Report for Hillgrove Resources Ltd

Germination Research on Selected Taxa for the Kanmantoo Restoration Technology Project

Progress Report August 2015

South Australian Seed Conservation Centre

Botanic Gardens of South Australia

Summary

The aim of the project is to assist the rehabilitation of indigenous plant communities and the revegetation of disturbed areas according to the Kanmantoo Copper Mines Environmental Management Program.

Seed from 19 seed collections obtained from EBS Ecology were screened for viability and seed purity. The results showed that 18 of the seed collections tested had high viability (>50%) determined by x-ray imaging and cut testing. Germination experiments are in progress for all of these species; these results as well as seedling photos, will be presented in the next report.

Successful germination protocols for *Lomandra effusa* have been developed and germinating seeds were used to trial a method for growing seedlings for restoration at Hillgrove Kanmantoo Copper Mine. Using this method total of 400 seedlings have been successfully grown at the Botanic Gardens of South Australia Mt Lofty nursery. Further seed germination is underway to bulk up the seedling numbers to 1,500 by next year. *Themeda triandra* seedlings are also being grown at Mt Lofty Nursery. Further experiments were conducted on the germination of *Lomandra effusa* as well as L. *multiflora* ssp. *dura* and *L. densiflora* as they also occur in *Eucalyptus odorata* woodland. It was found that germination in *L. densiflora* and *L. multiflora* ssp. *dura* increased after treatment with smoke water. Wetting and drying treatments designed to mimic intermittent summer rainfall had a positive effect on the germination of all the species tested.

Soil samples taken from different depths at the Hillgrove site were tested for seedling emergence in the Adelaide Botanic Garden Nursery. The results from three sites are shown and it was found that 79 % of nondormant seeds germinated from the top 50 mm of soil. This information will assist management decisions regarding weed seed deposits and their possible removal from the site.

Information from several species that occur in vegetation communities that will be restored at the site has been loaded onto the Seeds of South Australia website (http://saseedbank.com.au/). We aim to add the data from this project to the website after pressing and lodging a specimen of each species in the Sate Herbarium. This website will be a useful reference for the rehabilitation team at the Kanmantoo site as well as a way to share information with the wider community.

Introduction

Lomandra effusa is a component of five of the vegetation communities recorded from the Kanmantoo Copper Mine site (EBS Ecology survey); including the threatened ecological communities, *Eucalyptus odorata* woodland and *Lomandra effusa* grassland. The propagation of *Lomandra* species for large scale restoration presents some difficulty as vegetative propagation can be unreliable and seed germination is often reported to be slow and/or sporadic. However, the most cost effective method of producing *Lomandra effusa* seedlings is through seed germination which would provide the best outcome for maintaining genetic diversity and ensure long term sustainability of the population on the rehabilitation sites. One of the initial aims of this project is to experiment with different approaches to propagate *Lomandra* species from the site, including novel germination techniques and traditional nursery methods.

Another aim of the project is to assist the success of restoration by assessing seed quality, and identifying dormancy mechanisms in seed collections. These experiments are still in progress and not all of the results are available to date. Seed collected from 21 species were obtained from EBS and have been assessed to determine the purity of the seed collection and the viability of the seed. This study will deliver information about the condition of the seeds held in storage that will be used for restoration and provide feedback for seed collectors in the future. Seeds from the collections provided last year were sown into pots and the seedlings were photographed to document the appearance of each species as the adult leaves emerged. These images will be useful for monitoring revegetation after direct seeding at the Hillgrove site.

Soil samples were collected from an area of the Hillgrove Kanmantoo mine site that will be scraped to remove the soil weed seed bank before seeding and planting the area with local vegetation. The aim of this study was to determine how the soil seeds bank is fractionated through the top 100 mm of soil. Soil seed banks have been investigated in various habitats and seed densities are reported to be high beneath disturbed areas and arable fields (Leck et al, 1989). The distribution of seeds in the soil profile will depend on the seed size and shape as well as the soil structure and particle size. Wind, water, mechanical disturbance (digging or ploughing), animal foraging and insect activity can also effect seed dispersal and thereby influence the structure of the soil seed bank profile.

It has generally been reported that most of the seeds in the soil seed bank in arable grasslands occur within the top few cm of soil. Weed seeds measured in a no tillage system in Wisconsin found that 60% of seeds were in the top 1 cm of soil and decreased logarithmically to a depth of 19 cm (Yenish et al, 1992). Another study

from a Mediterranean grassland found that 98.9% of viable weed seeds were situated in the top centimetre of soil with a significant fall in the number of seeds as the depth increased (Traba et al, 2004).

Information about species from the *Eucalyptus odorata* grassy woodland and Iron-grass grassland communities that are the focus of the restoration program at the Kanmantoo site is currently being compiled into the Seeds of South Australia website (saseedbank.com.au). Information about seed collecting, cleaning and germination is presented on this site as well as detailed photographs of plants and seeds. Information sheets for 12 species have been attached to the end of this report.

Materials and Methods

Seed Collection

Seed collections of *Lomandra effusa* were made from Hill (Hillgrove Kanmantoo site) and Frahn's scrub. Two seed collections were made at different times from Frahn's Scrub to compare the effect of collection time on maturity and seed viability. Seeds from *Lomandra densiflora* from Frahn's Scrub and *Lomandra multiflora* ssp *dura* from Finniss Oval were also collected for testing as they form part of the *Eucalyptus odorata* woodland vegetation community. The collections used for testing are listed below:

Collections of *Lomandra effusa*

Hartley - 15.12.2011 (collection from Phil Druce)

Frahn's Scrub – 20.12.2012

Hillgrove Hill - 22.11.2013

Frahn's Scrub – 23.10.2013

Frahn's Scrub – 22.11.2013

Frahn's Scrub – 21.11.2014

Lomandra densiflora

Frahn's scrub - 02.12.2013

Lomandra multiflora* ssp *dura

Finniss Oval - 08.01.2014

Seed collections from other species were carried out by EBS from the seed orchard area at Kanmantoo or from nearby areas of remnant Grassy Peppermint Box woodlands or *Lomandra effusa* tussock lands was carried out by EBS.

Seed Cleaning and Quantification

Seed batches were initially weighed and then the amount of seed in the sample was estimated after cleaning the seed and comparing the weight of pure seed to the whole sample weight. Seed cleaning was done using a combination of sieving and aspiration to remove twigs and other plant material from the collections. Alternatively, when cleaning to pure seed was difficult, a purity test was performed where 1g of the sample was weighed out and the seed in that subsample was picked out and weighed to determine the percent purity

of the seed batch. The weight of one seed was quantified by weighing five replicates of 20 seeds to determine the average weight per seed. The following formula was used to calculate the number viable seeds per kilogram of seed sample:

(1000/weight of 1 seed (g)) x (% viability/100) x (%purity/100) = number of viable seeds/kg

Seed Viability Testing

Seed viability was tested using the following methods.

X-ray: Seeds were x-rayed using a Faxitron X-ray MX-20 Specimen Radiography System. Up to 50 seeds were aligned onto an adhesive strip to capture an x-ray image. The images of the seeds were scored as viable where the seed appeared to be filled. X-ray is a non-destructive test that can assess seed fill for large numbers of seeds in a seed lot.

Cut Testing: Twenty seeds were dissected with a scalpel and aligned with adhesive and photographed under a dissecting microscope fitted with a camera. Seeds containing full white or cream endosperm and whole embryos were scored as viable. Cut testing was used to confirm the results of the x-ray.

Seedling Photos

Seeds were sown directly into potting soil and grown outside on benches under daily irrigation. Photos were taken as the seedlings developed to show the morphology of the young plant, typically when the first few adult leaves had opened.

Germination Screening

Lomandra seeds were put through a range of germination tests to assess germination capacity for different seed collections. The treatments used are described in Table 1.

The experiment plates were set up as follows:

A total of 50 seeds were used for each treatment. After treatment the seeds were placed onto moist sterile sand in Petri dishes and sealed with a thin strip of cling wrap. Plates were incubated at 15˚C with a 12 hour photoperiod. The plates were scored on a weekly basis for up to 16 weeks. Germination was scored when the radicle had grown to at least half the length of the seed coat, and the germinated seeds were removed after scoring.

Table 1. List of treatments used for germination experiments and further information about the rationale for using each treatment.

Treatment	**Method**	**Rationale**
Control	No treatments were applied to seeds before plating.	The control shows the germination response of untreated seeds.
Gibberellic Acid (GA)	Seeds soaked in a solution of GA dissolved in water. GA concentration ranged from 250 to 1000 mg/L and soaking duration ranged from 24 h to 72 h.	GA is a plant hormone that has many roles in plant growth and development. GA is used to alleviate physiological dormancy and promote germination in seeds.
Hydrogen Peroxide	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.
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 to 10% (v/v) before treating seeds.	Chemicals present in smoke have been shown to trigger germination in some species that are fire responsive.
After Ripening	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.

Stratification	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.
Wet/Dry Cycling	Seeds were placed in Petri dishes with sterile sand at 15˚C or 30˚C constant temperature. During incubation Petri dishes were wet on a twice weekly basis for 5 hours then allowed to dry out.	Wetting and drying simulates the effect of intermittent rainfall on the soil seed bank environment. Episodes of rainfall will cause the seeds to undergo several wetting and drying cycles before germination.
Constant Temperature 15˚C Incubator	Incubator set at 15˚C with a 12 h photoperiod.	Used as an alternative to diurnal cycling, underdeveloped embryos may grow faster at one optimal temperature. Constant temperatures are more likely to occur in nature when there are periods of constant, dense cloud cover.
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.
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.
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.

Lomandra effusa Planting Trial

The planting trial was set up on a rocky ridge top rising from a cropping paddock along the haul road into Hillgrove Kanmantoo Copper mine. *Lomandra effusa* seeds collected from Frahn's Scrub (22.11.2013) were used for the trial. The seeds were given one of three treatments as indicated below, and then dried and stored at room temperature before planting:

- T1 Control
- T2 GA (1000 mg/L) 24 h
- T3 Smoke Water (10% (v/v)) 24 h

The trial was planted on the 14th of May 2015 into dampened soil (due to morning rains ~1mm). The soil was loosened with a mattock and the top 2-4 cm removed with a shovel to take off the weed layer. A 50 mm square mesh grid was laid onto the soil and 36 seeds were lightly buried (approx. 0.5 cm deep) in a 6 x 6 row column-design as follows:

	A	**B**	**C**	**D**	**E**	**F**
1	T1	T2	T3	T2	T3	T1
2	T3	T1	T2	T1	T2	T3
3	T2	T3	T1	T3	T1	T2
4	T1	T2	T3	T2	T3	T1
5	T3	T1	T2	T1	T2	T3
6	T2	T3	T1	T3	T1	T2

After burial a cage was secured over the top with tent pegs to deter herbivores as shown in Figure 1. This was replicated 8 times along the ridge top, so that 96 seeds from each treatment were planted on the site making a total of 288 seeds planted in the trial. Emerging plants were scored after 10 weeks.

Figure 1. Tools used to set up the *Lomandra effusa* planting trial.

Soil Sampling Experiment

Collection of Soil Samples

Soil samples for Replicate 1 of the sampling experiment were taken on the 2nd of May 2014 from the paddock adjacent to the seed production area that has previously been used for cropping and grazing for many years. Soil samples were taken from 10 sites within the paddock that were selected at random. Soil samples for replicates 2 and 3 were taken on the 12th of March 2015 from two paddocks that also had a history of grazing and cropping. Soil samples were taken from 10 sites selected at random within each paddock. Soil samples were extracted using the equipment shown in Figure 2 A. Firstly the mattock was used to loosen the soil. The spade was used to cut a smooth face into the soil profile Figure 2 B. The metal tube (10 cm diameter) was then hammered into the soil up to 25 mm then the trowel was inserted below the tube which was lifted out and the soil from inside the tube was placed into a zip-lock bag. The metal tube was replaced and hammered in another 25 mm to take the second sample. This was repeated until four samples had been removed from the site, each one from an increased depth. The samples corresponded to the first 0-25 mm of the soil profile, then 25-50 mm, 50-75 mm, 75-100 mm and 100-125 mm.

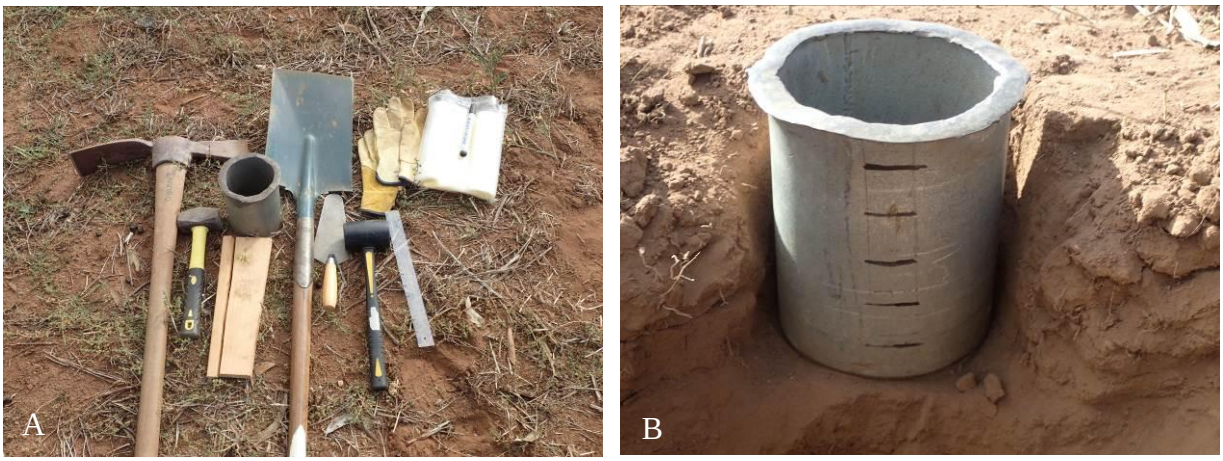


Figure 2. Soil sample collection. A) Tools used for sampling; B) Soil profile to 150 mm.

Sample Preparation

Soil samples were stored in a dry potting shed in for eleven days after collection. Samples were then processed at the Adelaide Botanic Gardens (ABG) Nursery. A sample of 150 g was weighed out from each bag and spread onto a tray containing wet, heat sterilized (autoclaved) sand as shown in Figure 3. The sample size allowed a thin layer (~ 5 mm) of soil to be spread over the damp sand. The utensils were cleaned after weighing each sample to avoid contamination between samples. The trays were placed in the glass house under irrigation (misted daily for 10 mins). Emerging plants were scored on a weekly basis, with monocots and dicots counted separately. Control trays contained sand only.

Figure 3. Sample preparation at the ABG nursery.

Results and Discussion

Testing Seed Viability: EBS Collections Year 3

Seed batches from 19 species provided by EBS for year 3 of this project were cleaned and quantified to determine the amount of pure seed (% purity) in the collections and the estimated number of viable seeds per kilogram using the methods described. Seed viability was investigated using x-ray imaging and cut testing and the results are shown in Table 2. The x-ray image shows whether the seeds are full which indicates that seed is viable. The x-ray technique is useful for detecting nonviable seed that is underdeveloped or predated. A sample of seeds that appeared to be viable and nonviable from the x-ray screen were also cut test to verify the x-ray result. Examples of viable and nonviable whole seeds and cut seeds are shown in Appendix 1. The x-ray images used to determine seed viability have been compiled and are shown in Appendix 2.

The majority of the seed collections had a high percentage of viable seeds. One exception was *Olearia pannosa* that had 24% viability. However, collections with low viability are not uncommon for this species as they can be prone to predation. From twelve collections made by the seed centre the seed viability was low to average, ranging from 15% to 70%. Viable seeds should be fat, hard and a dark red-brown colour (see photos shown on www.saseedbank.com.au).

The collection of *Themeda triandra* (EBSKAN118) had a marked improvement in seed viability (84%) compared to the previous collections (EBSKAN90 and EBSKAN38) that were analysed in previous years (28% and 18% respectively). This is likely to be due to the timing of collection when, the seeds were mature. Although the purity of the sample was low at 3% the seed that was collected had good viability. Similarly both the Eucalyptus species had low % purity which is unavoidable for most Eucalypts as they release chaff and seeds that are similar in size from the capsules.

Table 2. Species list showing viability results, % purity of the bulk seed mix and estimated number of viable seeds per kilogram of bulk seed mix.

#	Batch No	Species	% Viability	% Purity	# Viable seeds/kg
1	EBSKAN 21	*Acacia paradoxa*	98	100	71,594
2	EBSKAN 20	*Acacia pycnantha*	96	100	46,692
3	EBSKAN 133	*Atriplex semibaccata*	80	75	142,500
4	EBSKAN 113	*Austrodanthonia sp*	78	100	1,003,860
5	EBSKAN 112	*Austrostipa nodosa*	100	100	500,500
6	EBSKAN 132	*Cullen australasicum*	98	100	15,659
7	EBSKAN 110	*Eucalyptus calycogona*	98	6	14,984
8	EBSKAN 95	*Eucalyptus phenax ssp phenax*	94	14	6,509
9	EBSKAN 96	*Eucalyptus socialis*	90	3	922,131
10	EBSKAN 131	*Eutaxia microphylla*	72	100	11,472
11	EBSKAN 115	*Goodenia pinnatifida*	94	100	183,163
12	EBSKAN 126	*Hardenbergia violacea*	96	100	47,434
13	EBSKAN 128	*Helichrysum leucopsideum*	56	100	32,902
14	EBSKAN 122	*Kennedia prostrata*	100	100	11,052
15	EBSKAN 127	*Lotus australis*	100	100	313,971
16	EBSKAN 124	*Olearia pannosa* (ssp *pannosa*)	24	100	10,253
17	EBSKAN 125	*Podolepis rugata*	62	100	72,009
18	EBSKAN 118	*Themeda triandra*	84	3	125,466
19	EBSKAN 114	*Vittadinia blackii*	86	100	81,362

Seedling Photos

The images of young seedlings grown from species provided in year 1 and 2 are shown in Appendix 3. The images show the young leaves emerging and will be useful for monitoring seedling emergence after direct seeding. Most of the species have distinctive features at an early stage. However the grass seedlings look very similar at this stage. Seeds have been sown for this species set and photos will be included in the final report.

Germination testing

Initial germination testing of *Lomandra effusa*

Initial tests were done with *Lomandra effusa* seed collected from Hartley (15th December 2011). The experiments included a range of temperatures and treatments, and the results after 11 weeks are summarised in Table 3. A combination of cool germination temperatures and gibberellic acid appeared to be an effective treatment for stimulating germination. The highest germination level (70%) was recorded from a treatment that was soaking in water for 24 h, followed by soaking in gibberellic acid (1000 mg/L) and incubating at static temperature 15°C. The leaching treatment reduced the amount of mould associated with the seed surface during incubation on the agar plates.

Table 3. Summary of results from the initial experiment.

Treatment	**% Germination**
winter	18
spring	3
24 h soak in GA (250mg/L) winter	48
24 h soak in GA (250mg/L) spring	0
24 h soak in 10% smoked water soak and GA (250mg/L), winter incubator	50
Heat shock 90°C 15 mins, 24 h soak in 10% smoked water and GA (250mg/L), winter incubator	3
24 h soak in GA (250mg/L) 15°C constant	30
24 h soak in GA (1000mg/L) 15°C constant	70

Subsequently, a second experiment was set up to test three seed collections using cool conditions and gibberellic acid, the results of this experiment are shown in Table 4.

Table 4. Results from the second experiment comparing germination from three seed collections with and without gibberellic acid in winter conditions.

Seed collection	Treatment	% Germination
Hartley 15.12.2011	Winter incubator	22
	24 h soak in GA (250mg/L), winter incubator	42
Kanmantoo 13.11.2012	Winter incubator	0
	24 h soak in GA (250mg/L), winter incubator	0
Frahns 20.12.2012	Winter incubator	28
	24 h soak in GA (250mg/L), winter incubator	50

There appeared to be a viability issue was with the seed collection from Kanmantoo as no germinating seeds were observed. These seeds were firm and filled, yet they appeared paler than the collections from Hartley and Frahns. Close examination of the seeds showed that the hilum and the micropyle had not fully developed on the seeds from Kanmantoo (Figure 4). The Kanmantoo collection was made in November and it appeared likely that the seeds were harvested before they were fully ripe and this affected their ability to germinate. This information is very important for seed collectors, the seeds do not appear to mature post collection and so must be harvested when they have fully ripened.

Figure 4 Close up of *Lomandra effusa* seeds collected from Frahns Scrub 20th December 2012 (top) and Kanmantoo 13th November 2012 (bottom). Arrows indicate the hilum and micropyle, both were well developed in the seeds collected from Frahn's Scrub in December. Scale bars = 1mm.

Another experiment was set up to test the effect of gibberellic acid on the germination of *Lomandra effusa* seeds from three collections (Table 5). No seeds germinated from the first collection from Frahn's scrub, made on the 23rd of October 2013. However, seeds collected a month later on the 22nd of November had high levels of germination in the control (62%) and after treatment with gibberellic acid (74%). Images of the collections from Frahn's scrub and Hillgrove in 2013 are shown in Figure 5, the differences in the colour of the seeds and the dark hilum can be observed with the naked eye.

The germination results confirmed that *Lomandra effusa* seeds collected before maturity are not viable and demonstrate the importance of collecting fully mature *Lomandra* seed.

Table 5. Summary of Results for *Lomandra effusa* seeds from different collections treated with water or gibberellic acid (1000 mg/L).

Collection	Treatment	Germination (%)
Frahn's scrub 23.10.13	24 h soak in water	0
	24 h soak in GA (1000mg/L)	0
Frahn's scrub 22.11.13	24 h soak in water	62
	24 h soak in GA (1000mg/L)	74
Hillgrove 22.11.13	24 h soak in water	42
	24 h soak in GA (1000mg/L)	56

Figure 5. Seeds of *Lomandra effusa* collected from Frahn's scrub (A and B) and Hillgrove (C).

The importance of collecting mature *Lomandra effusa* seed.

The difference in seed maturity between two collections of *Lomandra effusa* is shown in Figure 4. Mature *Lomandra* seeds have the key features of having an all over darker colour which can range from light brown to grey, whilst immature seeds are a pale, creamy colour. The immature seeds have been shown not to germinate and are highly susceptible to fungal infection, as is often the case with nonviable seed. Mature seeds are capable of germination and have a darkened hilum, and a conspicuous micropyle. Since the differences in the colour of the seeds and the dark hilum can be observed with the naked eye, it is recommended that seeds be assessed with a visual check on collection. The capsules should be darkened and

have started to split with evidence of mature seed inside. *Lomandra effusa* is likely to be mature in late November to late December in areas near the Hillgrove site, depending on seasonal conditions.

Germination of different *Lomandra* species occurring in Grassy Peppermint Box Woodland in response to gibberellic acid, smoke water and wet/dry cycling.

This experiment was designed to test the effect of different treatments on the germination of different species of *Lomandra*. The effects of soaking in gibberellic acid (GA) or smoke water (SW) were assessed along with dry after ripening at 15˚C and 30˚C and cold or warm stratification with wet/dry cycling are shown in Figure 6. The cycling treatments were applied to simulate the wetting and drying effects of intermittent rainfall. The seed collections used for this experiment are listed in Table 6 below.

Table 6. Provenance and seed collection times for different *Lomandra* species.

Species	**Collection Site**	**Collection Date**
Lomandra effusa	Frahn's scrub	20.12.2012
Lomandra effusa	Frahn's scrub	21.11.2014
Lomandra densiflora	Frahn's scrub	02.12.2013
Lomandra multiflora ssp *dura*	Finnis Oval	02.01.2014

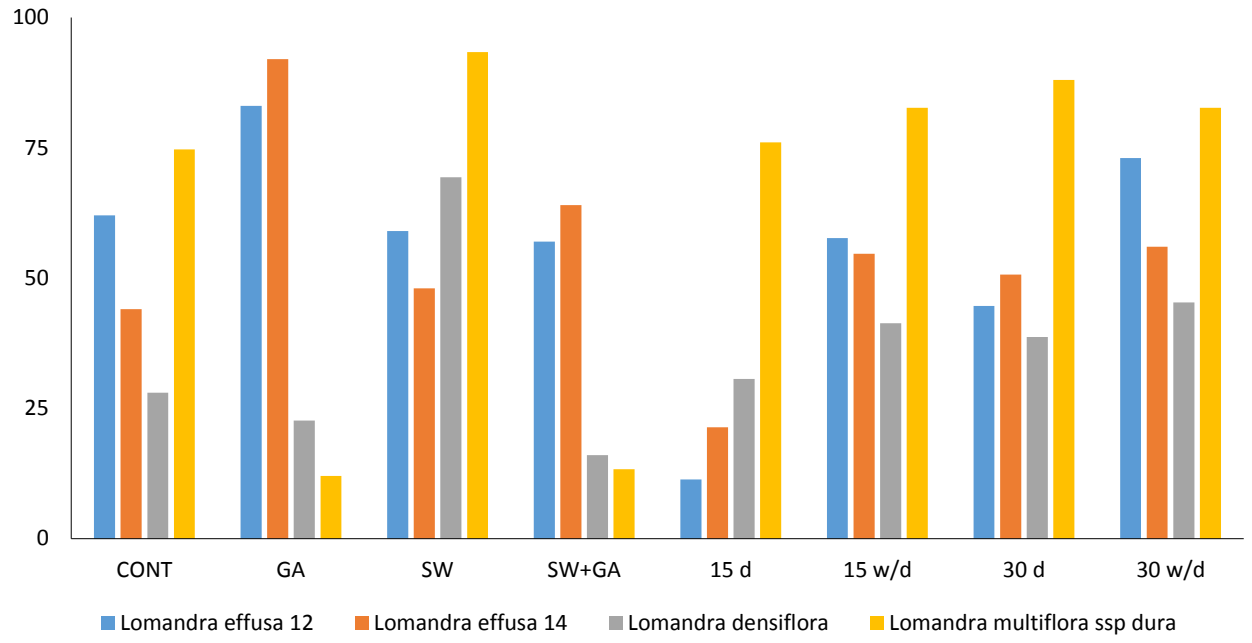


Figure 6. *Lomandra* seeds from different species were treated for 72 h with solutions of water (CONT); gibberellic acid (1000 mg/L) (GA); smoke water 10% (v/v) (SW); or a combination of GA and SW. Other treatments were incubated for 6 weeks at 15°C in dry sand (15 d); 15°C in dry sand that was wet once a week then allowed to dry (15 w/d); 30°C in dry sand (30 d) and 30°C in dry sand that was wet once a week then allowed to dry (30 w/d).

The species with the highest germination results across the range of treatments was *Lomandra multiflora* ssp *dura*. These seeds appeared to be mostly nondormant as the 75 % germinated in the control treatment. The high concentration of GA (1000 mg/L) appeared to inhibit germination in *Lomandra densiflora* and *Lomandra multiflora* ssp *dura*, however, for both these species germination was enhanced by treatment with smoke water. In contrast, *Lomandra effusa* seeds from two collections had increased germination levels after treatment with GA. The wet/dry cycling treatments at 30°C enhanced the germination above the levels in the control for all of the species tested. This treatment was designed to mimic the wetting and drying at warm temperatures that the seeds would experience over summer once they were released from the parent plant and subjected to summer rains interspersed with dry periods. Cycling between dry after ripening and warm stratification had a positive effect on germination.

The *Lomandra effusa* seeds collected in 2012 had higher levels of germination in the control treatment than the collection in 2014, which suggests that after ripening may play a role in alleviating dormancy for this species.

Lomandra effusa Preliminary Nursery Trial for dividing mature plants

Seven plants of varying sizes of *Lomandra effusa* were dug up from the Kanmantoo Copper mine site (June 2013) from an area marked for clearance to grow in pots for testing methods of vegetative propagation. These were placed into pots and are being kept in the Mount Lofty Nursery and all had survived transplanting after 6 months.

The plants have now been growing in pots for just over a year and five of them are still in a healthy condition. However, division has not been attempted as there is no sign of new growth emerging to date. These plants will continue to be monitored for new growth over the coming months.

Provision of *Lomandra effusa* seedlings

Germinating seeds of *Lomandra effusa* have been planted into small biodegradable pots and then transferred to tube-stock pots in the nursery at the Mount Lofty Botanic Gardens. Initial trials have found the method to be successful and 400 healthy seedlings are currently growing that will be available for planting at the Hillgrove Kanmantoo site. Seed was sourced from Hillgrove and from the nearby (approx. 6 km) Frahn's scrub. Large scale seed germination has now begun in order to provide 1,500 seedlings in tube stock for restoration of the Hillgrove Kanmantoo Coppermine Site. Viable *Themeda triandra* seeds have been sorted from the collection provided by EBS and will also be propagated in the nursery.

Lomandra effusa On-site Planting Trial

Positive early results were obtained from the planting trial at the Hillgrove Kanmantoo site. Emerging seedlings were scored 10 weeks after planting and 9 seedlings were observed starting to protrude from the soil. Five had been treated with smoke water, two with GA and two had no treatment. The emerging seedlings will continue to be scored on a monthly basis to determine whether this method would be useful for revegetation of *Lomandra effusa*.

Soil Sampling and Seedling Emergence

Soil was taken from up to 4 different depths from three padocks: paddock 1 in 2014 and 5 depths in paddock 2 and 3 in 2015. A total of thirty samples were taken as 10 replicates were sampled from each paddock. The soil samples were then spread out into trays that were lightly irrigated in the glasshouse at the Botanic Gardens of Adelaide.

Seedlings were first observed within the first week after spreading out the soil samples into seedling trays. In general, the monocotyledon species were the first to emerge. Seedling emergence had peaked by four weeks and only approximately 2% of new germinants were observed in the last two weeks of scoring. Seedling trays and emerging seedlings are shown in Figure 7. By definition, seeds that are nondormant germinate within 30 days but seedlings from dormant seeds may take longer to emerge (Baskin and Baskin, 2004). These seeds may require stratification at warm or cool temperatures to break dormancy.

Table 7 shows the number of seedlings that emerged from the soil samples after six weeks. Of the total number of seedlings 79 % emerged from the top two sampling depths, indicating that a large portion of the soil seed bank resides within the top 50 mm of soil. Figure 8 shows a graph of the total number of seedlings per replicate site at each depth that was sampled. A total of 462 monocotyledon seedlings were recorded and all of these appeared to be from the Gramineae family. Surprisingly, the total number of dicotyledon seedlings was also 462, indicating that overall, equal numbers of dicots and monocots were observed. However, there is likely to be a number of dormant seeds that have not germinated during the time frame of this experiment.

Table 7. Number of seedlings emerged from soil samples. 10 samples were taken from each of three paddocks. The three replicates shown are from the 3 paddocks sampled in 2014 (rep1) and 2015 (reps 2&3). The number of monocotyledon, dicotyledon and total seedlings are shown from each replicate (ns – not sampled).

Monocot seedlings	**Rep 1**	**Rep 2**	**Rep 3**
Depth	#	#	#
0-25 mm	110	169	8
25-50 mm	44	56	6
50-75 mm	26	14	6
75-100 mm	9	7	2
100-125	ns	1	4

Dicot seedlings	**Rep 1**	**Rep 2**	**Rep 3**
Depth	#	#	#
0-25 mm	39	119	66
25-50 mm	21	55	38
50-75 mm	16	23	27
75-100 mm	10	15	17
100-125	ns	6	10

Total seedlings	**Rep 1**	**Rep 2**	**Rep 3**
Depth	#	#	#
0-25 mm	150	288	74
25-50 mm	65	111	44
50-75 mm	42	37	33
75-100 mm	19	22	19
100-125	ns	7	14

Figure 7. A) Soil samples spread out into seedling trays and placed in the glass house under irrigation. B) Seedlings emerging from soil samples taken from different depths. Soil from two replicates are shown (front and back) at different depths shown from right to left 0-25 mm , 25-50 mm, 50-75 mm, 75-100 mm.

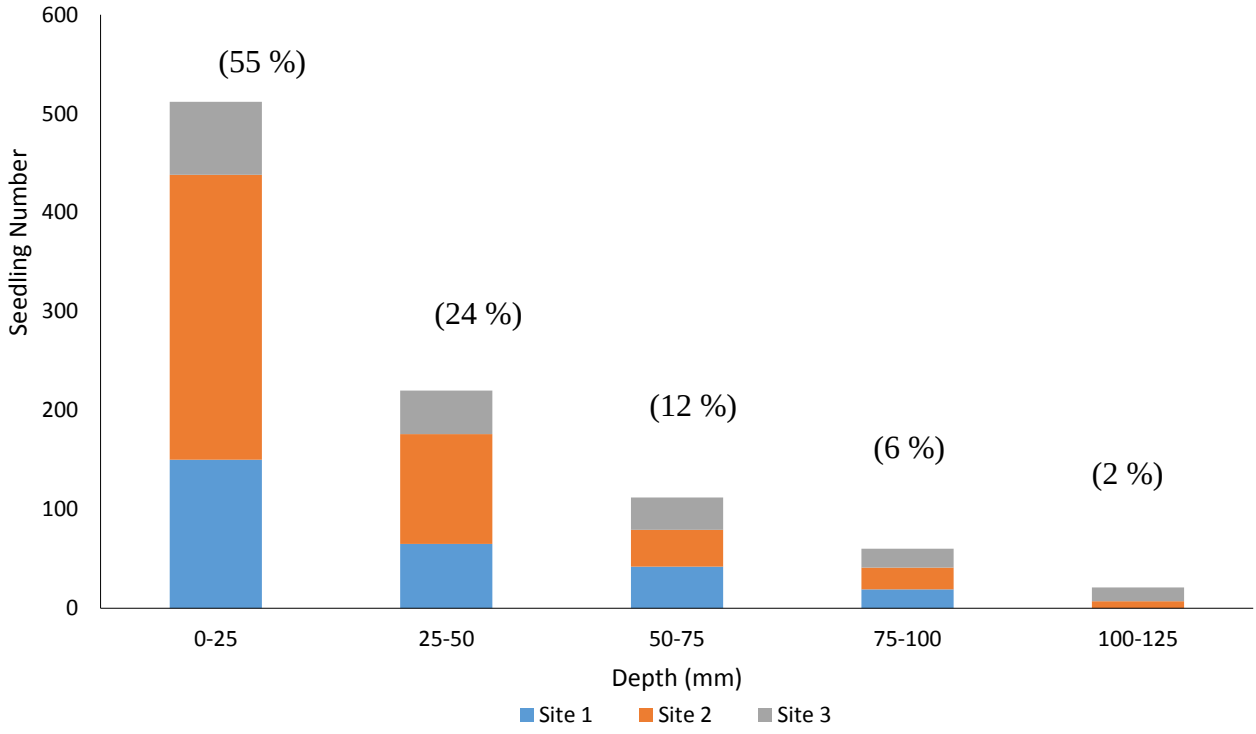


Figure 8. Total number of seedlings observed from 3 sample sites that emerged from different soil depths. The percent of the total seedlings that emerged is shown in parenthesis for each depth.

Seeds of South Australia

Several of the species from the plant communities at Hillgrove have been loaded onto the Seeds of South Australia website (saseedbank.com.au) and printed versions have been compiled in Appendix 3. We aim to load more of these species on the website and will add the information from the germination experiments from this project. Since we have obtained and lodged a specimen in the State Herbarium for all of the plant species entered on the website we will do the same for the plants that relate to the EBS seed collections before entering the data.

References

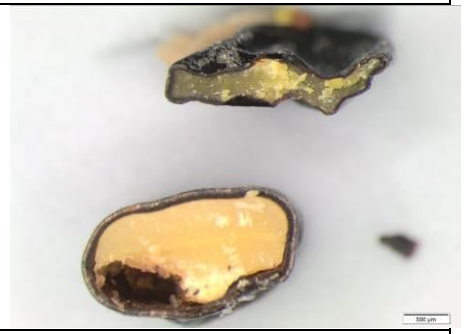
Leck MA, Parker VT, Simpson RL. (1989) Ecology of soil seed banks. Academic Press, London.

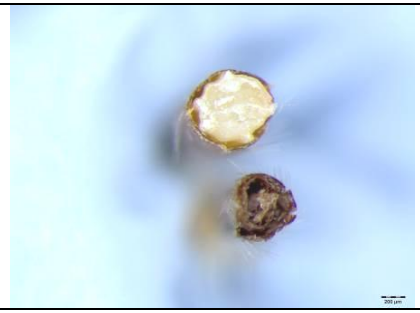
Baskin, J. and Baskin, C. (2004) A classification system for seed dormancy. *Seed Science Research* 14, 1-16.

Traba J, Azcárate FM, Peco B. (2004) From what depth do seeds emerge? A soil seed bank experiment with Mediterranean grassland species. *Seed Science Research* 14:297-303.

Yenish JP, Doll JD, Buhler DD. (1992) Effects of tillage on vertical distribution and viability of weed seed in soil. *Weed Science* 40: 429-433.

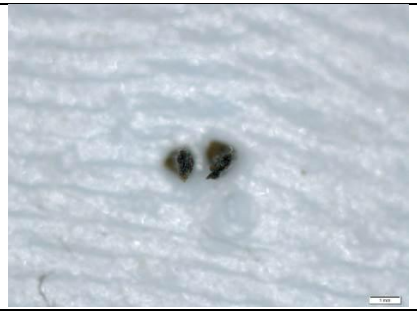
Appendix 1 Images of viable and nonviable seeds for the species provided in year 3.

Viable Seed	Nonviable Seed	Viable seed - cross section	Nonviable seed – cross section
Acacia paradoxa EBSKAN21			
			
Acacia pycnantha EBSKAN20			
			
Atriplex semibaccata EBSKAN133			
			

Viable Seed	Nonviable Seed	Viable seed - cross section	Nonviable seed – cross section
Austrodanthonia sp EBSKAN113			
			
Austrostipa nodosa EBSKAN112			
			
Cullen australasicum EBSKAN132			
			

Viable Seed	Nonviable Seed	Viable seed - cross section	Nonviable seed – cross section
Eucalyptus calycogona EBSKAN110			
			
Eucalyptus phenax ssp. phenax EBSKAN95			
			
Eucalyptus socialis EBSKAN96			
			

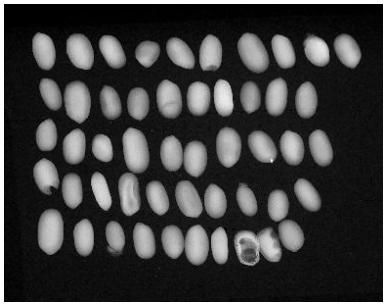
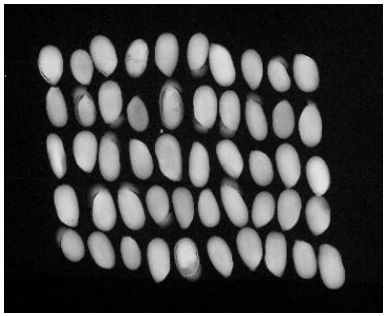
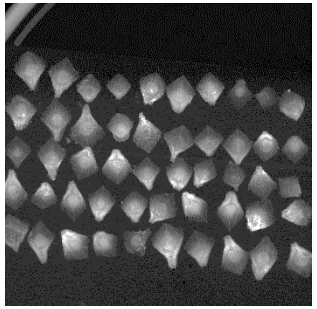
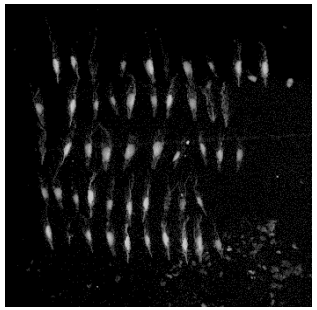
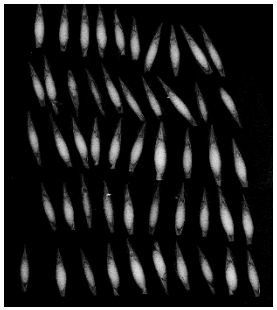
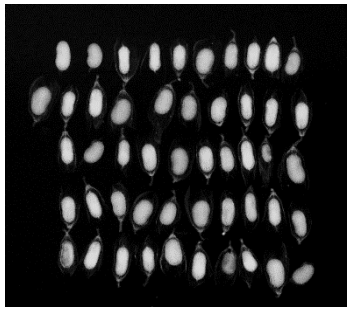
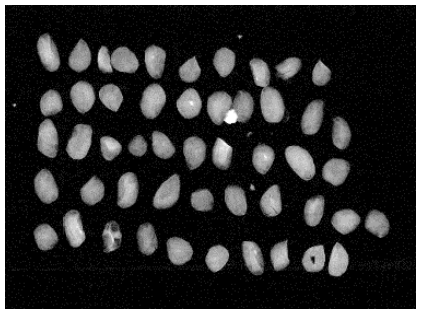
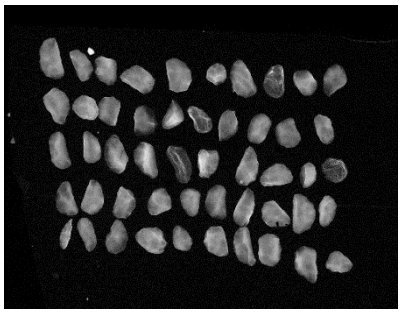
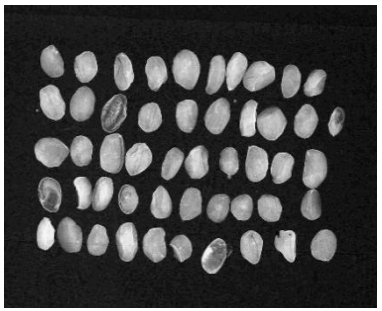
Viable Seed	Nonviable Seed	Viable seed - cross section	Nonviable seed – cross section
Eutaxia microphylla EBSKAN131			
			
Goodenia pinnatifida EBSKAN115			
			
Hardenbergia violacea EBSKAN126			
			

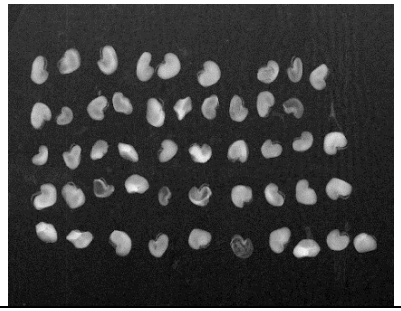
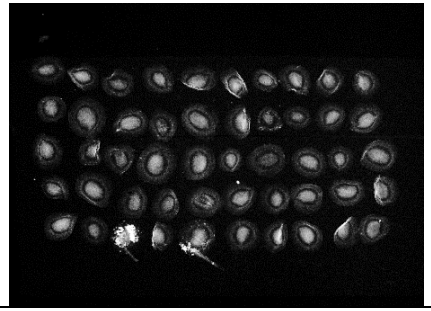
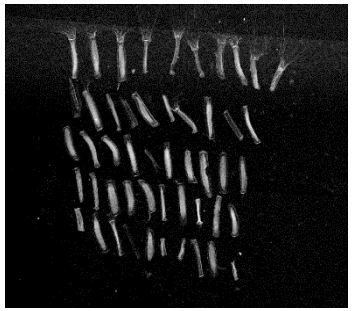
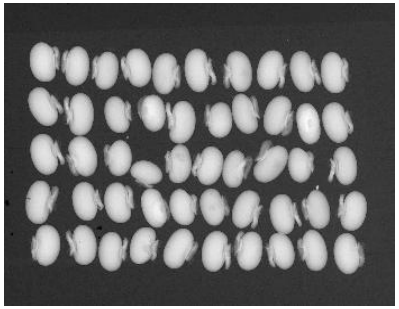
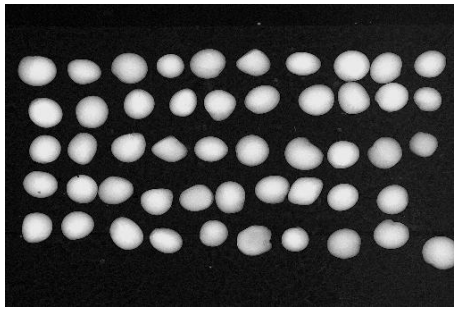
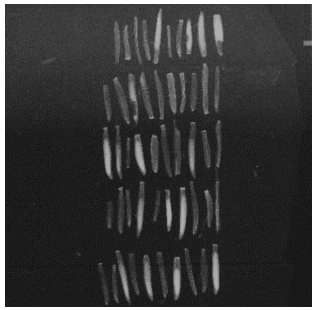
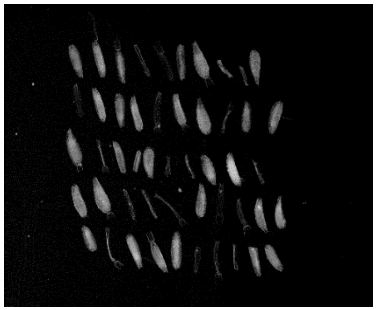
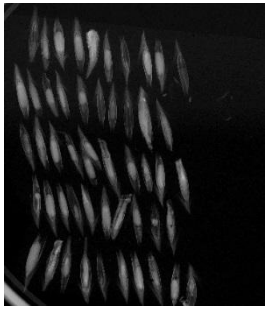
Viable Seed	Nonviable Seed	Viable seed - cross section	Nonviable seed – cross section
Helichrysum leucopsideum EBSKAN128			
			
Kennedia prostrata EBSKAN122			
			
Lotus australis EBSKAN127			
			

Viable Seed	Nonviable Seed	Viable seed - cross section	Nonviable seed – cross section
Olearia pannosa EBSKAN124			
			
Podolepis rugata EBSKAN125			
			
Themeda triandra EBSKAN118			
			

Viable Seed	Nonviable Seed	Viable seed - cross section	Nonviable seed – cross section
Vittadinia blackii EBSKAN114			
			

Appendix 2 X-ray images of seeds provided in year 3.

Acacia paradoxa EK21 (98%) 	*Acacia pycnantha* EK20 (96%) 	*Atriplex semibaccata* EK113 (80%) 
Austrodanthonia sp EK113 (78%) 	*Austrostipa nodosa* EK112 (100%) 	*Cullen australasicum* EK132 (98%) 
Eucalyptus calycogona EK110 (98%) 	*Eucalyptus phenax ssp. phenax* EK95  (94%)	*Eucalyptus socialis* EK96 (90%) 

Eutaxia microphylla EK131 (72%) 	*Goodenia pinnatifida* EK115 (94%) 	*Hardenbergia violacea* EK126 (96%) 
Helichrysum leucopsideum EK128 (56%) 	*Kennedia prostrata* EK122 (100%) 	*Lotus australis* EK127 (100%) 
Olearia pannosa EK124 (24%) 	*Podolepis rugata* EK125 (62%) 	*Themeda triandra* EK118 (84%) 

Vittadinia blackii EK114 (86%)

Appendix 3. Images of seedlings grown from seeds provided in years 1 and 2.

Acacia pycnantha

Allocasuarina verticilliata

Anthosachne scabra

Aristida behriana

Arthropodium strictum

Atriplex semibaccata

Austrodanthonia sp

Austrostipa blackii

Austrostipa elegantissima

Austrostipa nodosa 	*Bursaria spinosa* 	*Callitris gracilis* 
Chrysocephalum apiculatum 	*Clematis microphylla* 	*Convolvulus remotus* 
Dodonaea viscosa 	*Einadia nutans* 	*Enchylaena tomentosa* 

Goodenia pinnatifida	*Ptilotus spathulatus*	*Velleia paradoxa*
		
Vittadinia blackii	*Vittadinia cuneata*	*Vitadinnia megacephala*