination of gibberellic acid and smoke water or a combination of dry heat and gibberellic acid. The most effective treatment tested for *Calytrix tetragona* was dry heat and smoke water. These species appeared to respond to the fire cues heat and smoke, but in some cases there was an increase in germination when used in combination with gibberellic acid. The germination response to chemicals in smoke can be dependent on the seed dormancy status, which may be effected by a variety of environmental signals (Baker et al, 2005). In this study gibberellic acid was used to alleviate dormancy in combination with fire cues and resulted in higher germination rates for some species.

Physical Dormancy

Some plant families have a type of dormancy known as physical dormancy, where water is prevented from imbibing the embryo by a water impermeable seed coat (Baskin et al 2000; Baskin and Baskin 2004). The outer layer of the seed coat is usually hard and may be waxy. Dormancy is broken when water penetrates the seed coat, which can happen in nature over time, through crazing, scarification, heat, weathering or insect damage. In the laboratory or nursery germination can be initiated by scarification or treatment with hot water. Hot water ruptures the water gap in the seed coat and allows water uptake into the embryo. Plant species used in this project with physical dormancy were *Lasiopetalum baueri*, *Pomaderris paniculosa* ssp*. paniculosa*, *Spyridium subochreatum* and *Spyridium fontis-woodii*. Only a small number of *Spyridium fontis-woodii* was collected and the control experiment was omitted for this species in lieu of conserving the seeds in the seed bank. For the other species a marked difference between the control and treatment by nicking or hot water was observed. Physical dormancy is relatively simple to alleviate using these methods.

Difficult to Germinate

The species that were the most difficult to germinate were *Astroloma conostephioides* and *Astroloma humifusum.* These species have underdeveloped embryos, a woody endocarp and display morphophysiological dormancy which can be complex and difficult to alleviate. Methods to overcome this type of dormancy include stratification at warm or cool temperatures, cycling between seasonal temperatures and the use of chemical compounds such as smoke water and gibberellic acid. Germinating seeds emerged at very low rates from fruits. When germination occurred the endocarp split to allow germinating seeds to emerge (Figure 3). We found that generally the woody endocarp is a barrier to germination in *Astroloma* and further work is needed to find a more rapid method of excising seed from the endocarp or rupturing the endocarp in such a way that the embryo is not damaged and the seed is able to push out of the woody structure. It appears that the endocarp restricts full imbibition and therefore expansion of the embryo. Seeds of *A. conostephioides* and *A. humifusum* germinated readily after being removed from the physical confinement of the surrounding fruit and treatment with gibberellic acid. Further experiments are required to try and find a method suitable for larger scale experiments in the nursery. These results will be included on the seeds of South Australia web site as they become available.

Figure 3. Germinating *Astroloma humifusum* seeds emerging from the woody endocarp.

Interpretation of Germination Results

A summary of the most effective treatments used in the germination experiments and general advice regarding common problems with propagation for each species is shown in Table 4. These guidelines are a good starting point for growers to consider before commencing germination of these species. Results will vary depending on the initial seed viability, seed storage conditions, temperatures used for germination and concentration of chemicals applied to the seeds.

Table 3. Summary of germination results showing controls and the most effective treatments.

No	Species	Treatment	Germination (%)
1	*Adriana quadripartita*	Control; spring/autumn GA (1000 mg/L) continuous; spring/autumn	0 60
2	*Astroloma conostephioides*	Hydrogen Peroxide; spring/autumn Seed excised from the endocarp; GA (500 mg/L); 15 °C constant temp	4 85
3	*Astroloma humifusum*	Hydrogen Peroxide; winter Seed excised from the endocarp; GA (500 mg/L); 15 °C constant temp	0 85
4	*Boronia coerulescens* ssp. *coerulescens*	Control; winter Leaching (48 h); GA (500 mg/L) with Smoke Water (10 % (v/v)) for 24 h; winter	0 38
5	*Calytrix tetragona*	Control; spring/autumn Dry heat (90 °C for 15 min); GA (250 mg/L) with Smoke Water (10% (v/v)) for 24 h; spring/autumn	0 62
6	*Exocarpos sparteus*	Leaching (48 h); winter GA (400 mg/L) for 48 h; winter	0 36

No	Species	Treatment	Germination (%)
7	*Exocarpos syrticola*	Leaching (48 h); winter GA (400 mg/L) for 48 h; AR 20 °C for 3 weeks; winter	0 72
8	*Frankenia pauciflora* var. *gunnii*	Control; winter GA (250 mg/L) continuous; winter	96 100
9	*Gahnia filum*	Control; spring/autumn Nicked seed coat; Leaching (7 d), spring/autumn	88 90
10	*Hibbertia riparia*	Control; winter GA (500 mg/L) with Smoke Water (10% (v/v)) for 24 h; spring/autumn	0 62
11	*Hibbertia sericea*	Control; winter GA (500 mg/L) with Smoke Water (10% (v/v)) for 24 h; spring/autumn	2 40
12	*Lasiopetalum baueri*	Control: winter Nicked seed coat; winter	2 100
13	*Leucopogon parviflorus*	Hydrogen Peroxide; spring/autumn Hydrogen Peroxide; Smoke Water (10% (v/v)) for 24 h; GA (1000 mg/L) for 72hrs; spring/autumn	0 100
14	*Lomandra densiflora*	Control; 15 °C constant temperature Hydrogen peroxide; Smoke Water (10% (v/v)) for 24 h; 15 °C constant 12 h photoperiod	16 69
15	*Lomandra effusa*	Control; 15 °C constant temperature 12 h photoperiod Hydrogen peroxide; GA (1000 mg/L) for 72 h; 15 °C constant 12 h photoperiod	62 92
16	*Lomandra juncea*	Control; 15 °C constant temperature Hydrogen peroxide; GA (1000 mg/L) for 72 h; 15 °C constant 12 h photoperiod	6 35
17	*Lomandra leucocephala* ssp. *robusta*	Control; 15 °C constant temperature 12 h photoperiod Hydrogen peroxide; GA (1000 mg/L) for 72 h; 15 °C constant 12 h photoperiod	0 32
18	*Lomandra mutliflora* ssp. *dura*	Control; 15 °C constant temperature 12 h photoperiod Hydrogen peroxide; Smoke Water (10% (v/v)) for 24 h; 15 °C constant 12 h photoperiod	78 93
19	*Muehlenbeckia adpressa*	Control; spring/autumn	No germination after 34 days
20	*Muehlenbeckia gunnii*	Control; spring/autumn STRAT 6 weeks spring/autumn; winter	81 94
21	*Nitraria billardierei*	Leaching (15 d); spring/autumn Leaching (15 d); Dry heat (120 °C) for 2min; GA (1000 mg/L) for 72 h; spring/autumn	28 48

No	Species	Treatment	Germination (%)
22	*Pimelea glauca*	Control; spring/autumn	No germination after 34 days
23	*Pomaderris paniculosa* ssp. *paniculosa*	Control; winter Wet heat (100 °C) for 30 sec; winter	4 72
24	*Spyridium fontis-woodii*	Control; spring/autumn Wet heat (95 °C) for 30 sec; spring/autumn	NA 70
25	*Spyridium subochreatum*	Control; spring/autumn 4 weeks then transferred to winter Nicked seed coat; winter	0 84
26	*Tecticornia indica* ssp. *leiostachya*	Control; spring/autumn GA (250mg/L) continuous; spring/autumn	72 70
27	*Thomasia petalocalyx*	Control; winter Nicked seed coat; winter	4 94

NA – not available.

Table 4. Summary table of the seed germination data and advice for collection and/or germination of each species.

Species	Best treatment for germination	Advice for this species
Adriana quadripartita	Treat seeds with GA for increased germination levels. Germinate seeds in spring through to autumn.	Seeds may require cold stratification prior to germination warmer temperatures, spring/autumn and summer.
Astroloma conostephioides	Germination rates were very slow for this species. Some germination was observed during winter in a nursery experiment where seeds were placed in potting soil in the previous summer. Germination was observed within 2-3 weeks after seeds were removed from the woody endocarp or the endocarp had been cracked, and treated with GA.	This species can be propagated through cuttings using a perlite:peat (90:10) propagation mix and application of rooting hormone. Excise the seeds from the woody fruit and treat with gibberellic acid for rapid germination. Care must be taken not to damage the seed during this process. Seedling growth in potting mix may be slow.
Astroloma humifusum	Germination has been very slow for this species. Some germination was observed during winter in a nursery experiment where seeds were placed in potting soil during the summer of the year before. Germination was observed within 2-3 weeks after seeds were removed from the woody endocarp and treated with GA. Care must be taken not to damage the seed during this process.	This species can be propagated through cuttings, using a perlite: peat (90:10) propagation mix and application of rooting hormone. Excise the seeds from the woody fruit and treat with gibberellic acid for rapid germination. Care must be taken not to damage the seed during this process. Seedling growth in potting mix may be slow.
Boronia coerulescens ssp. *coerulescens*	Treat seeds with GA and diluted smoke water for increased germination.	Seeds may require stratification in combination with fire cues to germinate.
Calytrix tetragona	Germination increased after dry heat (15 min; 90 °C) and diluted smoke water in combination with GA. Germination rates were slow with 50 % of seeds germinating after 79 days.	Viability is a likely issue for this species. The seeds collected had low viability mainly due to predation. Seeds with drill holes will be non-viable. Seeds may require stratification in combination with fire cues to germinate.
Exocarpos sparteus	Treat seeds with GA for increased germination results. Seeds are most likely to germinate during winter.	Viability is a likely issue for this species. Collect shiny brown nuts that are filled with endosperm, check by cutting open some seeds. Viable seeds were collected from emu scats. Seeds may require stratification to germinate.

Species	Best treatment for germination	Advice for this species
Exocarpos syrticola	Treat seeds with GA for increased germination results. Seeds are most likely to germinate during winter.	Viability is a likely issue for this species. Collect shiny dark coloured nuts that are filled with endosperm, check by cutting open some seeds. Seeds may require stratification to germinate.
Frankenia pauciflora var. *gunnii*	No treatment is required for this species. High levels of germination (100%) were observed in the control test incubated under winter temperature conditions.	Using a hand lens, check that viable seeds are present in the capsules during collection.
Gahnia filum	No treatment is required for this species. High levels of germination (88%) were observed in the control test incubated under spring temperature conditions.	Check that seeds are viable on collection.
Hibbertia riparia	Treat seeds with GA and diluted smoke water for increased germination.	May be difficult to collect large numbers of seed numbers as capsules dehisce when seed is ripe. Seeds may require stratification in combination with fire cues to germinate.
Hibbertia sericea	Treat seeds with GA and diluted smoke water for increased germination.	May be difficult to collect large numbers of seed numbers as capsules dehisce when seed is ripe. Seeds may require stratification in combination with fire cues to germinate.
Lasiopetalum baueri	Break physical dormancy by nicking the seed coat or try hot water treatment before sowing.	Predation is a likely issue for this species. Avoid collecting fruit with evidence of predation. These seeds have physical dormancy, the seed coat requires modification by abrasion, nicking or heat shock to disrupt the water impermeable layers.
Leucopogon parviflorus	Treat fruits with GA and use in combination with diluted smoke water and/or dry heat (100°C) for 2 min for increased germination results.	Viability is likely to be a problem for this species. Collect large white fruits and check that the seeds inside are filled by cutting open the fruits. Seeds may require stratification in combination with fire cues to germinate.

Species	Best treatment for germination	Advice for this species
Lomandra densiflora	Treat seeds with hydrogen peroxide (30%) and diluted smoke water for increased germination results. 69% of seeds germinated after this treatment and incubation at 15°C.	Collect ripe seed when fruits have begun to split open. Immature seed will not germinate.
Lomandra effusa	A reasonable level of germination (44%) was observed in the control test incubated at 15 ˚C. Treatment with GA will increase germination levels (up to ~90%).	Collect ripe seed when fruits have begun to split open. Immature seed will not germinate.
Lomandra multiflora ssp. *dura*	No treatment is required for this species, high levels of germination (75%) were observed in the control test incubated at 15 ˚C. Germination level was increased after treatment with diluted smoke water (93%).	Collect ripe seed when fruits have begun to split open. Immature seed will not germinate.
Lomandra leucocephala ssp. *robusta*	Low germination levels were observed in control experiments. Germination increased to 32% after treatment with GA.	Collect ripe seed when fruits have begun to split open. Immature seed will not germinate.
Lomandra juncea	Low germination levels were observed in control experiments. Germination increased to 35% after treatment with GA or GA and diluted smoke water.	Collect ripe seed when fruits have begun to split open. Immature seed will not germinate.
Muehlenbeckia gunnii	No treatment is required for this species, high levels of germination (94%) were observed in the control test incubated in a spring/autumn temperatures.	Collect yellow fruits containing mature, black seeds.
Nitraria billardierei	Germination of 28% of seeds was observed after leaching seeds in water for 2 weeks. Germination increased to 48% after treatment with GA and dry heat 120°C for ~2 mins.	The woody fruit surrounding the seed may inhibit germination. Leaching with water, treatment with GA and heat shock increased germination.
Pomaderris paniculosa ssp. *paniculosa*	Break physical dormancy with hot water treatment before sowing. Best germination rate (72%) was observed in winter conditions.	These seeds have physical dormancy, the seed coat requires modification by abrasion, nicking or heat shock to disrupt the water impermeable layers.

Species	Best treatment for germination	Advice for this species
Spyridium fontis-woodii	Break physical dormancy with hot water treatment before sowing. 70% of seeds germinated in spring conditions.	These seeds have physical dormancy and the seed coat requires modification by abrasion, nicking or heat shock to disrupt the water impermeable layers.
Spyridium subochreatum	Break physical dormancy with hot water treatment or nicking the seed. Highest germination (46%) was observed in winter conditions.	These seeds have physical dormancy, the seed coat requires modification by abrasion, nicking or heat shock to disrupt the water impermeable layers.
Tecticornia indica ssp. *leiostachya*	No treatment is required for this species, high levels of germination (72%) were observed in the control incubated in a winter environment.	Check that viable seed has been collected. The seed sits within wedge shaped fruits in between segments of the fruiting spike.
Thomasia petalocalyx	Break physical dormancy by nicking the seed coat or try hot water treatment before sowing.	Predation in this species can cause low viability. Avoid collecting fruits with evidence of predation. These seeds have physical dormancy, the seed coat requires modification by abrasion, nicking or heat shock to disrupt the water impermeable layers.

Vegetative Propagation of *Astroloma conostephioides* and *Astroloma humifusum*

The results from the *Astroloma* propagation experiment showed that good root development had occurred after seven months of cuttings being maintained on a heat bed in a moist environment. The strike rates (percentage of cuttings with good root development) from both species are shown below:

Nursery conditions	*Astroloma conostephioides*	*Astroloma humifusum*
With bottom heat	33%	20%
No bottom heat	44 %	10%

The rooted cuttings were potted into tube-stock pots and remain in good condition, however, they have been slow to put on new growth. Further experimentation is required using a range of different potting mixes and inoculation with ericoid mycorrhizae to improve the vigour of the rooted cuttings.

Discussion

Seed Viability

Testing seed viability is a crucial step, as non-viable seeds cannot germinate. Seeds can be non-viable for several reasons, including stress during development, predation, collection of immature seeds and inappropriate storage conditions. There is a high chance of collecting seed with low viability for some species that are commonly predated by insects or typically only develop a small number of viable seeds. In some cases, plants set larger amounts of viable seeds post fire in response to increased available nutrients and decreased competition from other plants. The post fire conditions are favourable for seed development, seedling establishment and plant survival.

Seeds that were collected from *Leucopogon parviflorus* along the Coorong had low viability (30%) mostly due to poor development and seed fill. Previous collections by the seed centre staff from the same region and same time of year had higher viability (up to 90%), showing that seed quality can vary between seasons for this species. Another species with low viability (18%) was *Calytrix tetragona*, the majority of the seed collection was predated which could be seen by small holes in the fruit. *Exocarpos sparteus* seeds collected from bushes at Ngarkat CP on Feb 5th 2014 had low viability (14%) but, interestingly, seed found in emu scat at the same location were 100% viable. It is possible that nonviable seeds were destroyed in the emu's gut or that the viability of the seeds was higher at the time of ingestion. Seeds collected post fire from the same population in January 2005 had high viability (90%).

The importance of testing seed viability is fundamental but is often overlooked. Revegetation through direct seeding is futile if non-viable seed is used. Seed can be collected, cleaned, stored, seeded and monitored for seedling emergence that will not occur if little viable seed was collected in the first place. The poor seedling emergence results may then be put down to lack of germination or a variety of other reasons. This amounts to a waste of resources and limits the opportunity to learn from seeding experiments and importantly, failure to establish plants. It is therefore recommended that seed viability is routinely checked on collection and quantified after the seed is cleaned.

Seed dissection is a readily available tool for estimating seed quality and correlates to the expected germination percentage in the sample. This can be done in the field or in a laboratory using small snips or other similar tools. A sample of cut seeds can be viewed with a hand lens or dissecting microscope. Images of viable cut seeds have been provided in this report to assist seed collectors to examine the quality of seeds in the field before collection (Figure 2).

Germination

The results from the project indicate that the majority of the target species are able to be propagated through seed for the purpose of revegetation. Most of the species listed are not currently used in revegetation due to difficulties in propagation or lack of information about seed collection and germination.

Members of Ericaceae commonly occur in Australian temperate woodlands and heathlands, however little is known about their mechanisms for dormancy and germination (Merritt et al 2007). Three widespread and ecologically important heath species (*Astroloma conostephioides*, *Astroloma humifusum*, *Leucopogon parviflorus*) were tested in this project. Ericaceae fruit is dispersed as a drupe or a capsule containing several seeds inside a woody endocarp. The seeds are known to be difficult to germinate due to underdeveloped embryos and physiological dormancy (morphophysiological dormancy). Several reports show that freshly dispersed Ericaceae seeds do not germinate under laboratory conditions (Dixon et al 1995, Allan et al 2004, Ooi et al 2006, Turner et al 2009).

Seeds of *Astroloma xerophyllum* that were kept in natural conditions took several seasons to germinate and were shown to have morphophysiological dormancy, indicating that underdeveloped embryos in the seed contributed to seed dormancy. However, successful germination was achieved after excising seed from the endocarp and treatment with gibberellic acid (Turner at al 2009). This was also the case for the two *Astroloma* species tested in this project (Figure 4). Both species had high germination with excised seeds and exposure to gibberellic acid. This suggests that the process of weathering and stratification combine to alleviate dormancy in nature (Figure 3). The challenge now is to speed up that process in the laboratory or nursery.

Figure 4. Germination of excised seeds of *Astroloma conostephioides*.

Members of the Ericaceae family are also known to have specific ericoid mycorrhizae associated with their roots that enables access to moisture and nutrients through the expansive associated mycorrhizal hyphae network. It would be interesting to test the effects of mycorrhizal inoculations on young plants growing in pots and in the field as it may be an important step in the long-term success of revegetating Ericaeae species.

Leucopogon species have also been shown to have morphophysiological dormancy, which was overcome by changes in seasonal temperatures. Ooi et al (2006) found that dormancy was not broken by fire cues but germination was enhanced by smoke treatment once dormancy was overcome

Leucopogon parviflorus seeds used in this study had high germination after treatments with fire cues dry heat and smoke water in combination with gibberellic acid.

Nitraria billardierei grows in saline environments and bears fleshy drupes that are reported to germinate after emu ingestion. The increase in germination was attributed to the removal of the salts accumulating in the fruit flesh that may be inhibitory (Noble and Whalley 1978; Waisel 1972). This species is known to be difficult to germinate In our experiments the optimum treatment was leaching for 15 days in several changes of water, then the seeds were dried and subjected to a brief heat shock (120°C for 2 minutes) followed by exposure to gibberellic acid. This treatment was likely to remove salts and other inhibitors and overcome the physiological dormancy requirement. Interestingly, no germination was recorded without leaching the seeds in any treatment.

Two species of *Exocarpos* were studied, *E. syrticola* grows in saline coastal areas and *E. sparteus* grows in mallee communities on sandy soils. There has been very little information recorded about the germination requirements of these species. It has been reported that seeds from *Exocarpos aphyllus* germinated after vacuum infiltration with gibberellins (Lovey and Jusaitis 1994). Both of the species we tested also had increased germination after exposure to gibberellic acid. These species have morphophysiological dormancy, which was partially alleviated by treatment with gibberellic acid.

Hibbertia is another genus with morphophysiological dormancy where germination is reported to be difficult, requiring complex regimes of after ripening, stratification, wet and dry cycles and smoke (Hidayati et al 2012). *Hibbertia* species are typically not used in restoration because the seeds are hard to collect as the fruiting capsules dehisce when seeds are ripe, and plants are difficult to propagate. In this study two species of *Hibbertia* germinated after treatment with smoke water and gibberellic acid. Forty per cent of *H. sericea* and 62% of *H. riparia* seeds germinated after treatment. This can be considered a good result for members of this genus.

Iron grass natural temperate grassland in South Australia is a critically endangered vegetation community, with patches occurring in the Murray Darling Depression bioregion. However, there is scant information published on propagation of the iron grass species that dominate these ecological communities (*L. effusa*, and *L. multiflora* ssp. *dura*). Germination of *Lomandra sonderi* was reported to be improved by removal of the pericarp and gibberellic acid (Plummer et al, 1995).

In this project seeds from five Lomandra species were collected and tested using a range of laboratory techniques to study germination requirements. Interestingly, the optimum germination treatments differed between the *Lomandra* species. *L. multiflora* ssp. *dura* had high germination in the control and after smoke water treatment but was inhibited by gibberellic acid application. However, germination in *L. effusa* was enhanced after treatment with gibberellic acid. *L. densiflora* had high germination after treatment with smoke water and/or gibberellic acid.

L. leucocephala ssp. *robusta* and *L. juncea* were the most difficult to germinate and both grow in sandy soils. *L. juncea* had the highest germination after treatment with aerated water followed by gibberellic acid. It is possible that some inhibitors may have been leached out from the testa during this treatment, resulting in increased germination. *L. leucocephala* ssp. *robusta* had highest germination after prolonged dry heat (50°C 24 h), which may also have broken down germination inhibitors in the seed.

The germination experiments were conducted under laboratory conditions and results will vary from germination tests done in other conditions (nursery/garden/field), especially with different seed collections from that species. The information provided should be used as a guide when collecting and germinating seeds for revegetation and habitat restoration projects.

Propagation

Striking cuttings can be an effective method of propagation where seed germination is difficult. The propagation of *Astroloma* species through cuttings had a reasonable success rate, especially with *Astroloma conostephiodes*. The cuttings were taken in autumn and the propagation mix had a high percent of perlite (90%), and therefore good drainage. These may be important factors for success with striking cuttings. To provide a good representation of genetic diversity cuttings should be sourced from a large number (> 50) of individuals. The genetic diversity of populations established from cutting material can then increase through sexual reproduction between individuals once the plants are mature. The disadvantages of propagation through cuttings is that less genetic diverse than using seed and that root development can be less vigorous. The propagation *Astroloma* species may become possible through seed germination with more research into practical germination methods and investigating the role of mycorrhizal fungi in seedling establishment.

Information Sharing

The information compiled on the Seeds of South Australia website (Appendix 2) will be an ongoing resource that will continue to facilitate the propagation of these and other species from the CLLMM region. The data and images will continue to be updated as new information is obtained by the SASCC.

Future Work

We will continue experimenting with the species that have been difficult to germinate. In particular, the *Astroloma* species, to find a suitable technique of germinating seeds that can be readily adapted to nurseries. Progress in this area will be uploaded onto the Seeds of South Australia website.

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Appendix 1.

Methods and results from germination experiments conducted for the target species. Treatments are described in Table 1. The treatment with the highest level of germination for that species is shown in bold. **T0:** Number of days before first germinant observed. **T50:** Number of days to achieve 50% germination.

No	Species	Treatment	Germination % (Average)	T_0	T_{50}
1	*Adriana quadripartita*	Control; spring/autumn	0	N/A	N/A
		GA (250 mg/L) continuous; spring/autumn	48	14	N/A
		KNO_3 (100 mg/L) continuous; spring/autumn	8	28	N/A
		Wet heat (100 °C) for 1 min; spring/autumn	0	N/A	N/A
		Nicked seed coat; spring/autumn	20	28	N/A
		GA (500 mg/L) continuous; 20 °C constant temperature	16	15	N/A
		GA (1000 mg/L) continuous; 20 °C constant temperature	8	8	N/A
		GA (500 mg/L) continuous; spring/autumn	48	22	N/A
		GA (1000 mg/L) continuous; spring/autumn	**60**	**15**	**46**
		GA (500mg/L) continuous; summer	48	15	N/A
		GA (1000mg/L) continuous; summer	**60**	**15**	**36**

No	Species	Treatment	Germination % (Average)	T_0	T_{50}
2	*Astroloma conostephioides*	Hydrogen Peroxide; spring/autumn	4	119	N/A
		Hydrogen Peroxide; GA (1000 mg/L) for 72 h; spring/autumn	0	N/A	N/A
		Leaching (13 d); Hydrogen Peroxide; GA (1000 mg/L) for 72 h; spring/autumn	0	N/A	N/A
		Hydrogen Peroxide; Smoke Water (10% (v/v))for 24 h; GA (1000 mg/L) for 72 h; spring/autumn	0	N/A	N/A
		Aerosol smoke (15mins); Hydrogen Peroxide; GA (1000 mg/L) for 72hrs; spring/autumn	0	N/A	N/A
		Hydrogen Peroxide; winter	0	N/A	N/A
		Hydrogen Peroxide; AR 35 °C for 6 weeks; winter	0	N/A	N/A
		Hydrogen Peroxide; AR 35 °C for 6 weeks; GA (1000 mg/L) with Smoke Water (33 % (v/v) for 48 h; winter	0	N/A	N/A
		Hydrogen Peroxide; Wet/dry 35 °C for 6 weeks; winter	0	N/A	N/A
		Hydrogen Peroxide; Wet/dry 35 °C for 6 weeks; GA (1000 mg/L) with Smoke Water (33 % (v/v) for 48 h; winter	0	N/A	N/A
		Hydrogen Peroxide; AR summer for 8 weeks; winter	0	N/A	N/A
		Hydrogen Peroxide; AR summer for 8 weeks; GA (1000 mg/L) with Smoke Water (33 % (v/v)) for 48 h; winter	2	160	N/A

No	Species	Treatment	Germination % (Average)	T_0	T_{50}
	Astroloma conostephioides	Hydrogen peroxide; AR summer for 8 weeks; spring/autumn	0	N/A	N/A
		Hydrogen peroxide; AR summer for 8 weeks; GA (1000 mg/L) with smoke water (33 % (v/v)) for 48 h; spring/autumn	0	N/A	N/A
		Hydrogen peroxide; AR summer for 8 weeks; STRAT spring/autumn 10 weeks; winter	0	N/A	N/A
		Hydrogen peroxide; AR summer for 8 weeks; GA (1000 mg/L) with smoke water (33 % (v/v)) for 48 h; STRAT spring/autumn 10 weeks; winter	4	160	N/A
		Hydrogen peroxide; GA (900 mg/L); smoke water (10% (v/v)) 48 h; 15 °C constant temp	0	N/A	N/A
		Hydrogen peroxide; endocarp gently cracked; GA (900 mg/L); smoke water (10% (v/v)) 48 h; 15 °C constant temp	33	18	N/A
	(Seeds collected from Horsnell Gully CP December 2015 88% viable) ↓	Seed excised from the endocarp; 15 °C constant temp	30	28	N/A
		Seed excised from the endocarp; GA (500 mg/L) + smoke water (10% (v/v)); 15 °C constant temp	64	21	42
		Seed excised from the endocarp; GA (500 mg/L); 15 °C constant temp	**85**	**21**	**28**
3	*Astroloma humifusum*	Hydrogen peroxide; winter	0	N/A	N/A
		Hydrogen peroxide; AR 35 °C for 6 weeks; winter	0	N/A	N/A

No	Species	Treatment	Germination % (Average)	T_0	T_{50}
	Astroloma humifusum	Hydrogen peroxide; AR 35 °C for 6 weeks; GA (1000 mg/L) with smoke water (33 % (v/v)) for 48 h; winter	6	63	N/A
		Hydrogen peroxide; wet/dry 35 °C for 6 weeks; winter	6	63	N/A
		Hydrogen peroxide; wet/dry 35 °C for 6 weeks; GA (1000 mg/L) with smoke water (33 % (v/v)) for 48 h; winter	2	140	N/A
		Hydrogen peroxide; AR summer for 8 weeks; winter	0	N/A	N/A
		Hydrogen peroxide; AR summer for 8 weeks; GA (1000 mg/L) with smoke water (33 % (v/v)) for 48 h; winter	6	140	N/A
		Hydrogen peroxide; AR summer for 8 weeks; spring/autumn	0	N/A	N/A
		Hydrogen peroxide; AR summer for 8 weeks; GA (1000 mg/L) with smoke water (33 % (v/v)) for 48 h; spring/autumn	0	N/A	N/A
		Hydrogen peroxide; AR summer for 8 weeks; STRAT spring/autumn 10 weeks; winter	0	N/A	N/A
		Hydrogen peroxide; AR summer for 8 weeks; GA (1000 mg/L) with smoke water (33 % (v/v) for 48 h; STRAT spring/autumn 10 weeks; winter	6	84	N/A
		Hydrogen peroxide; GA (900 mg/L); smoke water (10% (v/v)) 48 h; 15 °C constant temp	0	N/A	N/A

No	Species	Treatment	Germination % (Average)	T_0	T_{50}
		Hydrogen peroxide; endocarp gently cracked; GA (900 mg/L); smoke water (10% (v/v)) 48 h; 15 °C constant temp	2	N/A	N/A
	(Seeds collected from Mt Pleasant March 2016, 89% viable)	**Seed excised from the endocarp; GA (500 mg/L); 15 °C constant temp**	**85**	**21**	**21**
4	*Boronia coerulescens*	Control; winter	0	N/A	N/A
		Leaching (48 h); GA (500 mg/L) with smoke water (10 % (v/v)) for 24 h; winter	**38**	**22**	**N/A**
		Control; spring/autumn	0	N/A	N/A
		Leaching (48 h); GA (500 mg/L) with smoke water (10 % (v/v)) for 24 h; spring/autumn	34	22	N/A
5	*Calytrix tetragona*	Control; spring/autumn	0	N/A	N/A
		GA (250 mg/L) for 24 h; spring/autumn	0	N/A	N/A
		GA (250 mg/L) with smoke water (10 % (v/v)) for 24 h ; spring/autumn	28	57	N/A
		Dry heat (90 °C for 15 min); GA (250 mg/L) with smoke water (10% (v/v)) for 24 h; spring/autumn	**62**	**41**	**79**
6	*Exocarpos sparteus*	Leaching (48 h); winter	0	N/A	N/A
		GA (400 mg/L) for 48 h; winter	**38**	**42**	**N/A**

No	Species	Treatment	Germination % (Average)	T_0	T_{50}
		GA (400 mg/L) with smoke water (20% (v/v)) for 48 h; winter	18	49	N/A
		Dry heat (120 °C) for 4 min; GA (400 mg/L) with smoke water (20% (v/v)) for 48 h; winter	15	49	N/A
7	*Exocarpos syrticola*	Fruit removal; GA (400mg/L) for 48hrs; AR 20°C for 3 weeks; winter	20	49	N/A
		GA (400 mg/L) for 48 h; AR 20 °C for 3 weeks; winter	**72**	**40**	**93**
		Aerosol smoke (15min); GA (400mg/L) for 48 h; AR 20 °C for 3 weeks; winter	32	49	N/A
		Leaching (48 h); winter	0	N/A	N/A
		GA (400 mg/L) for 48 h; winter	62	28	70
		GA (400 mg/L) with smoke water (20 % (v/v)) for 48 h; winter	68	28	63
		Dry heat (120 °C) for 4 min; GA (400 mg/L) with smoke water (20% (v/v)) for 48 h; winter	28	42	N/A
8	*Frankenia pauciflora* var. *gunnii*	Control; winter	96	7	14
		GA (250 mg/L) continuous; winter	**100**	**7**	**14**
		Control; spring/autumn	72	7	21

No	Species	Treatment	Germination % (Average)	T_0	T_{50}
		GA (250 mg/L) continuous; spring/autumn	78	7	21
9	*Gahnia filum*	Control; spring/autumn	88	29	51
		Nicked seed coat; spring/autumn	80	29	61
		Nicked seed coat; leaching (7 d), spring/autumn	**90**	**22**	**44**
		GA (250 mg/L) with smoke water (10% (v/v)) for 24 h; spring/autumn	74	29	51
11	*Hibbertia riparia*	Control; winter	0	N/A	N/A
		Nicked seed coat; winter	18	60	N/A
		GA (500 mg/L) for 24 h; winter	12	50	N/A
		GA (500 mg/L) with smoke water (10% (v/v)) for 24 h; spring/autumn	**62**	**43**	**89**
10	*Hibbertia sericea*	Control; winter	2	60	N/A
		Nicked seed coat; winter	14	60	N/A
		GA (500 mg/L) for 24 h; winter	26	43	N/A
		GA (500 mg/L) with smoke water (10% (v/v)) for 24 h; spring/autumn	**40**	**50**	**N/A**

No	Species	Treatment	Germination % (Average)	T_0	T_{50}
12	*Lasiopetalum baueri*	Control; winter	2	58	N/A
		Control; spring/autumn	2	63	N/A
		Nicked seed coat; winter	**100**	**7**	**7**
		Nicked seed coat; spring/autumn	72	14	36
13	*Leucopogon parviflorus*	Fruit removed; Control; sown 14/3/13	9	91	N/A
		Fruit removed; smoke water; sown 14/2/13	8	N/A	N/A
		Hydrogen peroxide; spring/autumn	0	N/A	N/A
		Hydrogen peroxide; GA (1000 mg/L) for 72hrs; spring/autumn	60	49	116
		Dry heat (100 °C) for 2 min; hydrogen peroxide; GA (1000 mg/L) for 72 h; spring/autumn	80	63	77
		Leaching (13 days); hydrogen peroxide; GA (1000 mg/L) for 72hrs; spring/autumn	40	70	N/A
		Hydrogen peroxide; smoke water (10% (v/v)) for 24 h; GA (1000 mg/L) for 72hrs; spring/autumn	**100**	**49**	**77**
		Dry heat (90˚C) for 15 mins; hydrogen peroxide; GA (1000mg/l) + smoke water for 48 hours; spring/autumn.	44	63	N/A

No	Species	Treatment	Germination % (Average)	T_0	T_{50}
14	*Lomandra densiflora*	Control; 15 °C constant temperature	16	61	N/A
		GA (1000 mg/L) for 24 h; 15 °C constant temperature 12 h photoperiod	32	61	N/A
		Hydrogen peroxide; 15 °C constant temperature 12 h photoperiod	28	35	N/A
		Hydrogen peroxide; GA (1000 mg/L) for 72 h; 15 °C constant 12 h photoperiod	23	28	N/A
		Hydrogen peroxide; smoke water (10% (v/v)) for 24 h; 15 °C constant 12 h photoperiod	69	28	49
		Hydrogen peroxide; GA (1000 mg/L) 72 h; smoke water (10% (v/v)) 24 h; 15 °C constant temperature 12 h photoperiod	14	28	N/A
		Hydrogen peroxide, GA (1000 mg/L) + smoked water (20% (v/v) 24 hrs; winter	**90**	**28**	**35**
		Hydrogen peroxide, GA (1000 mg/L) 24 hrs; winter	80	28	42
15	*Lomandra effusa*	Control; 15 °C constant temperature 12 h photoperiod	62	61	75
		GA (1000 mg/L) for 24 h; 15 °C constant temperature 12 h photoperiod	74	28	71
		Hydrogen Peroxide; 15 °C constant temperature 12 h photoperiod	44	28	N/A

No	Species	Treatment	Germination % (Average)	T_0	T_{50}
		Hydrogen peroxide; GA (1000 mg/L) for 72 h; 15 °C constant 12 h photoperiod	**90**	**35**	**42**
		Hydrogen peroxide; smoke water (10% (v/v)) for 24 h; 15 °C constant 12 h photoperiod	48	28	N/A
		Hydrogen peroxide; GA (1000 mg/L) for 72 h; smoke water (10% (v/v)) for 24 h; 15 °C constant 12 h photoperiod	65	35	56
16	*Lomandra multiflora* ssp. *dura*	Control; 15 °C constant temperature 12 h photoperiod	78	28	64
		GA (1000 mg/L) for 24 h; 15 °C constant temperature 12 h photoperiod	10	28	N/A
		Hydrogen peroxide; 15 °C constant temperature 12 h photoperiod	75	28	42
		Hydrogen peroxide; GA (1000 mg/L) for 72 h; 15 °C constant temperature 12 h photoperiod	12	28	N/A
		Hydrogen peroxide; smoke water (10% (v/v)) for 24 h; 15 °C constant temperature 12 h photoperiod	**93**	**28**	**42**
		Hydrogen peroxide; GA (1000 mg/L) for 72 h; smoke water (10% (v/v)) for 24 h; 15 °C constant temperature 12 h photoperiod	13	28	N/A
17	*Lomandra juncea*	Control; 15 °C constant temperature	6	28	N/A

No	Species	Treatment	Germination % (Average)	T_0	T_{50}
		GA (1000 mg/L) for 24 h; 15 °C constant temperature 12 h photoperiod	12	28	N/A
		Hydrogen peroxide; 15 °C constant temperature 12 h photoperiod	7	28	N/A
		Hydrogen peroxide; GA (1000 mg/L) for 72 h; 15 °C constant temperature 12 h photoperiod	35	35	N/A
		Hydrogen peroxide; smoke water (10% (v/v)) for 24 h; 15 °C constant 12 h photoperiod	7	28	N/A
		Hydrogen peroxide; GA (1000 mg/L) for 72 h; smoke water (10% (v/v)) for 24 h; 15 °C constant temperature 12 h photoperiod	35	28	N/A
		Hydrogen peroxide; aerated water 72 h; GA (1000 mg/L) for 72 h; 15 °C constant temperature 12 h photoperiod	**70**	**28**	**42**
		Dry heat 50 °C 24 h; hydrogen peroxide; GA (1000 mg/L) for 72 h; 15 °C constant temperature 12 h photoperiod	62	35	49
		Hydrogen peroxide; GA (1000 mg/L) for 72 h; 15 °C constant temperature 12 h photoperiod	60	28	56
		Hydrogen peroxide (3%) 72 h; GA (1000 mg/L) for 72 h; 15 °C constant temperature 12 h photoperiod	40	42	N/A
18	*Lomandra leucocephala*	Control; 15 °C constant temperature 12 h photoperiod	0	N/A	N/A

No	Species	Treatment	Germination % (Average)	T_0	T_{50}
		GA (1000 mg/L) for 24 h; 15 °C constant temperature 12 h photoperiod	2	56	N/A
		Hydrogen peroxide; 15 °C constant temperature 12 h photoperiod	1	28	N/A
		Hydrogen peroxide; GA (1000 mg/L) for 72 h; 15 °C constant 12 h photoperiod	44	42	N/A
		Dry heat (50 °C) 24 h; hydrogen peroxide; GA (1000 mg/L) 72 h; 15 °C constant temperature 12 h photoperiod	**50**	**49**	**94**
		Hydrogen peroxide; aerated water 72 h; GA (1000 mg/L) for 72 h; 15 °C constant temperature 12 h photoperiod	26	42	N/A
		Hydrogen peroxide; smoke water (10% (v/v)) for 24 h; 15 °C constant temperature 12 h photoperiod	0	N/A	N/A
		Hydrogen peroxide; GA (1000 mg/L) for 72 h; smoke water (10% (v/v)) for 24 h; 15 °C constant temperature 12 h photoperiod	12	28	N/A
19	*Muehlenbeckia adpressa*	Control; spring/autumn	No germination after 34 days		
		Nicked seed coat; spring/autumn	Results to be added to SoSA website		
20	*Muehlenbeckia gunnii*	Control; spring/autumn	81	31	41
		STRAT 6 weeks spring/autumn; winter	**94**	**21**	**41**

No	Species	Treatment	Germination % (Average)	T_0	T_{50}
		GA (250 mg/L) for 48 h; spring/autumn	92	14	31
21	*Nitraria billardierei*	Hydrogen peroxide (10%); spring/autumn	0	N/A	N/A
		Hydrogen peroxide (10%); winter	0	N/A	N/A
		Hydrogen peroxide (30%); spring/autumn	0	N/A	N/A
		Hydrogen peroxide (30%); winter	0	N/A	N/A
		Hydrogen peroxide (10%); GA (1000 mg/L) 3 d; spring/autumn	0	N/A	N/A
		Hydrogen peroxide (10%); GA (1000 mg/L) 3 d; winter	0	N/A	N/A
		Hydrogen peroxide (10%); GA (1000 mg/L) 3 d; spring/autumn	0	N/A	N/A
		Hydrogen peroxide (10%); GA (1000 mg/L) 3 d; winter			
		Leaching (15 d); spring/autumn	28	25	N/A
		Leaching (15 d); dry heat (120 °C) for 2min; spring/autumn	16	8	N/A
		Leaching (15 d); GA (1000 mg/L) for 72 h; spring/autumn	38	8	N/A
		Leaching (15 d); Dry heat (120 °C) for 2min; GA (1000 mg/L) for 72 h; spring/autumn	**48**	**8**	**N/A**

No	Species	Treatment	Germination % (Average)	T_0	T_{50}
22	*Pimelea glauca*	Control; spring/autumn Nicked; spring/autumn GA (500 mg/L) for 24 h; spring/autumn Smoke Water (10% (v/v)); for 24 h spring/autumn	No germination after 34 days Results to be added to SoSA website		
23	*Pomaderris paniculosa* ssp *paniculosa*	Control; winter	4	35	N/A
		Wet heat (100 °C) for 30 sec; winter	**72**	**20**	**35**
		Leaching (3 days); winter	6	20	N/A
		Dry Heat (120°C) 5 min; winter	38	20	N/A
		Control; spring/autumn	0	N/A	N/A
		Wet heat (100 °C) for 30 sec; spring/autumn	36	28	N/A
		Leaching (3 days); spring/autumn	4	62	N/A
		Dry Heat (120°C) 5 min; spring/autumn	34	28	N/A
24	*Spyridium fontis-woodii*	**Wet heat (95 °C) for 30 sec; spring/autumn**	**70**	**25**	**53**
25	*Spyridium subochreatum*	Control; spring/autumn 4 weeks then transferred to winter	0	N/A	N/A

No	Species	Treatment	Germination % (Average)	T_0	T_{50}
		Control; winter	2	51	N/A
		Nicked seed coat; spring/autumn 4 weeks then transferred to winter	46	10	N/A
		Nicked seed coat; winter	**84**	**7**	**21**
		Wet heat (100 °C) for 30 sec; spring/autumn 4 weeks then transferred to winter	24	61	N/A
26	*Tecticornia indica ssp. leiostachya*	**Control; spring/autumn**	**72**	**4**	**18**
		GA (250mg/L) continuous; spring/autumn	70	11	18
27	*Thomasia petalocalyx*	Control; winter	4	58	N/A
		Nicked seed coat; winter	**94**	**14**	**14**

Appendix 2.

Seeds of South Australia species page links.

Species	Seeds of South Australia species page link
Adriana quadripartita	http://saseedbank.com.au/species_information.php?rid=277
Astroloma conostephioides	http://saseedbank.com.au/species_information.php?rid=509
Astroloma humifusum	http://saseedbank.com.au/species_information.php?rid=510
Boronia coerulescens ssp. *coerulescens*	http://saseedbank.com.au/species_information.php?rid=705
Calytrix tetragona	http://saseedbank.com.au/species_information.php?rid=949
Exocarpos sparteus	http://saseedbank.com.au/species_information.php?rid=1928
Exocarpos syrticola	http://saseedbank.com.au/species_information.php?rid=1930
Frankenia pauciflora var. *gunnii*	http://saseedbank.com.au/species_information.php?rid=1956
Gahnia filum	http://saseedbank.com.au/species_information.php?rid=1977
Hibbertia riparia	http://saseedbank.com.au/species_information.php?rid=2309
Hibbertia sericea	http://saseedbank.com.au/species_information.php?rid=2310
Lasiopetalum baueri	http://saseedbank.com.au/species_information.php?rid=2530
Leucopogon parviflorus	http://saseedbank.com.au/species_information.php?rid=55
Lomandra densiflora	http://saseedbank.com.au/species_information.php?rid=2731
Lomandra effusa	http://saseedbank.com.au/species_information.php?rid=2732
Lomandra juncea	http://saseedbank.com.au/species_information.php?rid=2735
Lomandra leucocephala ssp. *robusta*	http://saseedbank.com.au/species_information.php?rid=2736
Lomandra mutliflora ssp. *dura*	http://saseedbank.com.au/species_information.php?rid=2740
Muehlenbeckia adpressa	http://saseedbank.com.au/species_information.php?rid=2989
Muehlenbeckia gunnii	http://saseedbank.com.au/species_information.php?rid=2993
Nitraria billardierei	http://saseedbank.com.au/species_information.php?rid=54
Pimelea glauca	http://saseedbank.com.au/species_information.php?rid=3344
Pomaderris paniculosa ssp. *paniculosa*	http://saseedbank.com.au/species_information.php?rid=3469
Spyridium fontis-woodii	http://saseedbank.com.au/species_information.php?rid=4835
Spyridium subochreatum	http://saseedbank.com.au/species_information.php?rid=4278
Tecticornia indica ssp. *leiostachya*	http://saseedbank.com.au/species_information.php?rid=4407
Thomasia petalocalyx	http://saseedbank.com.au/species_information.php?rid=4496