CLLMM Seed Research Project

Progress Report

December 2014

SOUTH AUSTRALIAN SEED CONSERVATION CENTRE

BOTANIC GARDENS OF SOUTH AUSTRALIA

Summary

Seed collections from 25 plant species have been made and collections for extra species are scheduled for December 2014 and beyond. The collections to date contain sufficient quantities of viable seed to conduct germination experiments. Germination experiments were set up for 20 species. High levels of germination (> 50%) were recorded from 11 species and experiments are still in progress for the remaining species. Vegetative propagation from cuttings was investigated for *Astroloma conostephioides* and *A. humifusum* and strike rates over 40% were measured for both species. This method of propagation may be a useful alternative to growing from seed as these species are notoriously difficult to germinate. Data recorded so far is presented in this report and is being incorporated in the Seeds of South Australia website (www.saseedbank.com.au) and will be 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 have been 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 the 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 SACC 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.

Methods

Seed collection, cleaning and quantification.

Plant populations with adequate seed set were located and seed collections were made at the appropriate time, when seeds were mature. The seeds were then 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 conditions of storage.

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 at home using small

snips or other similar tool, 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 the Table 1. A combination of treatments was used in several experiments and these are listed in sequential order in Table 3.

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

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.	Use to mimic extreme hot conditions that is required by some species to germinate eg, baking sand or bush fire.
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.

Treatment	Method	Rationale
Hydrogen Peroxide (H_2O_2)	Seeds soaked in hydrogen peroxide 30% (v/v) for 15 mins with gentle agitation, then rinsed 3 times with RO 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 and 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 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 would be 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 eg- 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.

Treatment	Method	Rationale
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 the 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 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 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 result

Results

Seed Collections and Seed Viability

Table 2 shows the list of seed collections from target species. Other species will be collected in the coming seasons. 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.

Table 2. Seed collection data and results from viability testing.

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 Highway, 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%

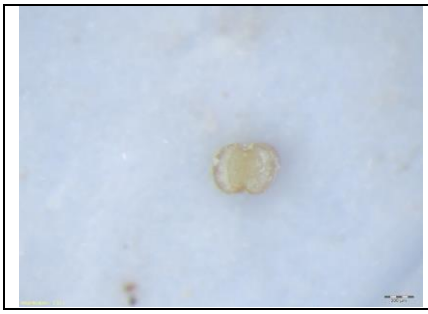
No	Family	Genus	Species	Date seed collected	Location	Seed number	Number seeds/g	Viability
12	Liliaceae	*Lomandra*	*juncea*	20-Nov-13	Monarto CP	1220	81	100%
13	Liliaceae	*Lomandra*	*multiflora ssp dura*	8-Jan-14	Finniss Oval	2964	60	100%
14	Myrtaceae	*Calytrix*	*tetragona*	20-Nov-13	Monarto CP	5660	602	18%
15	Polygonaceae	*Muehlenbeckia*	*gunnii*	23-Oct-13	Finniss - Milang Road	1140	73	100%
16	Rhamnaceae	*Spyridium*	*fontis-woodii*	30-Jan-13	Woods well	750	2230	74%
17	Rhamnaceae	*Spyridium*	*subochreatum*	7-Nov-13	Monarto CP	4100	2778	70%
18	Rhamnaceae	*Pomaderris*	*paniculosa*	To be collected Dec 2014	Tailem bend – Karoonda Rd	To be quantified		
19	Rutaceae	*Boronia*	*corulescens*	10-Oct-14	Cox Scrub	1040	339	62%
20	Santalaceae	*Exocarpos*	*sparteus*	5-Feb-14	Ngarkat CP (Emu Scats)	2100	45	100%
21	Santalaceae	*Exocarpos*	*syrticola*	30-Jan-13	Salt Creek	2400	40	95%
22	Sterculiaceae	*Lasiopetalum*	*baueri*	7-Nov-13	Ferries Macdonald CP	1300	685	52%

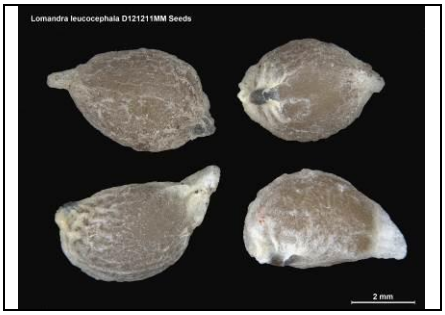
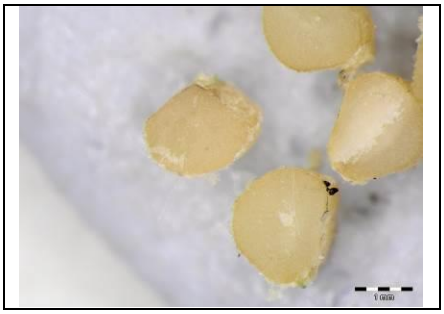
No	Family	Genus	Species	Date seed collected	Location	Seed number	Number seeds/g	Viability
23	Sterculiaceae	*Thomasia*	*petalocalyx*	11-Nov-13	Naracoorte Caves NP	900	722	16%
24	Thymelaeaceae	*Pimelea*	*glauca*	To be collected Dec 2014	Strathalbyn Cemetary	To be quantified		
25	Thymelaeaceae	*Pimelea*	*octophylla*	To be collected Dec 2014	Cox Scrub	To be collected		
26	Thymelaeaceae	*Pimelea*	*humilis*	To be collected Dec 2014	Cox Scrub	To be collected		
27	Zygophyllaceae	*Nitraria*	*billardierei*	28-Feb-13	Langhorne Creek - Wellington Rd	6200	16	96%

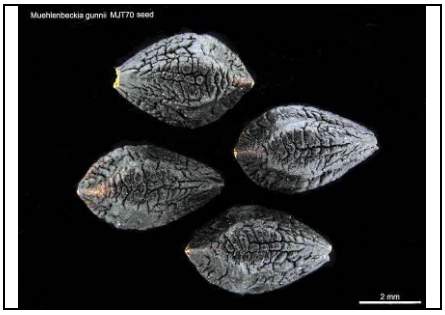
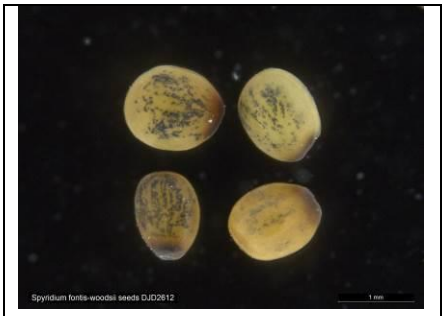
Figure 1 shows microscopic detail of viable seeds for the species that have been tested for germination. These images can be used as a guide during seed collection as a visual reference for what viable seed looks like.

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*		
Calytrix tetragona *Fruit with awns*		

Species	Seed Image	Viable Cut seed
Exocarpos sparteus *Nut*		
Exocarpos syrticola *Nut*		
Frankenia pauciflora var. gunnii *Seeds*		
Lasiopetalum baueri *Seeds*		
Leucopogon parviflorus		

Species	Seed Image	Viable Cut seed
Lomandra densiflora *Seeds*		
Lomandra effusa *Seeds*		
Lomandra muliflora ssp dura *Seeds*		
Lomandra leucocephala *Seeds*		
Lomandra juncea		

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

Species	Seed Image	Viable Cut seed
Thomasia petalocalyx		

Seed germination experiments

Experiments have been completed or are on-going for species that had sufficient seed collection last season. Initial results from the germination experiments from the SASCC are shown in Table 3.

The germination percentage is the proportion of seeds that germinated during the experiment period. The treatment with the highest level of germination for that species is shown in bold.

Table 3. Summary of germination experiments for collected seeds. Treatments are described in Table 2. Results shown are the average % germination for each treatment, T_0 is the number of days taken to observe the first germinating seed and T_{50} is the number of days for 50% of the seeds to germinate (N/A = Not applicable).

Species	**Treatment**	**Germination % (Average)**	**T_0**	**T_{50}**
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
	Boiling water (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
Astroloma conostephioides	Hydrogen Peroxide; spring/autumn	4	119	N/A

Species	Treatment	Germination % (Average)	T_0	T_{50}
	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	In progress		
	Hydrogen Peroxide; AR 35 °C for 6 weeks; winter	In progress		
	Hydrogen Peroxide; AR 35 °C for 6 weeks; GA (1000 mg/L) with Smoke Water (33 % (v/v) for 48 h; winter	In progress		
	Hydrogen Peroxide; Wet/dry 35 °C for 6 weeks; winter	In progress		
	Hydrogen Peroxide; Wet/dry 35 °C for 6 weeks; GA (1000 mg/L) with Smoke Water (33 % (v/v) for 48 h; winter	In progress		
	Hydrogen Peroxide; AR summer for 8 weeks; winter	In progress		
	Hydrogen Peroxide; AR summer for 8 weeks; GA (1000 mg/L) with Smoke Water (33 % (v/v)) for 48 h; winter	In progress		
	Hydrogen Peroxide; AR summer for 8 weeks; spring/autumn	In progress		
	Hydrogen Peroxide; AR summer for 8 weeks; GA (1000 mg/L) with Smoke Water (33 % (v/v)) for 48 h; spring/autumn	In progress		
	Hydrogen Peroxide; AR summer for	In progress		

Species	Treatment	Germination % (Average)	T_0	T_{50}
	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	In progress		

Species	Treatment	Germination % (Average)	T_0	T_{50}
Astroloma humifusum	Hydrogen Peroxide; winter	In progress		
	Hydrogen Peroxide; AR 35 °C for 6 weeks; winter	In progress		
	Hydrogen Peroxide; AR 35 °C for 6 weeks; GA (1000 mg/L) with Smoke Water (33 % (v/v)) for 48 h; winter	In progress		
	Hydrogen Peroxide; Wet/dry 35 °C for 6 weeks; winter	In progress		
	Hydrogen Peroxide; Wet/dry 35 °C for 6 weeks; GA (1000 mg/L) with Smoke Water (33 % (v/v)) for 48 h; winter	In progress		
	Hydrogen Peroxide; AR summer for 8 weeks; winter	In progress		
	Hydrogen Peroxide; AR summer for 8 weeks; GA (1000 mg/L) with Smoke Water (33 % (v/v)) for 48 h; winter	In progress		
	Hydrogen Peroxide; AR summer for 8 weeks; spring/autumn	In progress		
	Hydrogen Peroxide; AR summer for 8 weeks; GA (1000 mg/L) with Smoke Water (33 % (v/v)) for 48 h; spring/autumn	In progress		
	Hydrogen Peroxide; AR summer for 8 weeks; STRAT spring/autumn 10 weeks; winter	In progress		
	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	In progress		

Species	Treatment	Germination % (Average)	T_0	T_{50}
Calytrix tetragona	Control; spring/autumn	In progress		
	GA (250 mg/L) for 24 h; spring/autumn	In progress		
	GA (250 mg/L) with Smoke Water (10 % (v/v)) for 24 h ; spring/autumn	In progress		
	Dry heat (90 °C for 15 min); GA (250 mg/L) with Smoke Water (10% (v/v)) for 24 h; spring/autumn	In progress		
Exocarpos sparteus	Leaching (48 h); winter	In progress		
	GA (400 mg/L) for 48 h; winter	In progress		
	GA (400 mg/L) with Smoke Water (20% (v/v)) for 48 h; winter	In progress		
	Dry heat (120 °C) for 4 min; GA (400 mg/L) with Smoke Water (20% (v/v)) for 48 h; winter	In progress		
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 (15mins); GA (400mg/L) for 48 h; AR 20 °C for 3 weeks; winter	32	49	N/A
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

Species	Treatment	Germination % (Average)	T_0	T_{50}
Lasiopetalum baueri	Nicked seed coat; winter	**100**	7	7
Leucopogon parviflorus	Fruit removed; Control; sown 14/3/13	9	91	N/A
	Fruit removed; Smoke Water; sown 14/2/13	8		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
Lomandra densiflora	Control; 15 °C constant temperature 12 h photoperiod	16	61	N/A
	GA (1000 mg/L) for 24 h; 15 °C constant 12 h photoperiod	**32**	61	N/A
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

Species	Treatment	Germination % (Average)	T_0	T_{50}
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
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
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
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
Nitraria billardierei	Leaching (15 days); spring/autumn	28	25	N/A
	Leaching (15 days); dry heat (120 °C) for 2mins; spring/autumn	16	8	N/A
	Leaching (15 days); GA (1000 mg/L) for 72 h; spring/autumn	38	8	N/A
	Leaching (15 days); Dry heat (120 °C) for 2mins; GA (1000 mg/L) for 72 h; spring/autumn	**48**	8	N/A

Species	Treatment	Germination % (Average)	T_0	T_{50}
Spyridium fontis-woodsii	Wet heat (95 °C) for 30 sec; spring/autumn	**70**	25	53
Spyridium subochreatum	Control; spring/autumn	0	N/A	N/A
	Nicked seed coat; spring/autumn	4	7	N/A
	Wet heat (100 °C) for 30 sec; spring/autumn	0	N/A	N/A
Tecticornia indica ssp. leiostachya	Control; spring/autumn	**72**	4	18
	GA (250mg/L) continuous; spring/autumn	70	11	18
Thomasia petalocalyx	Nicked seed coat; winter	**94**	14	14

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

The results from the 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.

Interpretation of germination results

Table 4 summarises the most effective treatments used in the germination experiments and general advice regarding problems with propagation of each species.

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 results. Germinate seeds in warmer seasons.	Germination increased with application of GA and warmer temperatures, spring/autumn and summer.
Astroloma conostephioides	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. Tests are on-going for this species.	Germination rates have been low, however, this species responded well to propagation through cuttings.
Astroloma humifusum	Tests are on-going for this species.	Germination rates have been low, however, this species responded well to propagation through cuttings.
Calytrix tetragona	Tests are on-going for this species.	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	Tests are on-going for this species.	Viability is a likely issue for this species. Collect shiny brown nuts that are filled with endosperm, check by cutting open some seeds. Good viable seed was 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 were observed in the control test incubated in a winter environment.	Using a hand lens, check that seeds are present in the capsules during collection.
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.
Leucopogon parviflorus	Treat fruits with GA and use in combination with diluted smoke water and/or dry heat for 2 min for increased germination results.	Viability likely to be an issue for this species. Collect large white fruits and check that seeds are filled.
Lomandra densiflora	Tests are on-going for this species.	Be sure to collect ripe seed when fruits have begun to split open. Immature seed will not germinate.
Lomandra effusa	No treatment is required for this species, high levels of germination were observed in the control test	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
	incubated at 15 ˚C. Treatment with GA will increase germination levels.	
Lomandra muliflora ssp dura	No treatment is required for this species, high levels of germination were observed in the control test incubated at 15 ˚C.	Be sure to collect ripe seed when fruits have begun to split open. Immature seed will not germinate.
Lomandra leucocephala	Tests are on-going for this species.	Be sure to collect ripe seed when fruits have begun to split open. Immature seed will not germinate.
Lomandra juncea	Tests are on-going for this species.	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 were observed in the control test incubated in a spring/autumn environment.	Collect yellow fruits containing black seeds. Remove fruit flesh by wetting and rubbing through a sieve before storage.
Nitraria billardierei	Soak seeds in water for approx. 2 weeks changing the water frequently. To increase germination levels further treat with GA and heat shock seeds when dry for ~2 mins.	Germination levels were increased
Spyridium fontis-woodsii	Break physical dormancy with hot water treatment before sowing.	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	Tests are on-going for this species.	These seeds have combinational dormancy. The seed coat needs to be disrupted to over-come physical dormancy and other treatments are required to alleviate physiological dormancy.
Tecticornia indica ssp. leiostachya	No treatment is required for this species, high levels of germination 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.

Discussion

The results from the project so far indicate that many of the target species are able to be propagated 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 Project.

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 was light with good drainage and 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.

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.