CLLMM Seed Research Project

Progress Report

Picture - male flower spike of *Adriana quadripartita* (Woods Well Rd).

June 2015

SOUTH AUSTRALIAN SEED CONSERVATION CENTRE

BOTANIC GARDENS OF SOUTH AUSTRALIA

Summary

This report is a progress update 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 Coorong, Lower Lakes Recovery project, as part of the Commonwealth Bioremediation and Revegetation Project. The time frame for this project is from February 2013 to May 2016 and this report is the second year final report scheduled for June 2015.

Seed collections from 28 plant species have been made and collections for extra species are scheduled for the following season. The collections from 25 species contained sufficient quantities of viable seed for analysis, and germination experiments have been conducted for all of these species. Initial experiments are still in progress for four species, *Boronia coerulescens*, *Gahnia filum*, *Hibbertia crinita* and *H. riparia*. High levels of germination (> 40%) were recorded for 16 of the remaining 21 species. Further experiments will be conducted on 5 species that had lower germination levels (4 - 36%).

Recent experiments testing six *Lomandra* species and two *Astroloma* species are described in this report. High germination levels (69-93%) were achieved for four of the *Lomandra* species however, two species had lower levels of germination (32-35%). These species (*L. juncea* and *L. leucocephala*) will be tested with different treatments to try and improve germination rates. Of all the species tested throughout this project the two *Astroloma* species have been the most difficult to germinate. However, there has been some indication that temperature cycling in combination with gibberellic acid and smoked water alleviates dormancy. Further germination testing for these species will be undertaken in the next phase of the project.

Vegetative propagation from cuttings was investigated for *Astroloma conostephioides* and *A. humifusum* and strike rates of 44% and 20% were measured for each species respectively. This method of propagation may be useful for revegetation and further experiments will be conducted to try and improve the production of plants through cuttings.

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. A page will be generated for each species with photographic images of plants, flowers and seeds as well as information on seed collection and germination to facilitate the propagation of these plants by future practitioners.

Background

The purpose of this project is to investigate the germination and/or propagation requirements of key species identified by the Coorong Lower Lake Recovery Project group to support the Bioremediation and Revegetation Project. The plants identified as priority species for this project were selected because they are difficult to propagate. Research into germination and propagation methods is required in order to increase the number of species available for revegetation programs.

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 reflect the conditions seeds would experience in their natural habitat. A range of chemical and physical treatments are also used that can increase the level of 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, text books, practical experience and data from other seed banks. 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.

This project involves finding suitable propagation methods for 24 plant species that were identified as priority species for the region. In this report we present data for 21 species that have been tested to date. Experiments for four more species are currently in progress. 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 28 species (Table 2). 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 flesh 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.

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) = #seed/g.

Two species were added to the original target list:

Spyridium fontis woodsii 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

Testing seed viability is a crucial step as non-viable seeds will never germinate. Seeds can be non-viable for several reasons, for example; stress during development, predation, collection of immature seeds and inappropriate storage conditions.

Seed viability was estimated by x-raying 50 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 have been provided 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 was tested. Each treatment was applied to 50 seeds 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. An explanation of why the treatments were applied is also listed in Table 1. A combination of treatments was used in several experiments and these are listed 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.
Nicked seed coat	The outer layer of the seed coat was carefully nicked using	This process alleviates physical dormancy by allowing

Treatment	Method	Rationale
	a sharp scalpel to disrupt the water impermeable layers surrounding seeds with physical dormancy.	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.
Wet/Dry cycling	Seeds were placed in Petri dishes with sterile sand at a	Wetting and drying simulates the soil seed bank environment

Treatment	Method	Rationale
	specified temperature. During incubation seeds were wet on a weekly basis for 6 hours then allowed to dry out.	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 that had very low germination results to date.

An experiment was set up at the Mount Lofty Botanic Gardens Nursery on the 15th April 2014. Fresh cuttings were taken from Naracoorte caves National Park on the 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. A similar 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 will be 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 will provide 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

Table 2 shows the list of seed collections for target species. Sufficient quantities of seed for germination testing were collected from 25 out of the 28 species listed. Poor seed numbers were obtained from three species of *Pimelea*. The seeds of these plants are rapidly shed at maturity making the timing of seed collection critical. A different collection strategy will be used next season.

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

Figure 1 shows microscopic detail of viable seeds for the species that have been tested for germination. 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 what viable seed looks like. It is recommended that seeds are cut and examined 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 will not germinate.

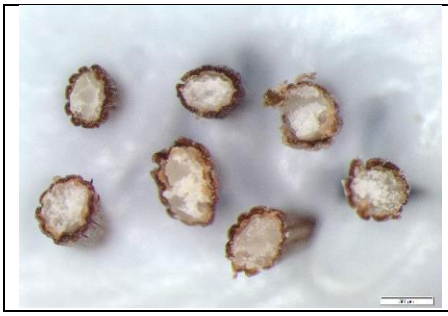
Table 2. Seed collection data and viability testing results.

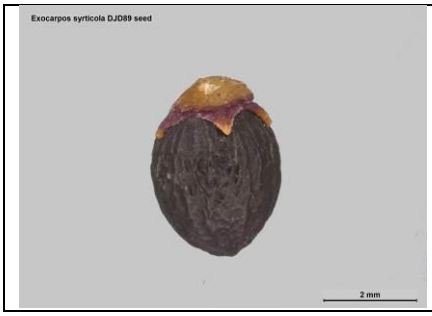
No	Family	Genus	Species	Date seed collected	Location	Seed number	Number seeds/g	Viability
1	Chenopodiaceae	*Tecticornia*	*indica ssp. leiostachya*	21-Feb-13	Salt Creek	4700	909	49%
2	Cyperaceae	*Gahnia*	*filum*	15-Nov-13		1500	725	80%
3	Dilleniaceae	*Hibbertia*	*crinita*	20- Oct-14	Bonney Reserve	1040	400	56%
4	Dilleniaceae	*Hibbertia*	*riparia*	27-Oct-14	Scott CP	1200	756	86%
5	Epacridaceae	*Astroloma*	*conostephioides*	4-Oct-12	Naracoorte Caves NP	1912	22	95%
6	Epacridaceae	*Astroloma*	*humifusum*	7-Nov-13	Frahns scrub	2960	14	92%
7	Epacridaceae	*Leucopogon*	*parviflorus*	30-Jan-13	Princess Hwy, Coorong	2876	54	30%
8	Euphorbiaceae	*Adriana*	*quadripartita*	30-Jan-13	Woods well	860	37	78%
9	Frankeniaceae	*Frankenia*	*pauciflora var. gunnii*	21-Feb-13	Salt Creek	66000	7692	92%
10	Liliaceae	*Lomandra*	*densiflora*	20-Nov-13	Frahns scrub	1500	74	100%
11	Liliaceae	*Lomandra*	*effusa*	22-Nov-13	Frahns scrub	2425	42	100%

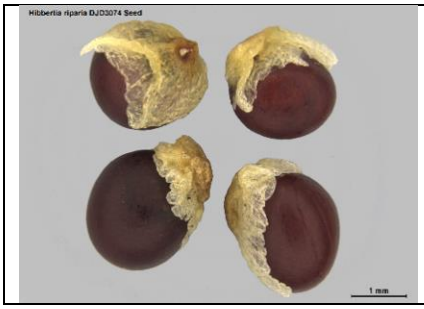
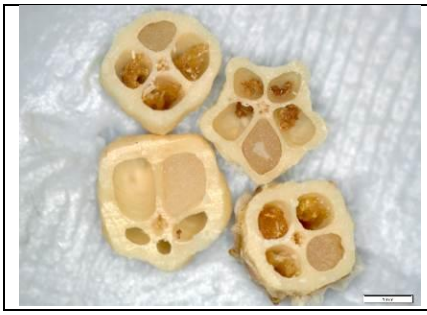
No	Family	Genus	Species	Date seed collected	Location	Seed number	Number seeds/g	Viability
12	Liliaceae	*Lomandra*	*juncea*	20-Nov-13	Monarto CP	1220	81	100%
13	Liliaceae	*Lomandra*	*leucocephala*	12-Dec-11	Langhorne Creek	5000	50	100%
14	Liliaceae	*Lomandra*	*multiflora ssp dura*	8-Jan-14	Finniss Oval	2964	60	100%
15	Myrtaceae	*Calytrix*	*tetragona*	20-Nov-13	Monarto CP	5660	602	18%
16	Polygonaceae	*Muehlenbeckia*	*gunnii*	23-Oct-13	Finniss - Milang Road	1140	73	100%
17	Rhamnaceae	*Spyridium*	*fontis-woodii*	30-Jan-13	Woods well	750	2230	74%
18	Rhamnaceae	*Spyridium*	*subochreatum*	7-Nov-13	Monarto CP	4100	2778	70%
19	Rhamnaceae	*Pomaderris*	*paniculosa*	10-Dec-14	Tailem bend – Karoonda Rd	5800	895	95%
20	Rutaceae	*Boronia*	*corulescens*	10-Oct-14	Cox Scrub	1040	339	62%
21	Santalaceae	*Exocarpos*	*sparteus*	5-Feb-14	Ngarkat CP	1470	45	10%
22	Santalaceae	*Exocarpos*	*syrticola*	6-Feb-14	Salt Creek	944	41	83%
23	Sterculiaceae	*Lasiopetalum*	*baueri*	7-Nov-13	Ferries Macdonald CP	1300	685	52%

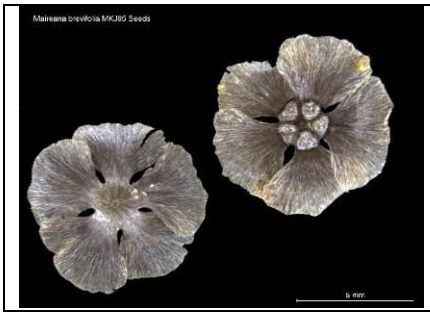
No	Family	Genus	Species	Date seed collected	Location	Seed number	Number seeds/g	Viability
24	Sterculiaceae	*Thomasia*	*petalocalyx*	11-Nov-13	Naracoorte Caves NP	900	722	16%
25	Thymelaeaceae	*Pimelea*	*glauca*	Low seed numbers	Strathalbyn Cemetery	To be collected		
26	Thymelaeaceae	*Pimelea*	*octophylla*	Low seed numbers	Cox Scrub	To be collected		
27	Thymelaeaceae	*Pimelea*	*humilis*	Low seed numbers	Cox Scrub	To be collected		
28	Zygophyllaceae	*Nitraria*	*billardierei*	28-Feb-13	Langhorne Ck - Wellington Rd	6200	16	96%

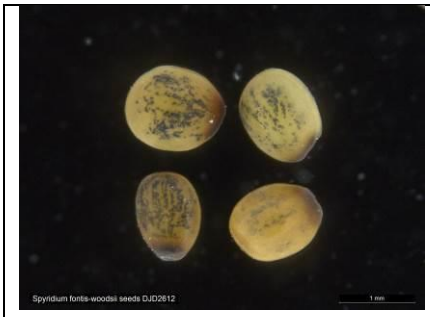
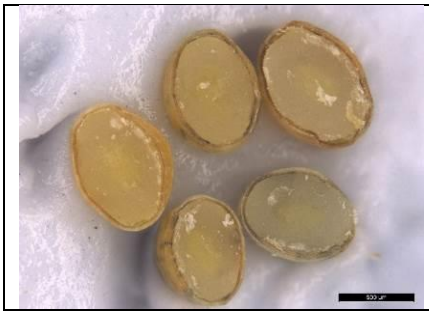
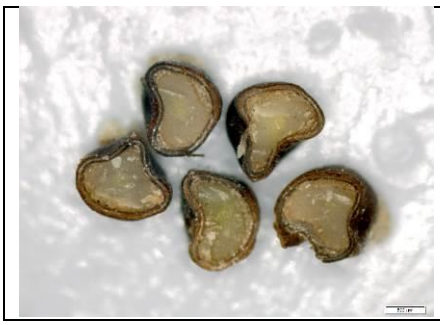
Figure 1. Images of seeds and fruits taken through a microscope and viable seeds cut open to show the inside of a healthy seed.

Species	Seed Image	Viable Cut seed
Adriana quadripartita *Seeds*		
Astroloma conostephioides *Woody drupe*		
Astroloma humifusum *Woody drupe*		
Boronia 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 crinita *Seeds*		

Species	Seed Image	Viable Cut seed
Hibbertia riparia *Seeds*		
Lasiopetalum baueri *Seeds*		
Leucopogon parviflorus *Woody drupe*		
Lomandra densiflora *Seeds*		
Lomandra effusa *Seeds*		

Species	Seed Image	Viable Cut seed
Lomandra muliflora ssp *dura* *Seeds*		
Lomandra leucocephala ssp *robusta* *Seeds*		
Lomandra juncea		
Maireana brevifolia *Fruit*		
Muehlenbeckia gunnii *Seeds*		

Species	Seed Image	Viable Cut seed
Nitraria billardierei *Woody drupe*		
Spyridium fontis-woodsii *Seeds*		
Spyridium subochreatum *Seeds*		
Tecticornia indica ssp. leiostachya *Seeds*		
Thomasia petalocalyx *Seeds*		

Germination Experiments

The results from germination experiments for 21 species have been 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 16 species were greater than 40% for at least one treatment which shows that successful methods of propagation through seed could be developed following the methods described. Experiments for four species, *Boronia coerulescens*, *Gahnia filum*, *Hibbertia crinita* and *H. riparia* commenced on the 17th of May and the final results will not be available for a few more weeks.

Five of the species had germination levels below 40%. These included the two species belonging to the genus *Astroloma*, two species of *Lomandra* and *Exocarpos sparteus*. All of these species have underdeveloped embryos 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. Different combinations of treatments will be tested in the next phase of the project to try and increase of germination rates for these species.

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 3. 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 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 results. Germinate seeds in spring through to autumn.	Germination increased with application of GA and 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. Laboratory results have been poor. Tests are on-going for this species.	Germination rates have been low, however, this species responded well to propagation through cuttings using a very light propagation mix and application of rooting hormone.
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. Laboratory results have been poor. Tests are on-going for this species.	Germination rates have been low, however, this species responded well to propagation through cuttings, using a very light propagation mix and application of rooting hormone.
Boronia coerulescens	Tests are in progress for this species.	
Calytrix tetragona	Germination responds to fire cues in this species. Dry heat (15 min; 90 °C) and diluted smoke water in combination with GA increased the level of germination. 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.
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.
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.
Frankenia pauciflora var. gunnii	No treatment is required for this species. High levels of germination (100%) were observed in the control test incubated in a winter environment.	Using a hand lens, check that viable seeds are present in the capsules during collection.

Species	Best treatment for germination	Advice for this species
Gahnia filum	Tests are in progress for this species.	
Hibbertia crinita	Tests are in progress for this species.	
Hibbertia riparia	Tests are in progress for this species.	
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.
Lomandra densiflora	Treat seeds with hydrogen peroxide (30%) and diluted smoke water for increased germination results. 69% of seeds germinated after this pretreatment after incubation at 15°C.	Be sure that the seed is ripe. Collect 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%).	Be sure that the seed is ripe. Collect when fruits have begun to split open. Immature seed will not germinate. After ripening appears to increase seed germination.
Lomandra muliflora 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%).	Be sure to collect ripe seed when fruits have begun to split open. Immature seed will not germinate.
Lomandra leucocephala	Germination levels have been reasonably low (maximum 32%) for this species. Further experiments will be conducted in the next phase of the project.	Be sure to collect ripe seed when fruits have begun to split open. Immature seed will not germinate.

Species	Best treatment for germination	Advice for this species
Lomandra juncea	Germination levels have been reasonably low (maximum 35%) for this species. Further experiments will be conducted in the next phase of the project.	Be sure to 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 black seeds. Remove fruit flesh by wetting and rubbing through a sieve before storage.
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.
Spyridium fontis-woodsii	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. Best germination rate (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 *A. humifusum*

The results from the *Astroloma* propagation experiment showed that good root development had occurred after 7 months of cuttings being maintained on a heat bed in a moist environment. The strike rates 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. A new experiment will be trialled this year using a range of different potting mixes to test whether the vigour of the rooted cuttings could be improved.

Discussion

Seed Viability

The importance of testing seed viability is fundamental but is often overlooked. The problem with non-viable seed is that, if not checked it can be collected, cleaned, stored, seeded and monitored for seedling emergence when the reality is that very little viable seed was collected in the first place. The poor seedling emergence results may then be put down to lack of germination for a variety of other reasons. This amounts to a waste of resources and the opportunity to establish plants in that year could be missed. It is therefore encouraged that seed viability is checked on collection and quantified after the seed is cleaned.

Germination

The results from the project so far indicate that the majority of the target species are able to be propagated through seed for the purpose of revegetation. The experiments have been conducted under laboratory conditions and results will vary from germination tests done in other (nursery/garden/field) conditions, especially with different seed collections from that species. The information provided should be used as a guide when collecting and germinating seeds for the bioremediation and revegetation 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 will increase through sexual reproduction between individuals once the plants are mature.

Information Sharing

The information compiled on the Seeds of South Australia website 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

For the last phase of this project we will focus on experimenting with the species that have been difficult to germinate. In particular the *Astroloma* species as they have had very low germination levels throughout the project so far. The other main objective is to make collections with sufficient viable seed of species from the genus *Pimelea* to test for germination efficiency.

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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.

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

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
		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			

		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	0 4	N/A 160	N/A N/A
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
		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

4	*Boronia coerulescens*	Control; winter	In progress		
		Leaching (48 h); GA (500 mg/L) with Smoke Water (10 % (v/v)) for 24 h; winter	In progress		
		Control; spring/autumn	In progress		
		Leaching (48 h); GA (500 mg/L) with Smoke Water (10 % (v/v)) for 24 h; spring/autumn	In progress		
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	**36**	42	N/A
		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
		GA (250 mg/L) continuous; spring/autumn	78	7	21
9	*Gahnia filum*	Control; spring/autumn	In progress		
		Nicked seed coat; spring/autumn	In progress		
		Nicked seed coat; Leaching (7 d), spring/autumn	In progress		
		GA (250 mg/L) with Smoke Water (10% (v/v)) for 24 h; spring/autumn	In progress		

10	*Hibbertia crinita*	Control; winter Nicked seed coat; winter GA (500 mg/L) for 24 h; winter GA (500 mg/L) with Smoke Water (10% (v/v)) for 24 h; spring/autumn	In progress In progress In progress In progress		
11	*Hibbertia riparia*	Control; winter Nicked seed coat; winter GA (500 mg/L) for 24 h; winter GA (500 mg/L) with Smoke Water (10% (v/v)) for 24 h; spring/autumn	In progress In progress In progress In progress		
12	*Lasiopetalum baueri*	Nicked seed coat; winter	**100**	7	7

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	**100**	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
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) for 72 h; Smoke Water (10% (v/v)) for 24 h; 15 °C constant 12 h photoperiod	14	28	N/A

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
		Hydrogen peroxide; GA (1000 mg/L) for 72 h; 15 °C constant 12 h photoperiod	**92**	21	35
		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 muliflora 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 12 h photoperiod	12	28	N/A
		Hydrogen peroxide; Smoke Water (10% (v/v)) for 24 h; 15 °C constant 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 12 h photoperiod	13	28	N/A

17	*Lomandra leucocephala*	Control; 15 °C constant temperature 12 h photoperiod	0	N/A	N/A
		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	**32**	28	N/A
		Hydrogen peroxide; Smoke Water (10% (v/v)) for 24 h; 15 °C constant 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 12 h photoperiod	12	28	N/A
18	*Lomandra juncea*	Control; 15 °C constant temperature	6	28	N/A
		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 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 12 h photoperiod	**35**	28	N/A

19	*Muehlenbeckia gunnii*	Control; spring/autumn	81	31	41
		STRAT 6 weeks spring/autumn; winter	**94**	21	41
		GA (250 mg/L) for 48 h; spring/autumn	92	14	31
20	*Nitraria billardierei*	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
21	*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

22	*Spyridium fontis-woodsii*	Wet heat (95 °C) for 30 sec; spring/autumn	**70**	25	53
23	*Spyridium subochreatum*	Control; spring/autumn 4 weeks then transferred to winter	0	N/A	N/A
		Nicked seed coat; spring/autumn 4 weeks then transferred to winter	**46**	10	N/A
		Wet heat (100 °C) for 30 sec; spring/autumn 4 weeks then transferred to winter	24	61	N/A
24	*Tecticornia indica ssp. leiostachya*	Control; spring/autumn	**72**	4	18
		GA (250mg/L) continuous; spring/autumn	70	11	18
25	*Thomasia petalocalyx*	Nicked seed coat; winter	**94**	14	14