Report for Hillgrove Resources Ltd

Seed Biology Research

Kanmantoo Restoration Project

Final Report 2016

South Australian Seed Conservation Centre

Government of South Australia

Botanic Gardens of SOUTH AUSTRALIA

Summary

The aim of this project was 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 61 seed collections from 44 species obtained from EBS Ecology were screened for viability and seed purity. Test results showed that the seed quality was generally high with 41 collections having greater than 75% viable seed. Six of the samples had below 30% viability. A total of 52 seed collections representing 44 species were screened for germination response to different treatments. Nine of the species had physical dormancy and high levels of germination were achieved after the seed coat was nicked. Twenty seven species were found to be nondormant and five had some level of physiological dormancy.

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 a total of 1000 seedlings have been successfully grown at the Botanic Gardens Mt Lofty nursery. A total of 1,500 *Themeda triandra* seedlings were also grown at the Mt Lofty Nursery to use at the mine site. 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/). 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.

Contents

Introduction

Project Objectives

- Screen seed collected from plant species grown in the seed production area, or from wild populations near the Kanmantoo site to determine the viability of the seed. These results will determine the condition of seed to be used for revegetataion and provide images of viable and nonviable seed as a reference tool when assessing other seed collections.
- Conduct a germination screen of the species to identify the conditions that favour germination. These results will provide useful information for preparing sowing mixtures and will indicate the best seasons for seed dispersal. Compilation of photographs of young seedlings to be used as a reference for monitoring seedling emergence in revegetation sites.
- To assess the effect of different treatments on the germination of *Lomandra effusa* seeds to gain a better understanding of the seed biology in order to assist propagation of seedlings for revegetation.
- Examine the quantity of seeds in the soil at different depths in the soil profile from pasture paddocks at the Kanmantoo site.

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 is not routine 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, and would also provide the best outcome for maintaining genetic diversity and ensure long-term sustainability of the revegetated sites.

The practise of soil scraping has recently been used as an effective way of removing weed seed and nutrient load from sites with a history of pasture and cropping. 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). 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.

Information about species from the Kanmantoo site has been compiled into the Seeds of South Australia website (saseedbank.com.au).

Materials and Methods

Seed Collection

Seed collections of *Lomandra effusa* were made from Nugent's Hill (Hillgrove Kanmantoo site) and Frahns scrub. Two seed collections were made at different times from Frahns Scrub to compare the effect of collection time on maturity and seed viability. Seeds from *Lomandra densiflora* from Frahns 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)

Frahns Scrub – 20.12.2012

Nugent's Hill - 22.11.2013

Frahns Scrub – 23.10.2013

Frahns Scrub – 22.11.2013

Frahns Scrub – 21.11.2014

December 2015 seed was not collected due to low seed set at Frahns Scrub and Nugent's Hill sites.

Lomandra densiflora

Frahns 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.

Species List and plant name changes

Notes about plant names used in this report.

- The genus *Austrodanthonia* is now named *Rytidosperma* in the SA Plant Census
- *Callitris preisii* ssp *verrucosa* has been changed to *Callitris verrucosa* in the SA Plant Census
- *Velleia paradoxa* is likely to be *Velleia arguta* as this is more commonly observed in the Frahns Scrub area (D. Duval pers. com.)
- *Elymus scaber has been changed to Anthosachne scabra* in the SA Plant Census

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 per cent 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

A range of experiments were set up to assess the germination capacity of seeds collected from different plant species. The treatments used are described in Table 1.

The experiment plates were set up as follows:

Germination of Lomandra

A total of 50 seeds were used for each treatment. After treatment the seeds were placed onto 1% agar or moist sterile sand in Petri dishes and sealed with a thin strip of cling wrap. Plates were incubated at various temperatures and photoperiods maintained in incubators. 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 germinated seeds were removed after scoring.

Germination of other species

Seeds collected by EBS were tested with a routine germination screen. A total of 50 seeds were used for treatment for 24h with or without GA before seeds were placed onto 1% agar and incubated in temperature controlled incubators programmed to mimic summer, winter and spring/autumn conditions. The plates were scored on a weekly basis for up to 10 weeks. Germination was scored when the radicle had grown to at least half the length of the seed coat, and 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	Wetting and drying simulates the effect of intermittent rainfall on the soil seed bank environment. Episodes

	dishes were wet on a twice weekly basis for 5 hours then allowed to dry out.	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 Frahns 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.

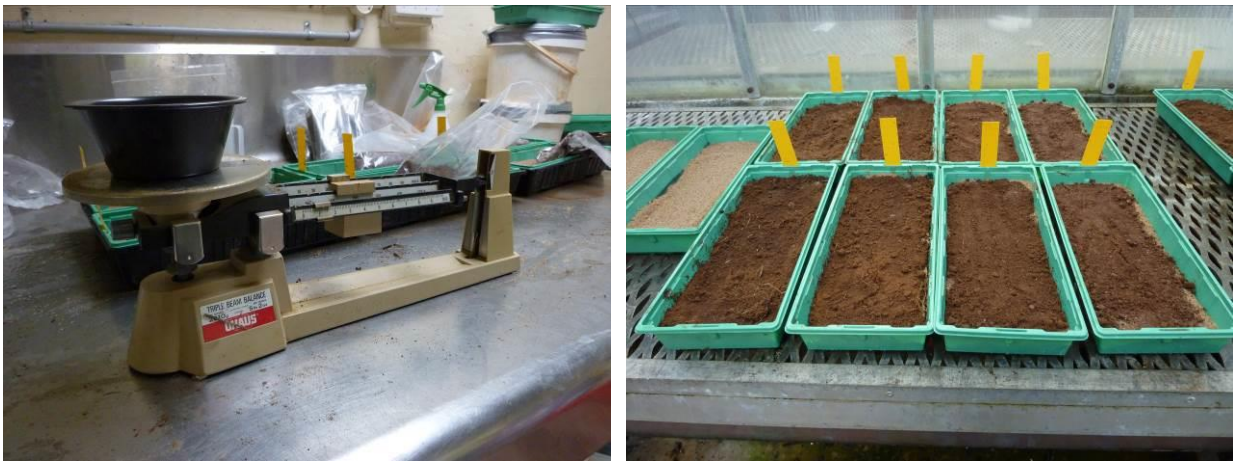

Figure 3. Sample preparation at the ABG nursery.

Results and Discussion

Testing Seed Collections

Seed Viability

A total of 61 seed batches collected from 44 species were provided by EBS during this project from 2013 to 2015. The seeds 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 filled with endosperm/embryo, indicating 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, with 41 having viability estimated at 75% or greater.

Six samples were estimated to have below 30% viable seeds, these were;

- *Aristida behriana* EBSKAN87 (10%)
- *Atriplex semibaccata* EBSKAN107 (14%)
- *Olearia pannosa* EBSKAN124 (24%)
- *Olearia sp* EBSKAN48 (4%)
- *Themeda triandra* EBSKAN90 (28%)
- *Themeda triandra* EBSKAN38 (18%)

However, collections with low viability are not uncommon for some *Olearia* species as they can be poorly developed and prone to predation. From twelve collections of *Olearia pannosa* ssp *pannosa* made by the seed centre the seed viability was low to average, ranging from 15% to 70%. Viable seeds can be assessed on collection and 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 (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 Eucalyptus species 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.

Project Year	Batch No	Species	% Viability	% Purity	# Viable seeds/kg
2015	EBSKAN21	*Acacia paradoxa*	98	100	71,594
2015	EBSKAN20	*Acacia pycnantha*	96	100	46,692
2014	EBSKAN58	*Acacia pycnantha*	80	91	30,834
2014	EBSKAN77	*Allocasuarina verticillata*	70	100	157,303
2014	EBSKAN98	*Anthosachne scabra*	80	1	1,361
2014	EBSKAN87	*Aristida behriana*	10	71	26,782
2013	EBSKAN37	*Aristida behriana*	74	65	172,589
2014	EBSKAN50	*Arthropodium strictum*	48	86	216,019
2014	EBSKAN133	*Atriplex semibaccata*	80	75	142,500
2015	EBSKAN107	*Atriplex semibaccata*	14	48	16,478
2014	EBSKAN80	*Atriplex semibaccata*	82	75	168,614
2015	EBSKAN113	*Austrodanthonia sp*	78	100	1,003,860
2014	EBSKAN91	*Austrodanthonia sp*	94	80	8,439,955
2013	EBSKAN36	*Austrodanthonia sp*	66	63	454,632
2013	EBSKAN39	*Austrostipa elegantissima*	78	26	143,879
2015	EBSKAN112	*Austrostipa nodosa*	100	100	500,500
2013	EBSKAN16	*Austrostipa nodosa*	82	16	145,778
2013	EBSKAN19	*Austrostipa sp*	56	27	52,857
2013	EBSKAN34	*Austrostipa sp*	68	30	88,696
2013	EBSKAN44	*Austrostipa sp*	80	69	107,873
2014	EBSKAN105	*Bursaria spinosa*	94	89	470,326
2013	EBSKAN68	*Bursaria spinosa*	96	93	507,236
2014	EBSKAN101	*Callitris gracilis*	42	91	28,264
2013	EBSKAN23	*Callitris preissii ssp verrucosa*	30	48	8,761
2014	EBSKAN104	*Chloris truncata*	46	79	1,559,657
2014	EBSKAN75	*Chrysocephalum apiculatum*	92	26	3,967,099

Project Year	Batch No	Species	% Viability	% Purity	# Viable seeds/kg
2013	EBSKAN43	*Clematis microphylla*	100	47	111,747
2014	EBSKAN102	*Convolvulus remotus*	94	100	61,486
2015	EBSKAN132	*Cullen australasicum*	98	100	15,659
2014	EBSKAN97	*Dodonaea viscosa*	100	99	102,659
2013	EBSKAN67	*Einadia nutans*	96	59	472,000
2014	EBSKAN103	*Enchylaena tomentosa*	40	66	46,739
2013	EBSKAN70	*Enchylaena tomentosa*	75	95	39,880
2015	EBSKAN110	*Eucalyptus calycogona*	98	6	14,984
2014	EBSKAN94	*Eucalyptus odorata*	100	25	1,452,861
2015	EBSKAN95	*Eucalyptus phenax ssp phenax*	94	14	6,509
2015	EBSKAN96	*Eucalyptus socialis*	90	3	922,131
2015	EBSKAN131	*Eutaxia microphylla*	72	100	11,472
2014	EBSKAN81	*Gonocarpus tetragynus*	40	60	518,432
2015	EBSKAN115	*Goodenia pinnatifida*	94	100	183,163
2014	EBSKAN83	*Goodenia pinnatifida*	90	57	210,428
2013	EBSKAN61	*Goodenia pinnatifida*	80	95	260,136
2015	EBSKAN126	*Hardenbergia violacea*	96	100	47,434
2015	EBSKAN128	*Helichrysum leucopsideum*	56	100	32,902
2015	EBSKAN122	*Kennedia prostrata*	100	100	11,052
2015	EBSKAN127	*Lotus australis*	100	100	313,971
2014	EBSKAN71	*Maireana brevifolia*	82	100	638,104
2015	EBSKAN124	*Olearia pannosa (ssp pannosa)*	24	100	10,253
2013	EBSKAN48	*Olearia sp*	4	93	47,874
2015	EBSKAN125	*Podolepis rugata*	62	100	72,009
2013	EBSKAN35	*Ptilotus spathulatus*	100	7	67,075
2015	EBSKAN118	*Themeda triandra*	84	3	125,466
2014	EBSKAN90	*Themeda triandra*	28	15	11,976
2013	EBSKAN38	*Themeda triandra*	18	16	10,256
2013	EBSKAN28	*Velleia paradoxa* (*arguta*)	95	85	164,082

Project Year	Batch No	Species	% Viability	% Purity	# Viable seeds/kg
2015	EBSKAN114	*Vittadinia blackii*	86	100	81,362
2013	EBSKAN29	*Vittadinia blackii*	98	27	264,600
2013	EBSKAN27	*Vittadinia cuneata*	88	93	741,578
2014	EBSKAN106	*Vittadinia sp mix*	100	60	727,393
2013	EBSKAN10	*Vittadinnia megacephala*	75	98	359,574
2014	EBSKAN86	*Wahlenbergia stricta*	90	100	58,427,577

Germination testing

A total of 52 seed batches collected from 41 different species were tested to assess germination response to different temperatures and the presence of the plant hormone gibberellic acid. Table 3 shows the results of seeds that were untreated or treated with GA (250 mg/L) for 24 h before incubation under conditions similar to winter, spring/autumn and summer (as described in Table 1). Germination graphs and explanatory comments about dormancy are shown in full in Appendix 3. Over half of the species (27 out of 41) fell into the nondormant category where seeds did not require any treatment to achieve a high level of germination.

Physiological dormancy was indicated in four of the species; *Austrostipa nodosa* EBSKAN16, *Austrostipa sp* EBSKAN119, *Maireana brevifolia* EBSKAN71 and *Podolepis rugata* EBSKAN125. Grass species may have an after ripening requirement before germination can occur (Adkins et al, 2002) that prevents germination during the warm seasons. It would be interesting to know the storage conditions for the EBSKAN 16 (year 1) and EBSKAN112 (year 3) collections of *Austrstipa nodosa* as the germination levels averaged approximately 45% and 80% respectively.

Germination levels for untreated *Maireana brevifolia* EBSKAN71 seeds averaged 18% and increased to a mean of 37% after treatment with GA. In the Chenopod family there can be dormancy imposed by the seed covering structures, in this case the perianth segments. We have found that germination increases in some *Maireana* species when these structures are removed.

The germination level for untreated *Podolepis rugata* EBSKAN125 seeds averaged 37%, and increased to 78% after treatment with GA. This result indicates that dormancy was alleviated after treatment with the plant hormone.

Physical dormancy is caused by a water-impermeable seed or fruit coat and has been found in members of 15 families of angiosperms (Baskin et al. 2000), including Leguminosae and Convolvulaceae. In general,

seeds with physical dormancy will germinate once the impermeable coat is disrupted and water penetrates the seed. There were 9 legume species and one *Convolvulus* species that were tested and all had high germination rates after seed nicking (Table 4). Graphs are shown in Appendix 3.

Table 3. Results of germination experiments on seed collections. Seeds were treated with GA (250 mg/L) before incubation under conditions similar to winter (W), spring/autumn (Sp) or summer (Su). (-) indicates not tested.

EBS#	Species	W	W GA	Sp	Sp GA	Su	Su GA
EBSKAN 77	*Allocasuarina verticillata*	56	-	60	-	62	-
EBSKAN 98	*Anthosachne scabra*	77	66	78	70	58	10
EBSKAN37	*Aristida behriana*	52	68	60	52	62	60
EBSKAN 50	*Arthropodium strictum*	96	96	78	82	4	0
EBSKAN80	*Atriplex semibaccata*	68	90	88	70	68	80
EBSKAN133	*Atriplex semibaccata*	78	-	76	-	58	-
EBSKAN36	*Austrodanthonia sp*	85	100	80	95	93	95
EBSKAN113	*Austrodanthonia sp*	66	-	86	-	78	-
EBSKAN39	*Austrostipa elegantissima*	78	72	80	70	54	72
EBSKAN12	*Austrostipa nodosa*	80	-	76	-	84	-
EBSKAN16	*Austrostipa nodosa*	42	42	40	38	40	44
EBSKAN34	*Austrostipa sp*	58	54	54	52	42	56
EBSKAN44	*Austrostipa sp*	90	88	84	100	58	62
EBSKAN19	*Austrostipa sp (blackii)*	14	20	18	22	14	2
EBSKAN68	*Bursaria spinosa*	94	56	86	50	0	2
EBSKAN23	*Callitris preissii ssp verrucosa*	16	10	24	9	0	0
EBSKAN101	*Callitris gracilis*	94	-	86	-	0	-
EBSKAN104	*Chloris truncata*	14	30	66	54	82	96

EBSKAN75	*Chrysocephalum apiculatum*	100	100	84	86	50	19
EBSKAN43	*Clematis microphylla*	94	80	96	80	8	14
EBSKAN67	*Einadia nutans*	98	100	98	100	70	100
EBSKAN70	*Enchylaena tomentosa*	63	51	56	45	9	12
EBSKAN110	*Eucalyptus calycogona*	82	-	76	-	84	-
EBSKAN94	*Eucalyptus odorata*	98	-	76	-	96	-
EBSKAN95	*Eucalyptus phenax ssp phenax*	94	-	94	-	90	-
EBSKAN96	*Eucalyptus socialis*	98	-	100	-	98	-
EBSKAN81	*Gonocarpus tetragynus*	34	48	54	74	42	48
EBSKAN61	*Goodenia pinnatifida*	96	84	84	94	78	80
EBSKAN115	*Goodenia pinnatifida*	92	-	100	-	78	-
EBSKAN128	*Helichrysum leucopsideum*	70	62	50	74	52	34
EBSKAN71	*Maireana brevifolia*	18	36	12	44	24	30
EBSKAN124	*Olearia pannosa* ssp. *pannosa*	6	10	4	2	0	0
EBSKAN48	*Olearia sp*	-	-	0	-	-	-
EBSKAN125	*Podolepis rugata*	12	62	56	86	42	86
EBSKAN35	*Ptilotus spathulatus*	90	-	93	-	100	-
EBSKAN38	*Themeda triandra*	3	3	13	15	15	8
Seed Centre	*Themeda triandra*	24	34	32	42	34	20
EBSKAN28	*Velleia paradoxa (arguta)*	94	100	94	96	18	46
EBSKAN29	*Vittadinia blackii*	78	92	92	94	74	98
EBSKAN114	*Vittadinia blackii*	98	-	86	-	78	-
EBSKAN27	*Vittadinia cuneata*	84	86	76	82	80	80
EBSKAN10	*Vittadinia megacephala*	78	82	76	58	48	56
EBSKAN86	*Wahlenbergia stricta*	76	68	64	12	0	6

Table 4. Seeds with physical dormancy were treated by nicking the seed coat (nick) before incubation under conditions similar to winter (W), spring/autumn (Sp) or summer (Su).

Batch number	Species	W	W nick	Sp	Sp nick	Su	Su nick
EBSKAN21	*Acacia paradoxa*	22	82	28	90	24	22
EBSKAN20	*Acacia pycnantha*	22	98	36	96	12	72
EBSKAN58	*Acacia pycnantha*	21	100	28	100	18	100
EBSKAN102	*Convolvulus remotus*	2	4	2	92	96	98
EBSKAN132	*Cullen australasicum*	14	100	12	100	2	100
EBSKAN97	*Dodonaea viscosa*	2	98	0	96	0	0
EBSKAN131	*Eutaxia micrphylla*	4	88	6	96	0	98
EBSKAN126	*Hardenbergia violacea*	2	100	4	100	0	46
EBSKAN122	*Kennedia prostrata*	2	86	0	94	2	14
EBSKAN127	*Lotus australis*	10	100	14	100	8	90

Seedling Photos

The images of young seedlings grown from species provided are shown in Appendix 4. 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.

Germination of *Lomandra* species.

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 5. 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 after soaking in gibberellic acid (1000 mg/L) and incubating at static temperature 15°C.

Table 5. 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 6.

Table 6. 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 with the seed collection from Kanmantoo as none of the seeds from that collection germinated. These seeds were firm and filled, yet they appeared paler than the collections from Hartley and Frahns Scrub. 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 Frahns 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 7). No seeds germinated from the first collection from Frahns 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 Frahns 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 7. Summary of Results for *Lomandra effusa* seeds from different collections treated with water or gibberellic acid (1000 mg/L).

Collection	Treatment	Germination (%)
Frahns scrub 23.10.13	24 h soak in water	0
	24 h soak in GA (1000mg/L)	0
Frahns 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 Frahns 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 8 below.

Table 8. Provenance and seed collection times for different *Lomandra* species.

Species	**Collection Site**	**Collection Date**
Lomandra effusa	Frahns scrub	20.12.2012
Lomandra effusa	Frahns scrub	21.11.2014
Lomandra densiflora	Frahns scrub	02.12.2013
Lomandra multiflora ssp. *dura*	Finnis Oval	02.01.2014

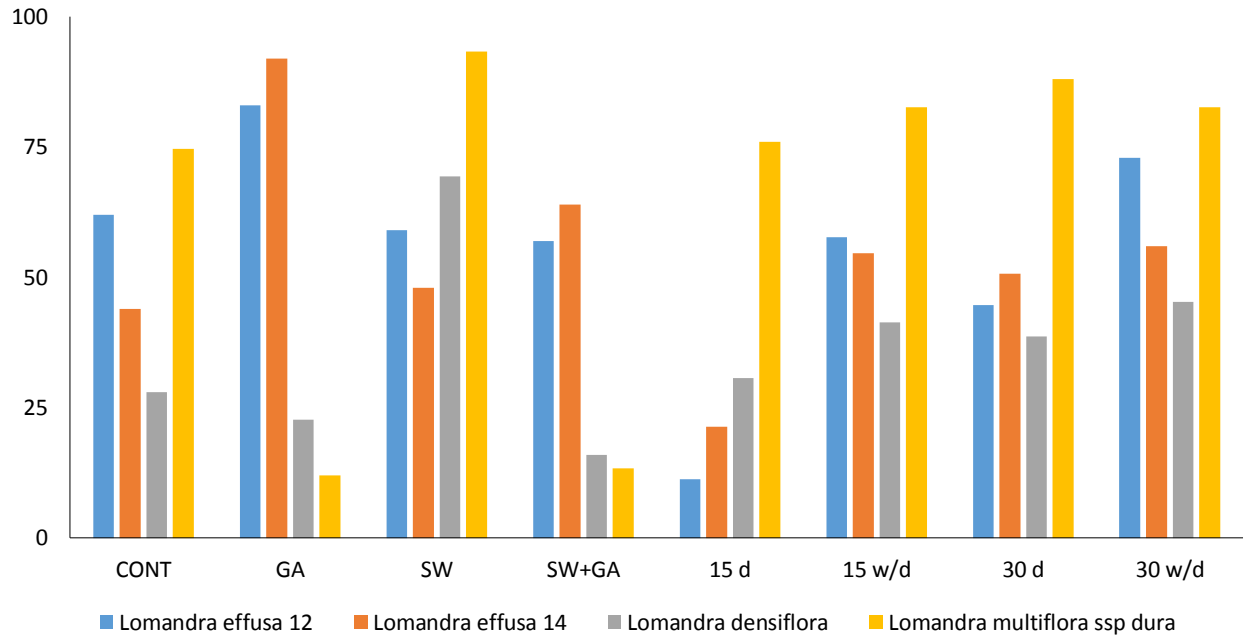

Figure 6. *Lomandra* seeds from different species were treated for 72 h with solutions of water (CONT); gibberellic acid (1000 mg/L) (GA); smoke water 10% (v/v) (SW); or a combination of GA and SW. Other treatments were incubated for 6 weeks at 15°C in dry sand (15 d); 15°C in dry sand that was wet once 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 been growing in pots for two years with five of them still in a healthy condition. However, the plants were not divided as there was no sign of new growth. It was concluded that this method of propagation would be very slow for old *Lomandra effusa* tussocks removed from the Hillgrove site.

Lomandra effusa **seedlings**

Germinating seeds of *Lomandra effusa* were planted into small biodegradable pots and then transferred to tube-stock pots in the nursery at the Mount Lofty Botanic Gardens. Seed was sourced from Hillgrove and from the nearby (approx. 6 km) Frahns Scrub and germinated in incubators in the seed lab and then potted on at the Mt Lofty nursery. Approximately 1,000 seedlings were potted into tube pots for restoration of the Hillgrove Kanmantoo Coppermine Site. Viable *Themeda triandra* seeds were sorted from the collection provided by EBS and 1,500 plants were propagated in the Mt Lofty Nursery to be planted at the Hillgrove site.

Themeda triandra seedlings grown for the project at Mt Lofty Botanic Gardens Nursery.

Lomandra effusa **On-site Planting Trial**

The planting trial at the Hillgrove Kanmantoo site was set up on 14th May 2015. A total of 288 seeds were planted Emerging seedlings were first observed 10 weeks after planting. The number of *Lomandra* seedlings observed in the first 15 weeks after planting are shown in Table 9. On the third visit to score the seedlings (24th of November 2015, ~27 weeks after planting) it was found that the emerging seedlings had all died, presumably due to lack of water. The results showed that several seeds germinated in scraped areas of the paddock after several weeks and that pretreatment with GA did not increase the number of seedlings emerging within the first 15 weeks. However, only approximately 9% of seeds germinated by week 15, it is unknown whether more seedlings would have emerged under irrigation. It would be recommended to commence any further trials earlier in the year and to use irrigation.

Table 9. Number of emerging seedlings in planting trial

Scoring Date	Week	T1 (control)	T2 (GA)	T3 (SW)
23/7/15	10	2	2	5
28/8/15	15	7	7	11

Soil Sampling and Seedling Emergence

Soil was taken from up to 4 different depths from 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 10 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 did not germinate during the time frame of this experiment.

Table 10. 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.

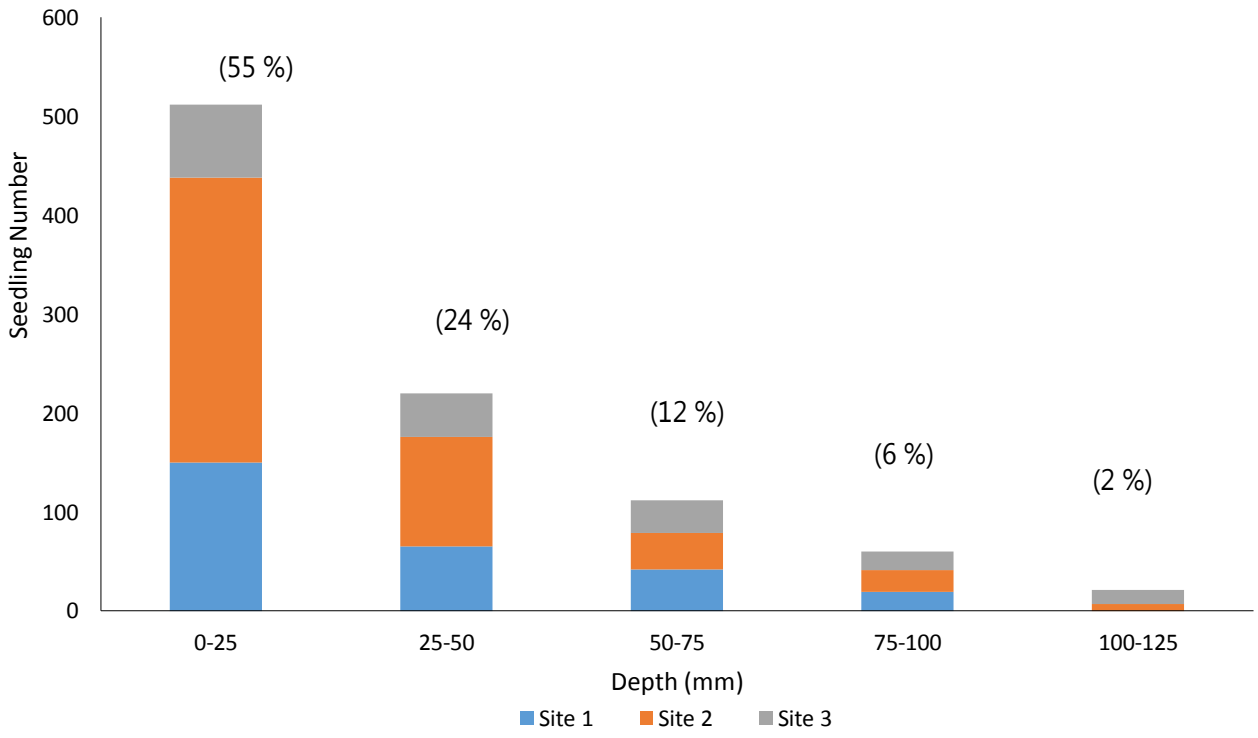

Figure 8. Total number of seedlings observed from 3 sample sites that emerged from different soil depths. The percent of the total seedlings that emerged is shown in parenthesis for each depth.

Seeds of South Australia website

Several of the species from the plant communities at Hillgrove have been loaded onto the Seeds of South Australia website (saseedbank.com.au).

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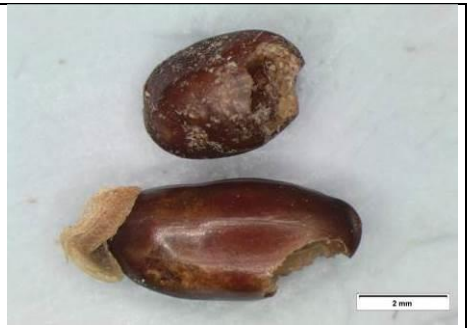
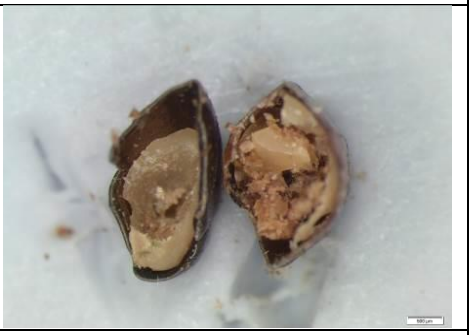
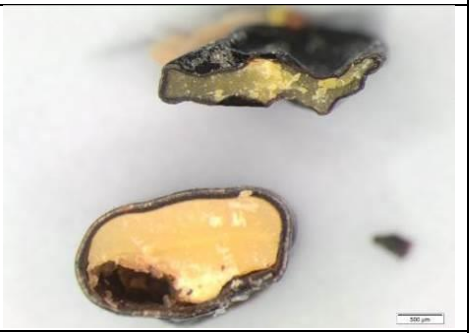
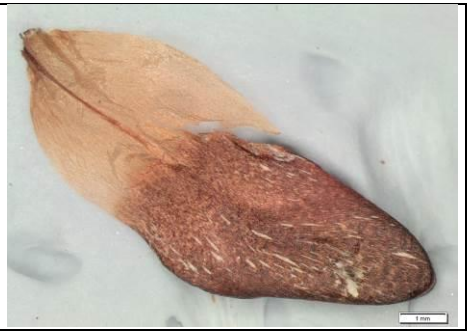
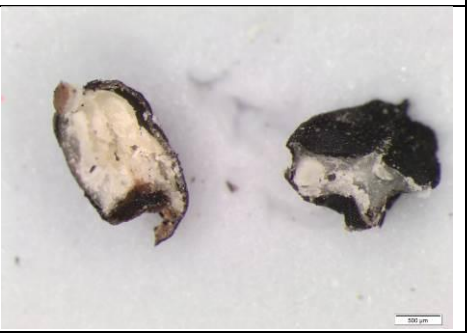
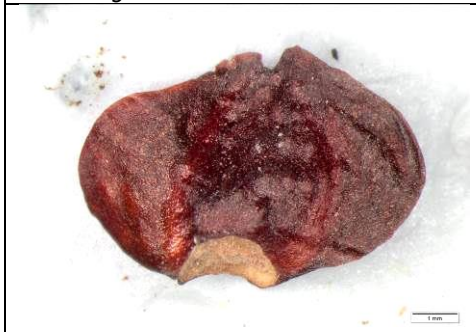
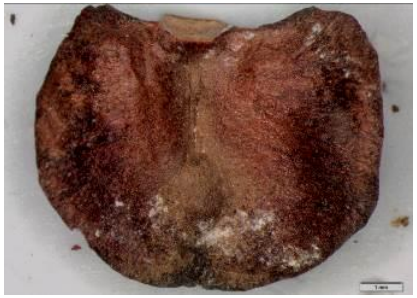
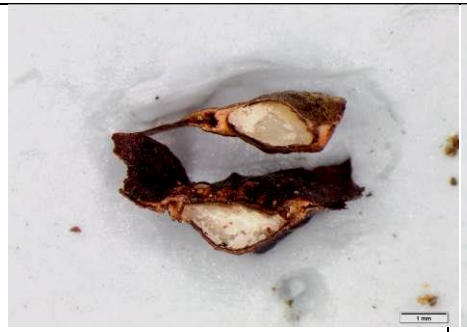
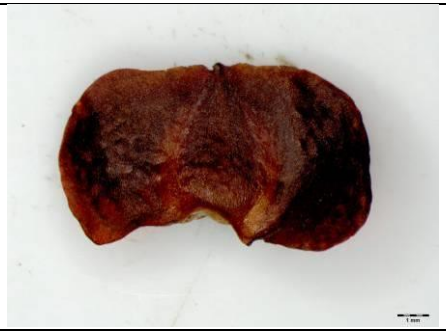
Appendix 1 Images of Viable and Nonviable Seeds

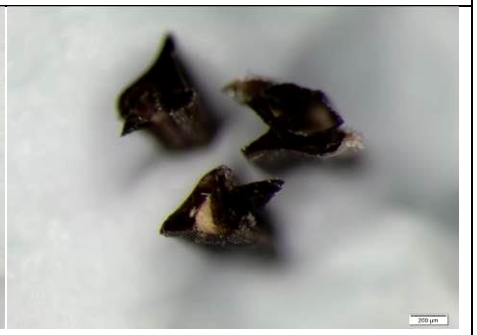
Viable Seed	Nonviable Seed	Viable seed - cross section	Nonviable seed – cross section
Acacia paradoxa EBSKAN21			
			
Acacia pycnantha EBSKAN20			
			
Allocasuarina verticilliata EBSKAN77			
			

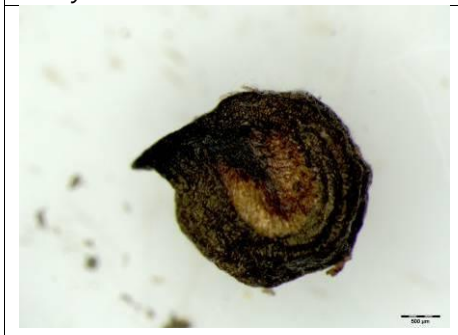
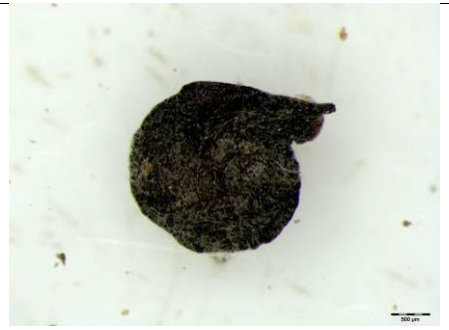
Viable Seed	Nonviable Seed	Viable seed - cross section	Nonviable seed – cross section
Anthosachne scabra EBSKAN98			
			
Aristida behriana EBSKAN37			
			
Arthropodium strictum EBSKAN50			
			

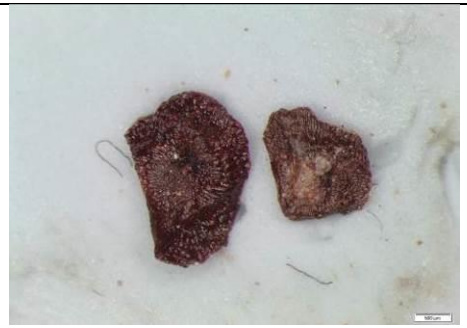
Viable Seed	Nonviable Seed	Viable seed - cross section	Nonviable seed – cross section
Atriplex semibaccata EBSKAN133			
			
Austrodanthonia sp EBSKAN113			
			
Austrostipa elegantissima EBSKAN39			
			

Viable Seed	Nonviable Seed	Viable seed - cross section	Nonviable seed – cross section
Austrostipa nodosa EBSKAN112			
			
Bursaria spinosa EBSKAN68			
			
Callitris gracilis EBSKAN101			
			

Viable Seed	Nonviable Seed	Viable seed - cross section	Nonviable seed – cross section
Callitris preissii ssp *verrucosa* EBSKAN23			
			
Chrysocephalum apiculatum EBSKAN75			
			
Clematis microphylla EBSKAN43			
			

Viable Seed	Nonviable Seed	Viable seed - cross section	Nonviable seed – cross section
Chloris truncata EBSKAN104			
			
Convolvulus remotus EBSKAN102			
			
Cullen australasicum EBSKAN132			
			

Viable Seed	Nonviable Seed	Viable seed - cross section	Nonviable seed – cross section
Dodonaea viscosa			
			
Einadia nutans EBSKAN67			
			
Enchylaena tomentosa EBSKAN70			
			

Viable Seed	Nonviable Seed	Viable seed - cross section	Nonviable seed – cross section
Eucalyptus calycogona EBSKAN110			
			
Eucalyptus odorata EBSKAN94			
			
Eucalyptus phenax ssp. phenax EBSKAN95			
			

Viable Seed	Nonviable Seed	Viable seed - cross section	Nonviable seed – cross section
Eucalyptus socialis EBSKAN96			
			
Eutaxia microphylla EBSKAN131			
			
Gonocarpus tetragynus EBSKAN81			
			

Viable Seed	Nonviable Seed	Viable seed - cross section	Nonviable seed – cross section
Goodenia pinnatifida EBSKAN115			
			
Hardenbergia violacea EBSKAN126			
			
Helichrysum leucopsideum EBSKAN128			
			

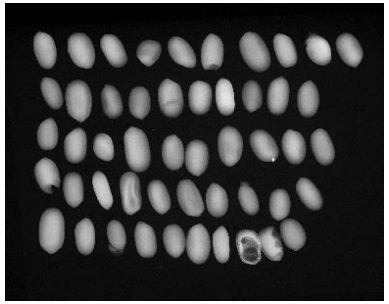
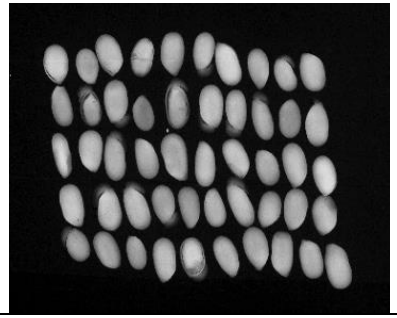
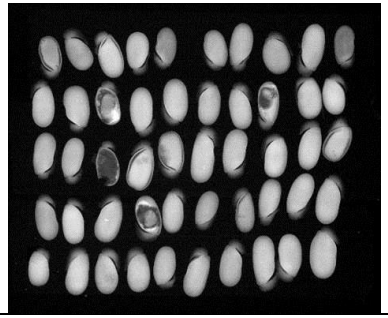
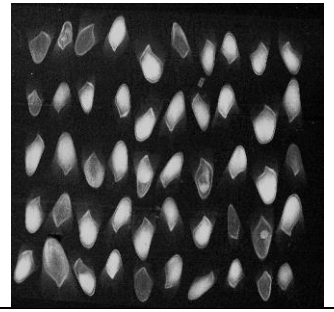
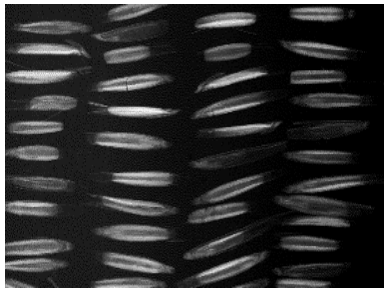
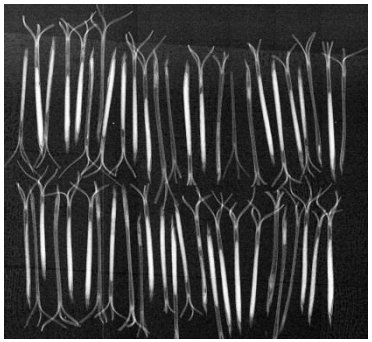
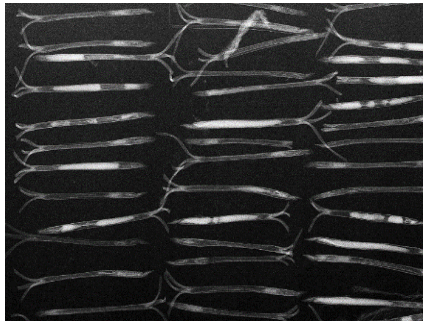
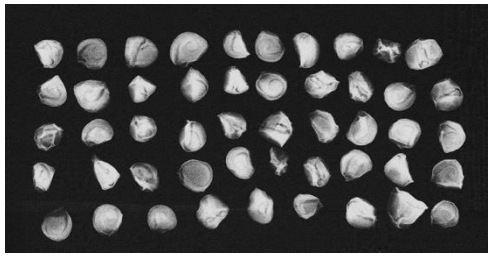
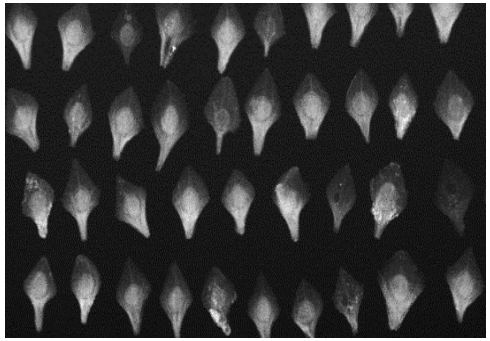
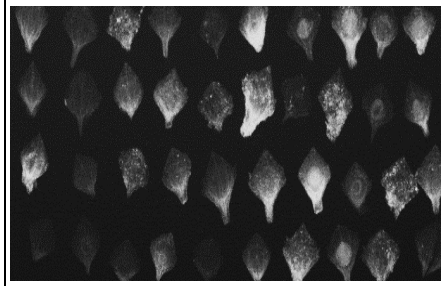
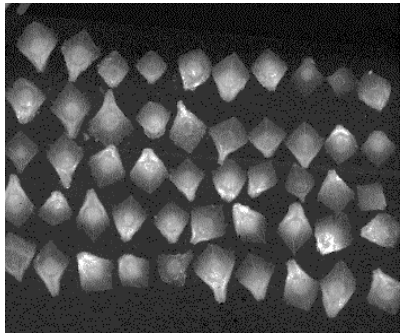
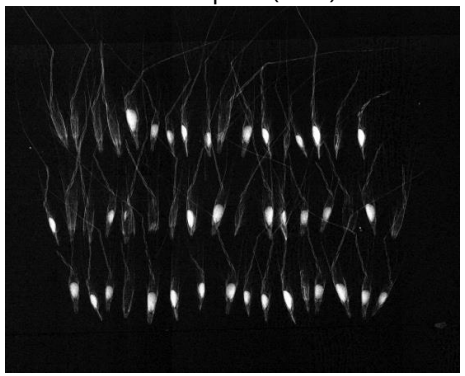
Viable Seed	Nonviable Seed	Viable seed - cross section	Nonviable seed – cross section
Kennedia prostrata EBSKAN122			
			
Lotus australis EBSKAN127			
			
Maireana brevifolia EBSKAN71			
			

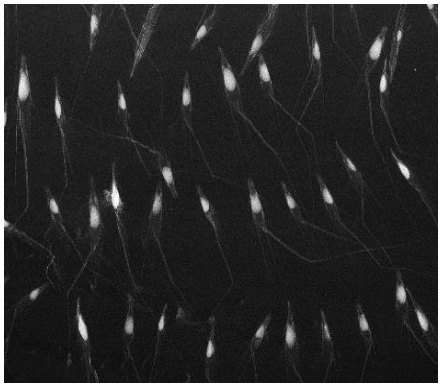
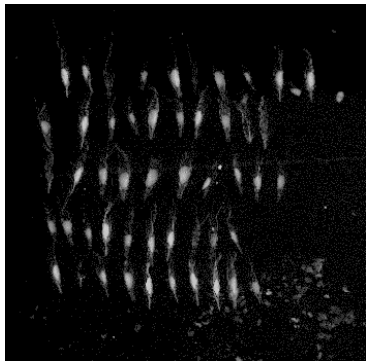
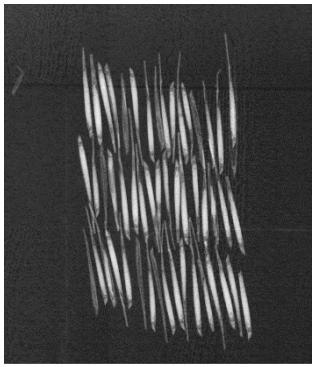
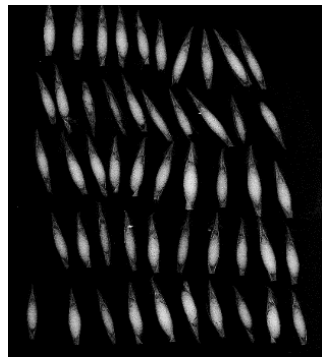
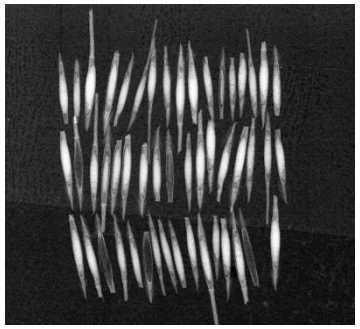
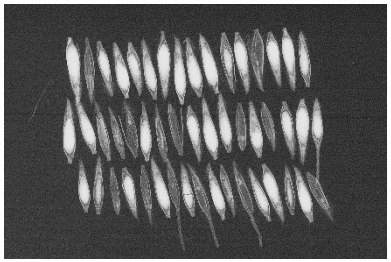
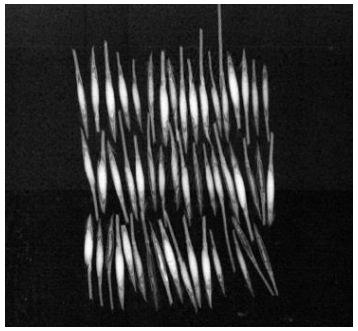
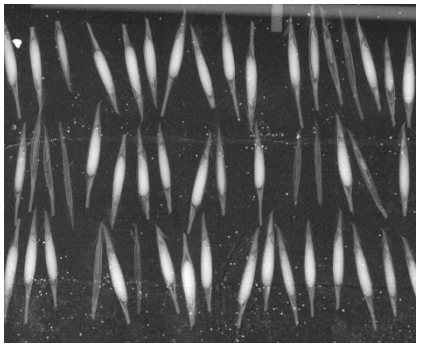
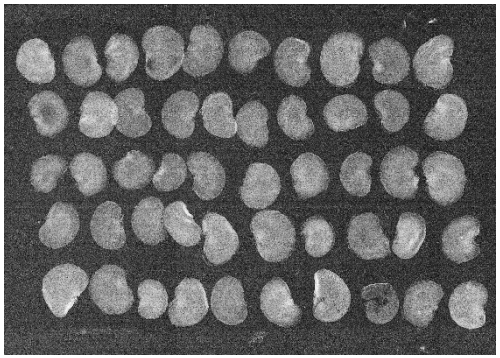
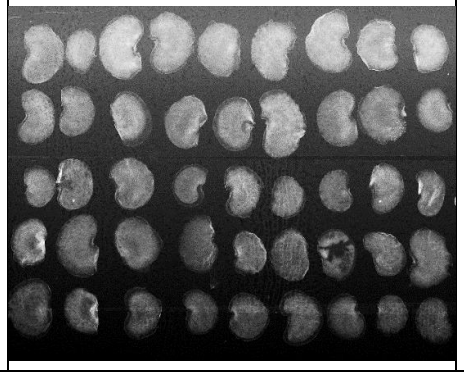
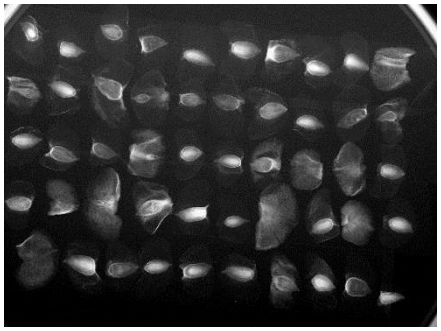
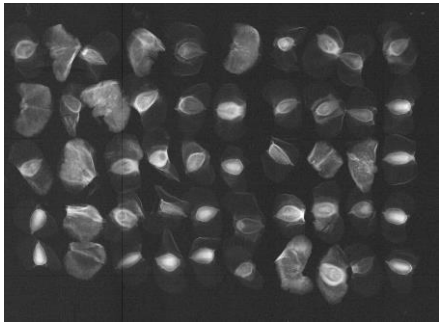
Viable Seed	Nonviable Seed	Viable seed - cross section	Nonviable seed – cross section
Olearia pannosa EBSKAN124			
			
Podolepis rugata EBSKAN125			
			
Ptilotus spathulatus EBSKAN35			
			

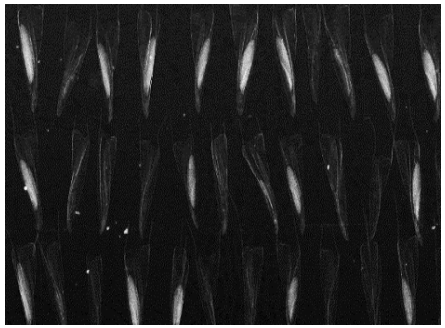
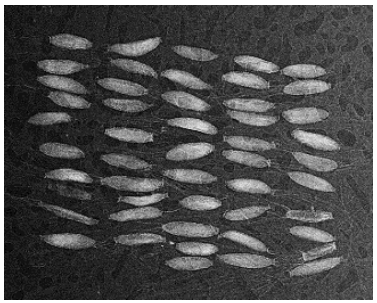
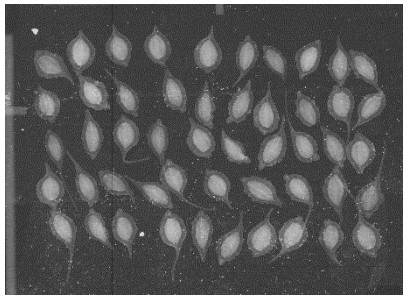
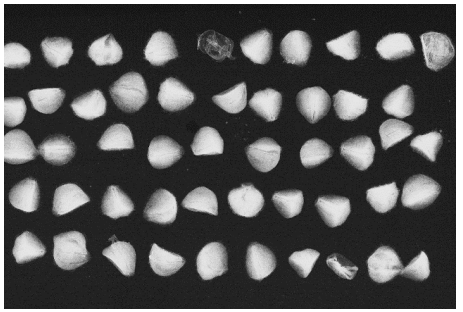
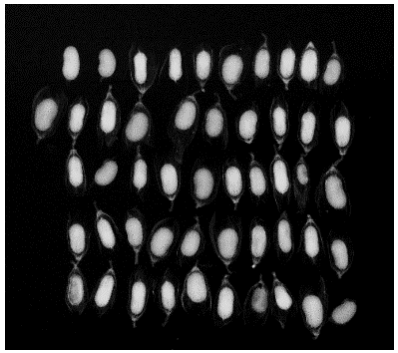
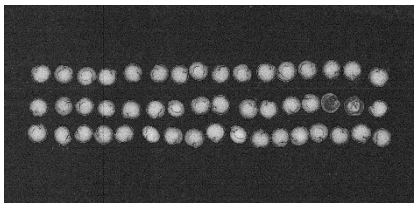
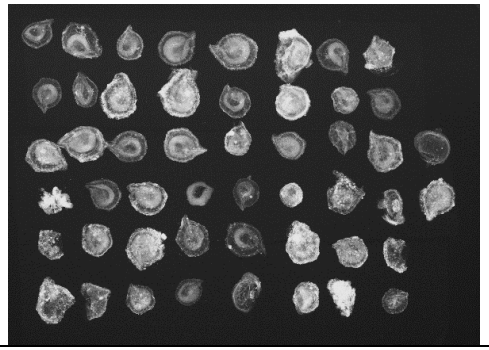
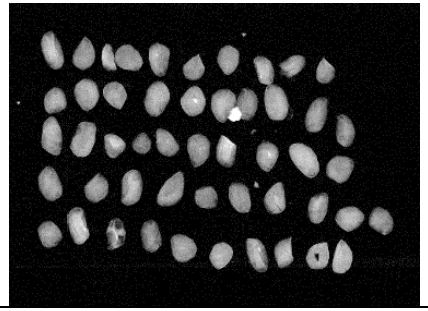
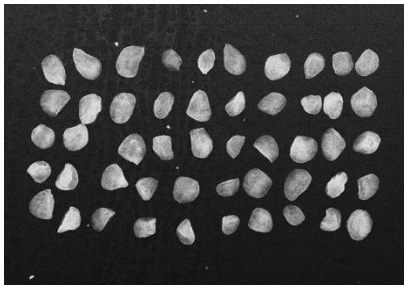
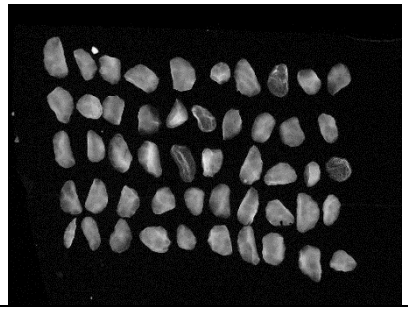
Viable Seed	Nonviable Seed	Viable seed - cross section	Nonviable seed – cross section
Themeda triandra EBSKAN118			
			
Velleia paradoxa (*arguta*) EBSKAN28			
			
Vittadinia blackii EBSKAN29			
			

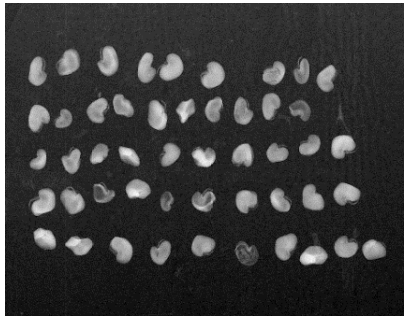
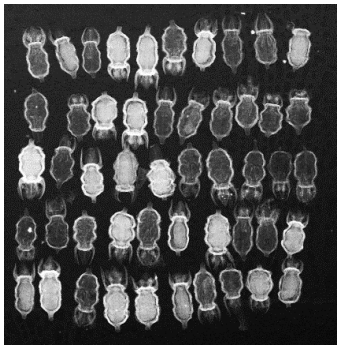
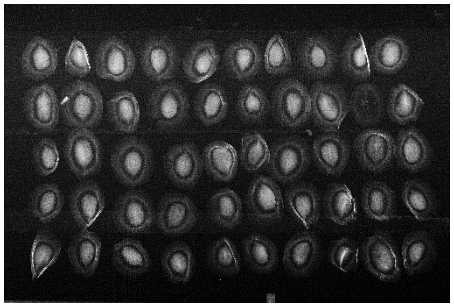
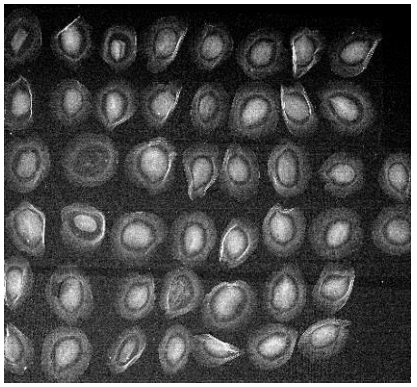
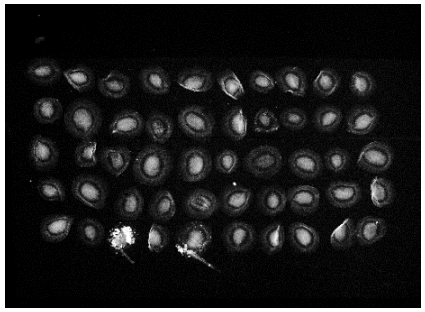
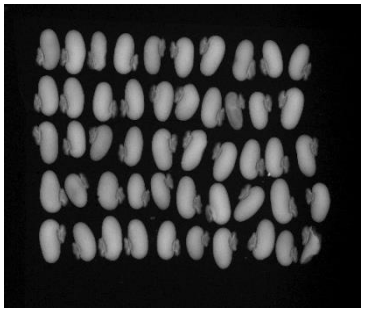
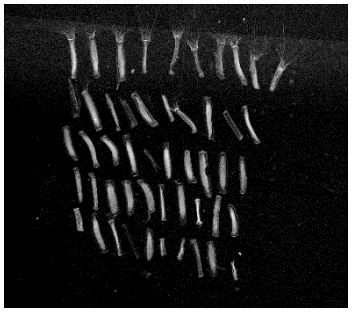
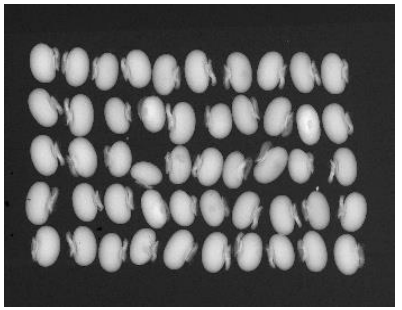
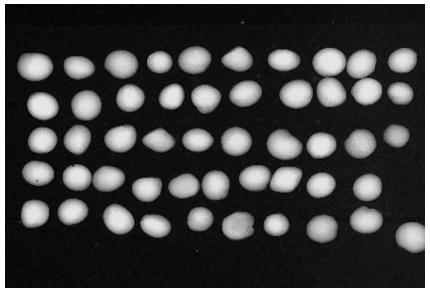
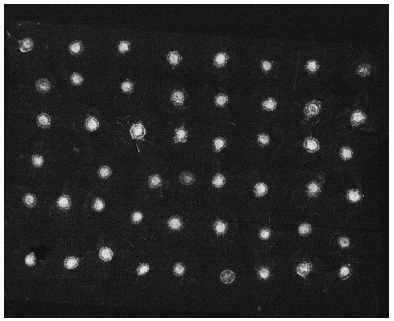
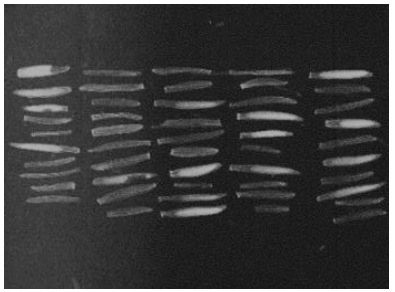
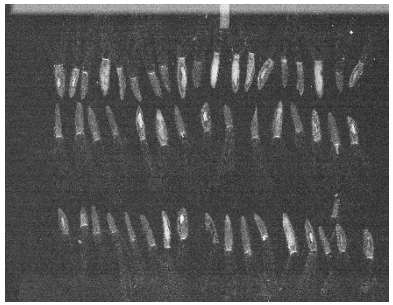
Viable Seed	Nonviable Seed	Viable seed - cross section	Nonviable seed – cross section
Vittadinia cuneata EBSKAN27			
			
Vittadinia megacephala EBSKAN10			
			
Wahlenbergia stricta EBSKAN86			
			

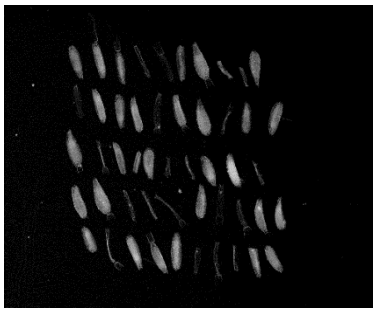
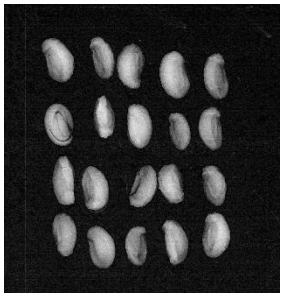
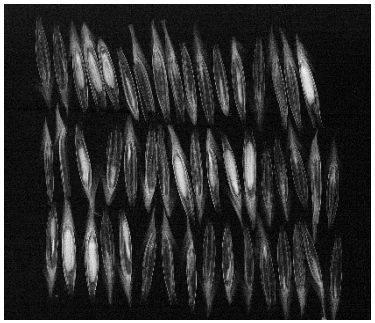
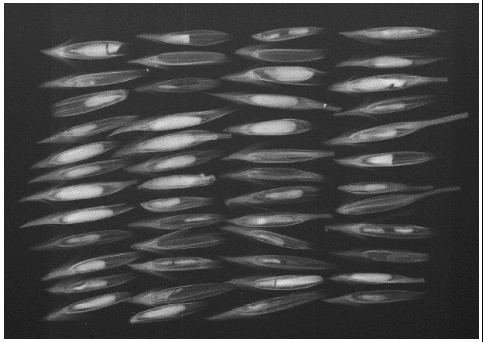
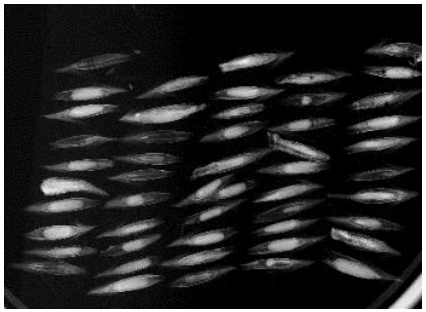
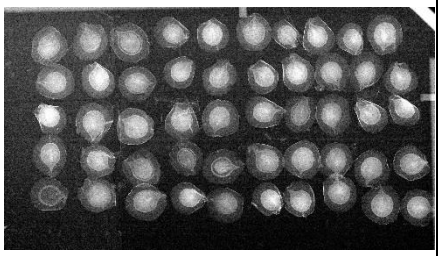
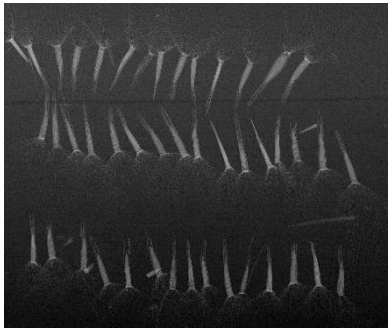
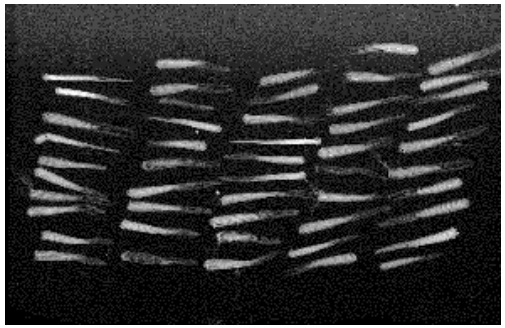
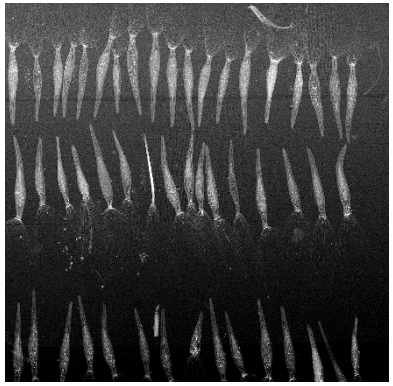
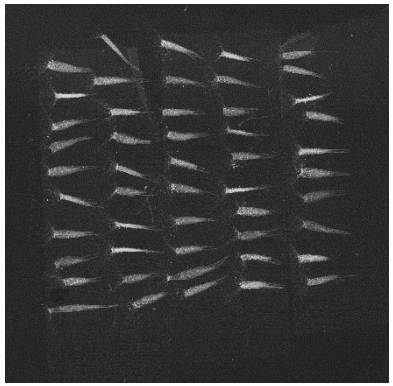
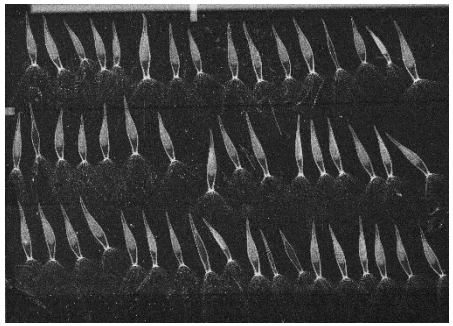
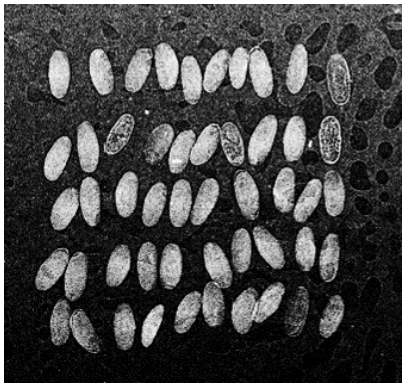
Appendix 2 X-ray Images of Seeds

Acacia paradoxa 21 (90%) 	Acacia pycnantha 20 (96%) 	Acacia pycnantha 58 (80%) 	Allocasuarina verticilliata 77 (70%) 
Anthosachne scabra 98 (80%) 	Aristida behriana 37 (74%) 	Aristida behriana 87 (10%) 	Arthropodium strictum 50 (48%) 
Atriplex semibaccata 80 (82%) 	Atriplex semibaccata 107 (14%) 	Atriplex semibaccata 133 (80%) 	Austrodanthonia sp 36 (66%) 

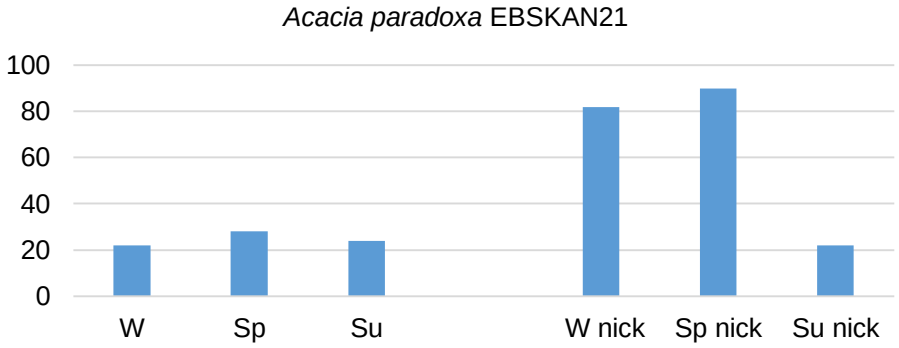
Austrodanthonia sp 91 (94%) 	Austrodanthonia sp 113 (78%) 	Austrostipa elegantissima39 (78%) 	Austrostipa nodosa 16 (82%) 
Austrostipa nodosa 112 (100%) 	Austrostipa sp 19 (56%) 	Austrostipa sp 34 (68%) 	Austrostipa sp 44 (80%) 
Bursaria spinosa 68 (96%) 	Bursaria spinosa 105 (94%) 	Callitris gracilis 101 (42%) 	Callitris preissii 23 (22%) 

Chloris truncata104 (46%) 	Chrysocephalum apiculata 75 (92%) 	Clematis microphylla 43 (100%) 	Convolvulus remotus 102 (94%) 
Cullen australasicum 132 (98%) 	Dodonaea viscosa 97 (100%) 	Einadia nutans 67 (96%) 	Enchylaena tomentosa 103 (40%) 
Eucalyptus calycogona 110 (98%) 	Eucalyptus odorata 94 (100%) 	Eucalyptus phenax 95 (94%) 	Eucalyptus socialis 96 (90%) 

Eutaxia microphylla 131 (72%) 	Gonocarpus tetragynus 81 (40%) 	Goodenia pinnatifida 61 (80%) 	Goodenia pinnatifida 83 (90%) 
Goodenia pinnatifida 115 (94%) 	Hardenbergia violacea 126 (96%) 	Helichrysum leucopsideum 128 (56%) 	Kennedia prostrata 122 (100%) 
Lotus australis 127 (100%) 	Maireana brevifolia 71 (82%) 	Olearia pannosa 124 (24%) 	Olearia sp 48 (4%) 

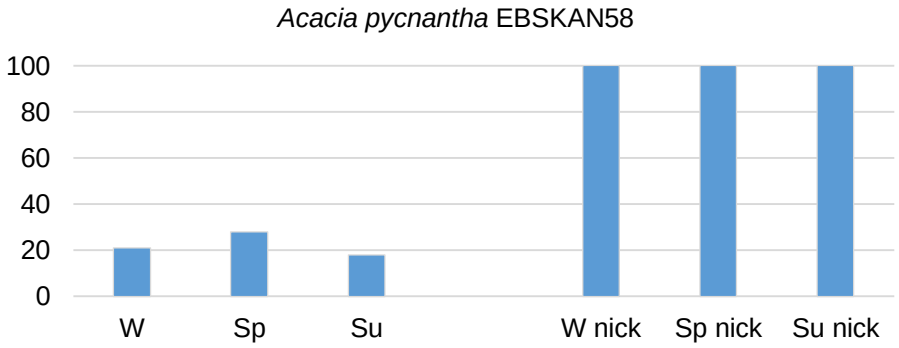
Podolepis rugata 125 (62%) 	Ptilotus spathulatus 35 (100%) 	Themeda triandra 38 (18%) 	Themeda triandra 90 (28%) 
Themeda triandra 118 (84%) 	Velleia paradoxa (arguta) 28 (95%) 	Vittadinia blackii 29 (98%) 	Vittadinia blackii 114 (86%) 
Vittadinia cuneata 27 (88%) 	Vittadinia 106 sp mix (100%) 	Vittadinia megacephala 10 (90%) 	Wahlenbergia stricta 86 (90%) 

Appendix 3. Graphs of Germination Experiments



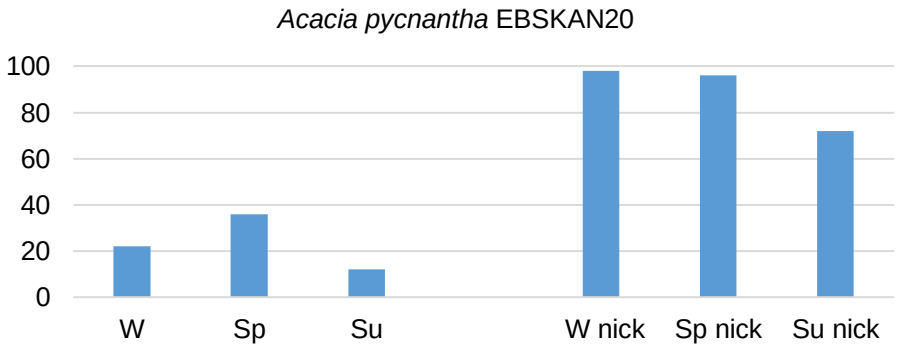
Viability = 98%

Acacia paradoxa seeds have physical dormancy which was broken when the seed coat was nicked. High levels of germination were achieved in the winter and spring incubators after seed nicking.



Viability = 80%

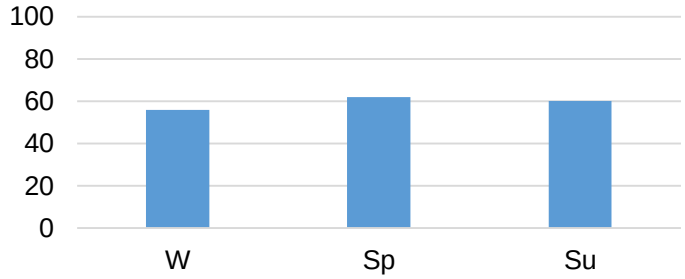
Acacia pycnantha seeds have physical dormancy which was broken when the seed coat was nicked. High levels of germination were achieved in all the incubators after seed nicking.



Viability = 96%

Acacia pycnantha seeds have physical dormancy which was broken when the seed coat was nicked. High levels of germination were achieved in all the incubators after seed nicking.

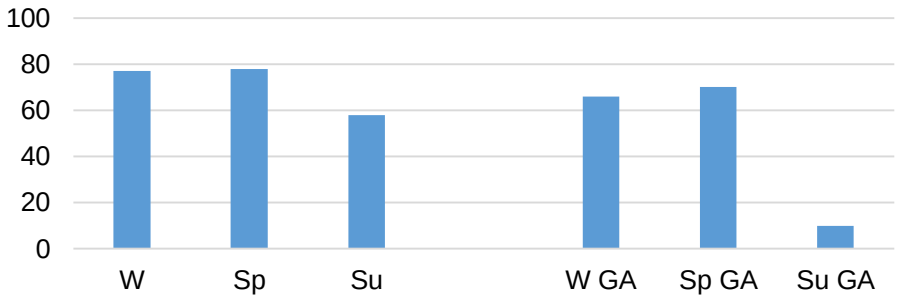
Allocasuarina verticillata EBSKAN77



Viability = 70%

High levels of germination were achieved in all incubators without GA. These seeds fall into the category of nondormant.

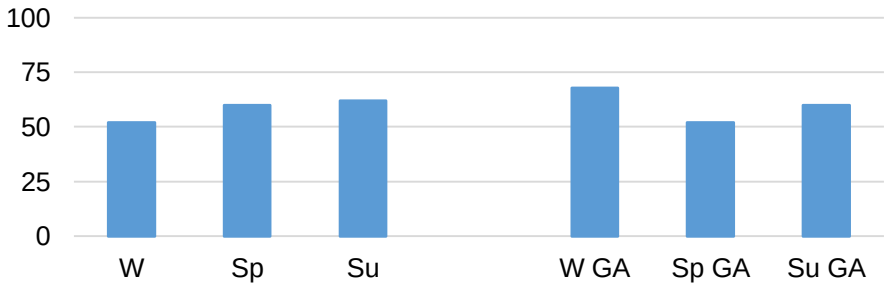
Anthosachne scabra EBSKAN98



Viability 80%

High germination levels with or without GA shows that the seeds are nondormant in winter and spring/autumn conditions.

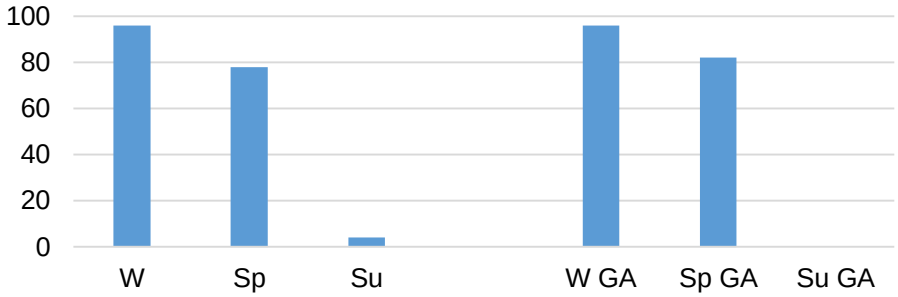
Aristida behriana EBSKAN37



Viability = 74 %

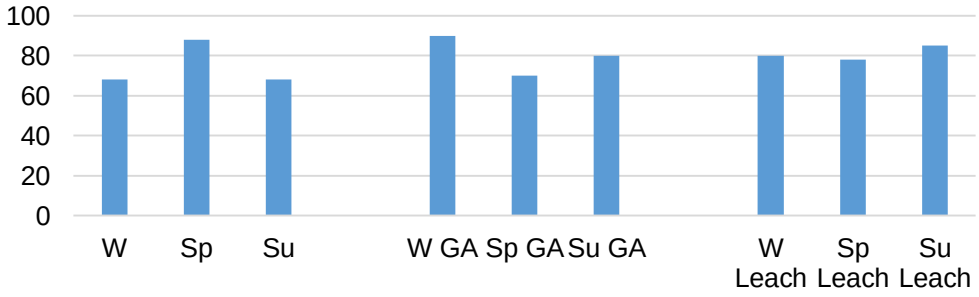
High levels of germination were achieved in all incubators with or without GA. These seeds fall into the category of nondormant and should germinate with sufficient moisture.

Arthropodium strictum EBSKAN50



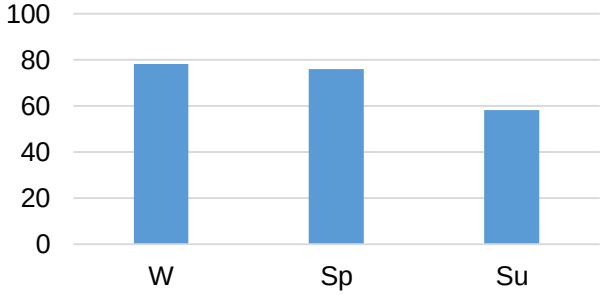
Viability = 100% viable seeds selected for germination experiments.
Seed lot viability = 48%
High germination levels with or without GA shows that the seeds are nondormant in winter and spring/autumn conditions. Low levels of germination in the summer incubator show that seeds did not germinate at higher temperatrures.

Atriplex semibaccata EBSKAN80

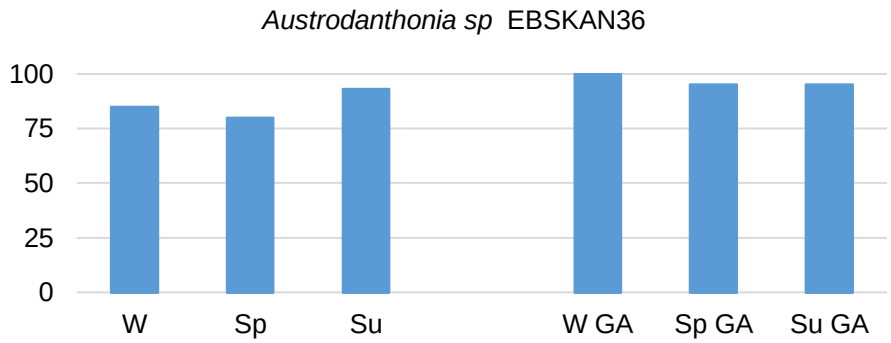


Viability = 82%
Mid to high levels of germination were achieved in all incubators with or without GA or leaching. These seeds fall into the category of nondormant.

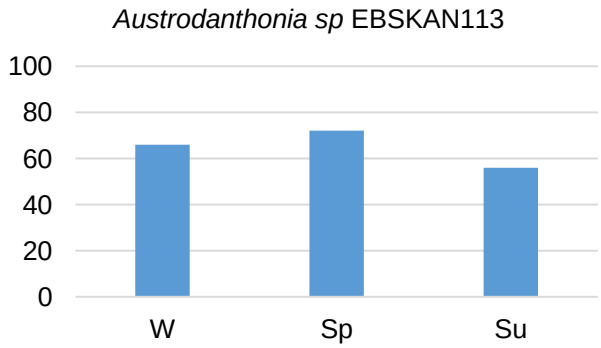
Atriplex semibaccata EBSKAN133



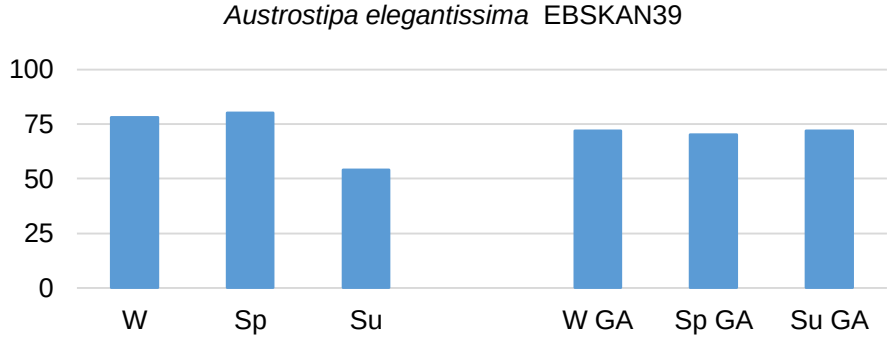
Viability = 80%
Mid to high levels of germination were achieved in all incubators without treatment. These seeds fall into the category of nondormant.



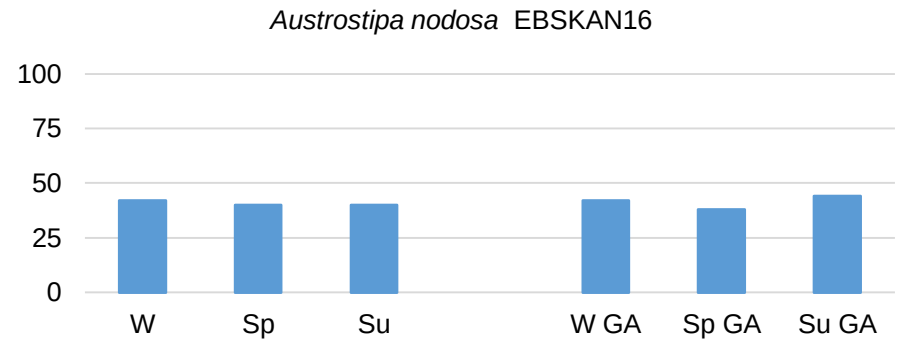
Viability = 66%
High levels of germination were achieved in all incubators with or without GA. These seeds fall into the category of nondormant.



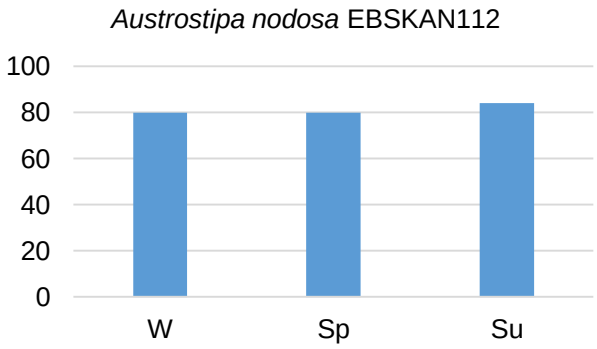
Viability = 78%
Mid to high levels of germination were achieved in all incubators with no treatment. These seeds fall into the category of nondormant.



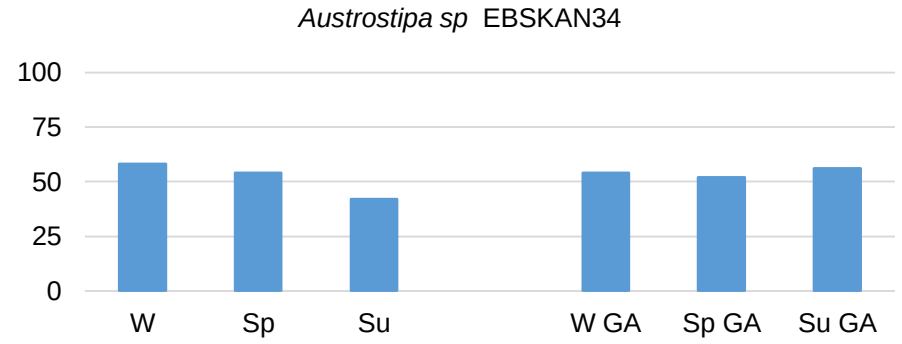
Viability = 78
High levels of germination were achieved in all incubators with or without GA. These seeds fall into the category of nondormant.



Viability = 82%
Low levels of germination were achieved in all incubators with or without GA. Approximately half of the seeds remained dormant.

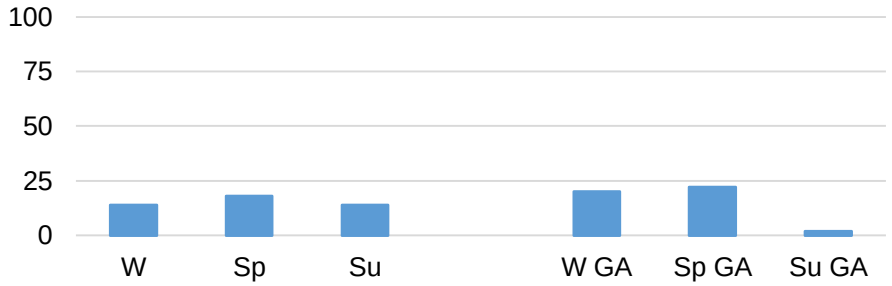


Viability = 100%
High levels of germination were observed in all incubators. The reduced level of dormancy in seeds from this batch may be due to after ripening.



Viability = 68%
High levels of germination were achieved in all incubators with or without GA. These seeds fall into the category of nondormant.

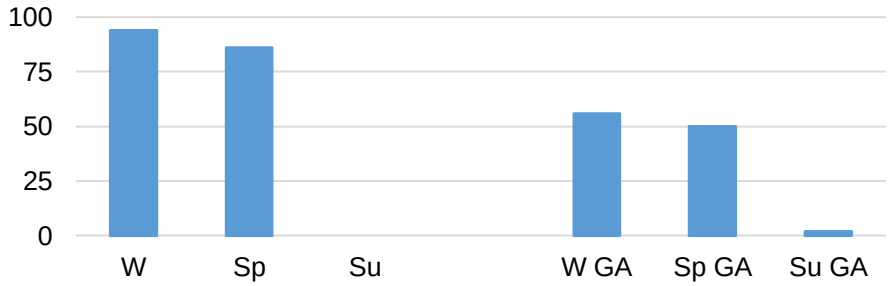
Austrostipa sp (*blackii*) EBSKAN19



Viability = 56%
Low levels of germination were achieved in all incubators with or without GA. Approximately 65 of the seeds remained dormant throughout this experiment.

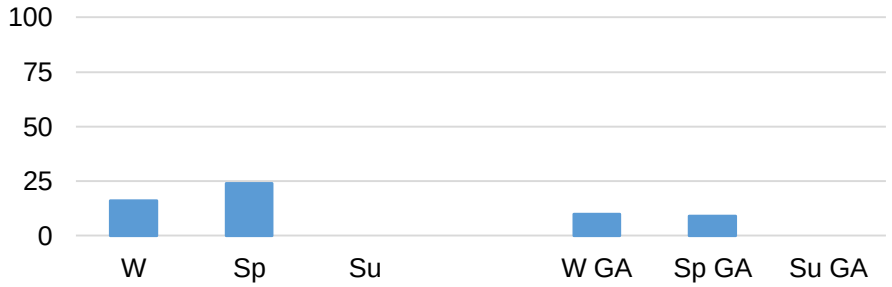
Low viability and dormancy in the seeds resulted in low levels of germination from seeds from this batch.

Bursaria spinosa EBSKAN68



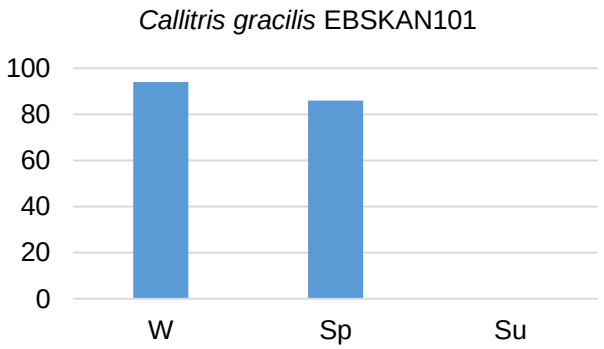
Viability = 96%
High levels of germination were achieved in the spring/autumn and winter incubators without GA. These seeds should germinate well in cooler seasons, with adequate moisture.

Callitris gracilis EBSKAN23



Viability = 30%
High levels of germination were achieved in the spring/autumn and winter incubators without GA. These seeds should germinate well in cooler seasons with adequate moisture.

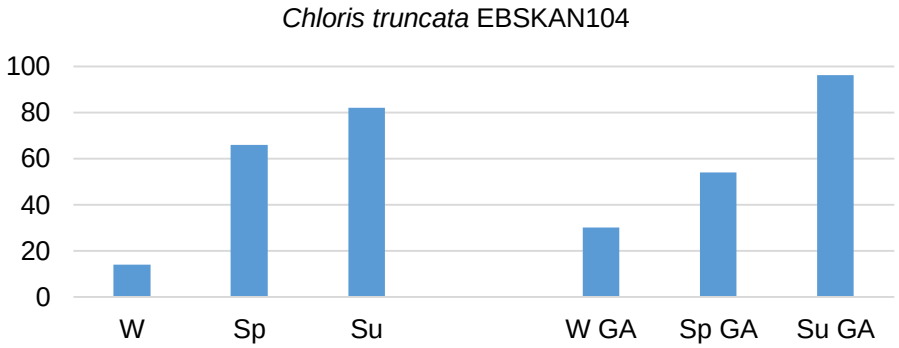
The main problem with these seeds is low viability, this is typical with this species and it is difficult to distinguish viable and nonviable seed without X-ray imaging or cut testing.



Viability = 100% viable seeds selected for germination experiments.

Seed lot viability = 42%

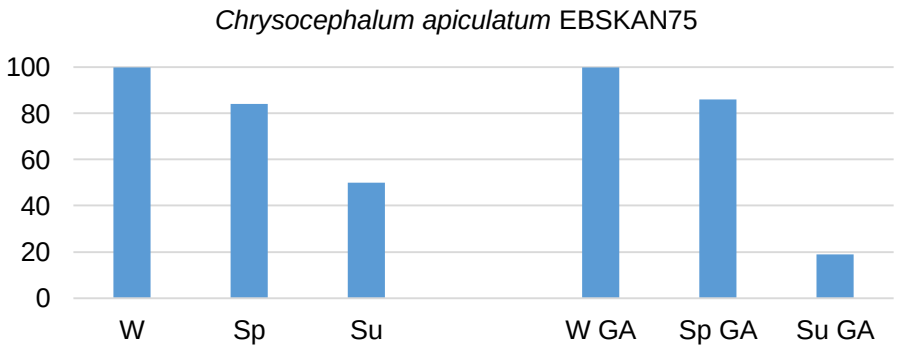
Viable seeds were selected for this experiment. High germination levels without GA shows that the seeds are nondormant in winter and spring/autumn conditions. Low levels of germination in the summer incubator show that seeds did not germinate at higher temperatrures.



Viability = 100% viable seeds selected for germination experiments.

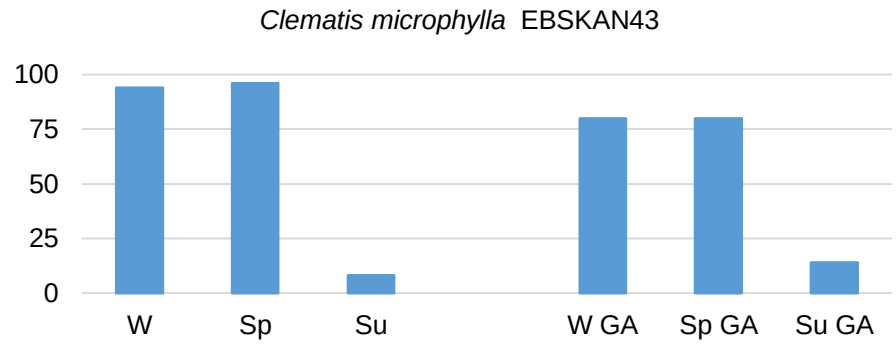
Seed lot viability = 46%

High germination levels with or without GA shows that the seeds are mostly nondormant in summer conditions. Low levels of germination in the winter incubator show that seeds did not germinate well at lower temperatrures. Germination increased after the application GA indicating that some of these seeds may have physiological dormancy

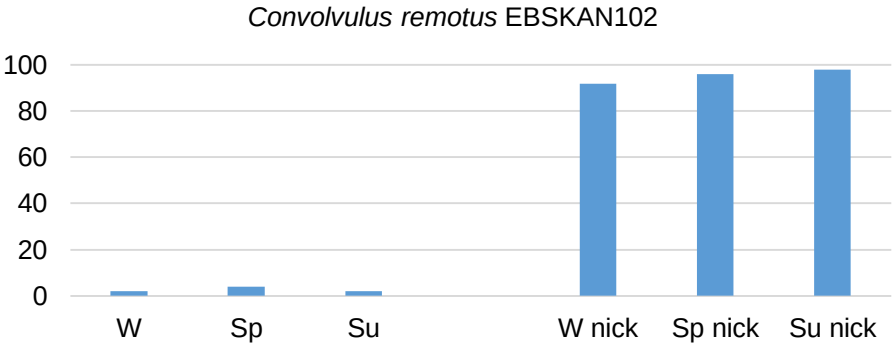


Viability = 92%

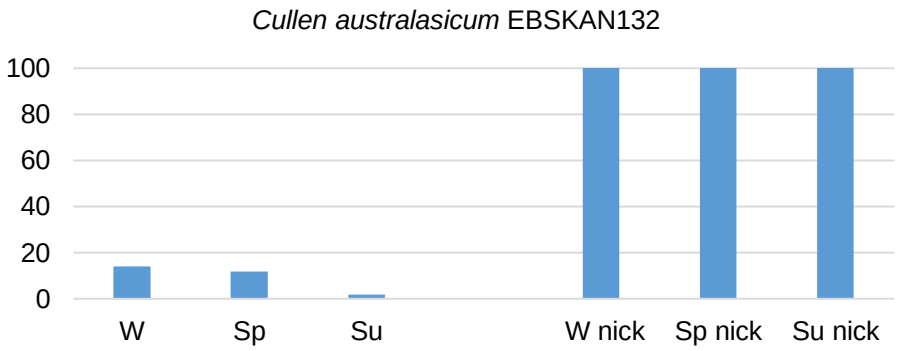
High germination levels with or without GA shows that the seeds are nondormant in winter and spring/autumn conditions. Low levels of germination in the summer incubator show that seeds did not germinate at higher temperatrures.



Viability = 100%
High levels of germination were achieved in the spring/autumn and winter incubators with or without GA. These seeds should germinate well in cooler seasons, with adequate moisture.

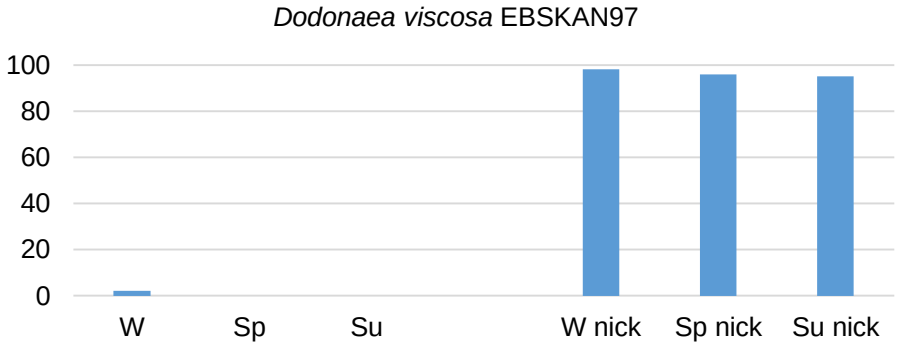


Viability = 100%
Convolvulus remotus seeds have physical dormancy which was broken when the seed coat was nicked. High levels of germination were achieved in all the incubators after seed nicking.



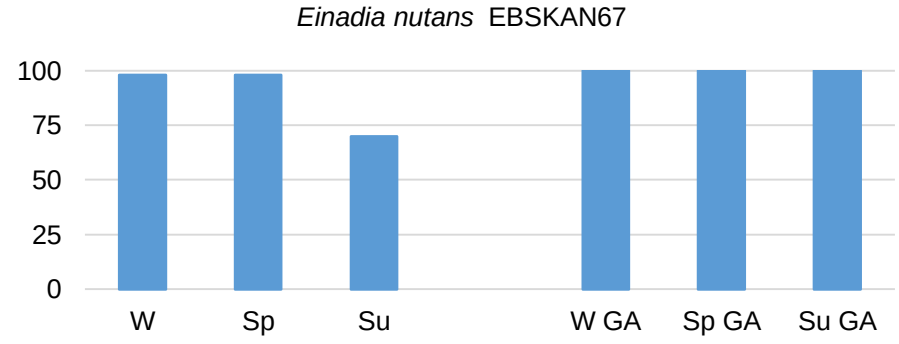
Viability = 98%

Cullen australasicum seeds have physical dormancy which was broken when the seed coat was nicked. High levels of germination were achieved in all the incubators after seed nicking.



Viability = 70%

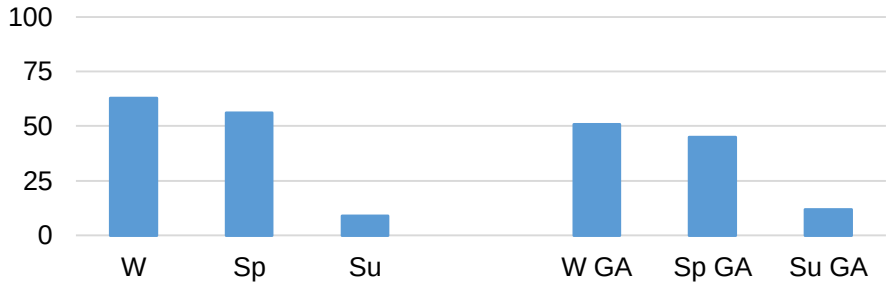
Dodonaea viscosa seeds have physical dormancy which was broken when the seed coat was nicked. High levels of germination were achieved in all the incubators after seed nicking.



Viability = 96%

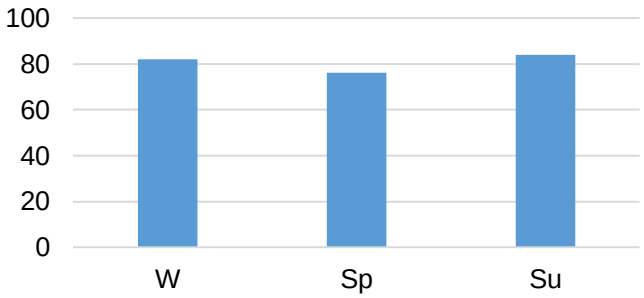
High levels of germination were achieved in the spring/autumn and winter incubators with or without GA. These seeds should germinate well in cooler seasons. A high portion of the seeds would also germinate in summer given sufficient moisture.

Enchylaena tomentosa EBSKAN70



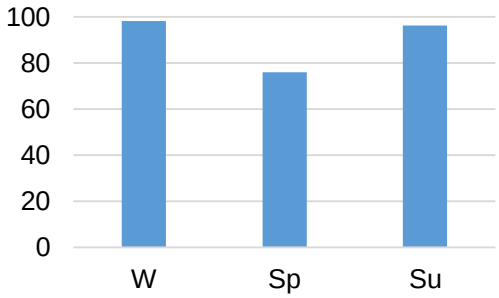
Viability = 75%
High levels of germination were achieved in the spring/autumn and winter incubators without GA. These seeds should germinate well in cooler seasons, given adequate moisture.

Eucalyptus calycogona EBSKAN110



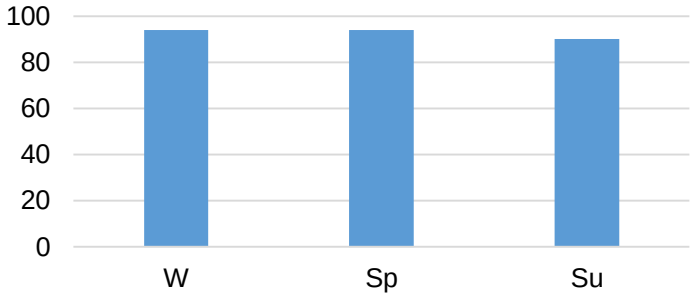
Viability = 98%
High levels of germination were observed in all incubators. These seeds fall into the category nondormant.

Eucalyptus odorata EBSKAN94



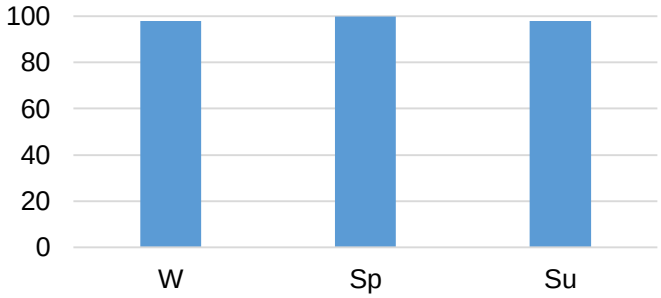
Viability = 100%
High levels of germination were observed in all incubators. These seeds fall into the category nondormant.

Eucalyptus phenax ssp. phenax EBSKAN95



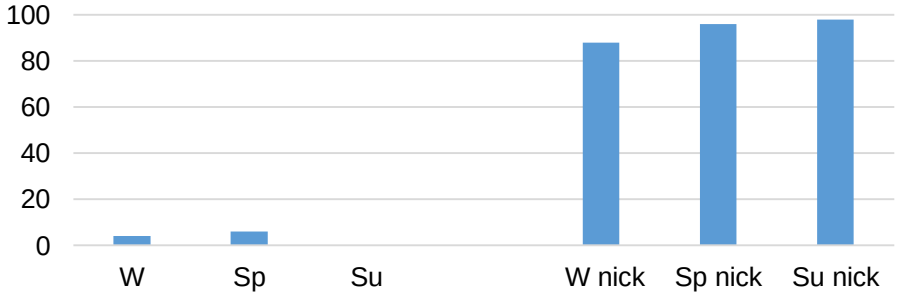
Viability = 94%
High levels of germination were observed in all incubators. These seeds fall into the category nondormant.

Eucalyptus socialis EBSKAN96

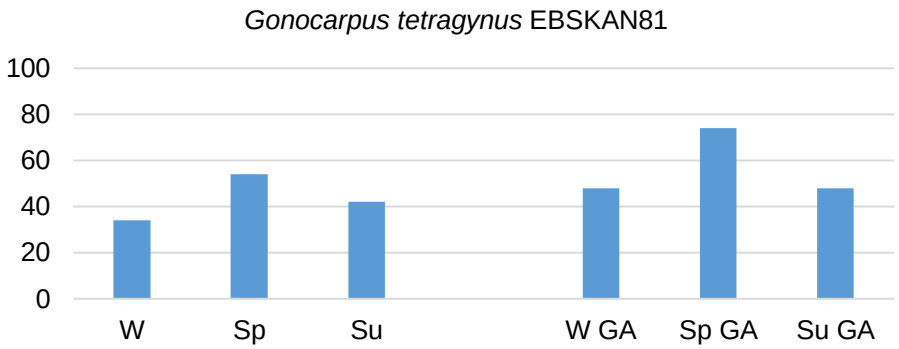


Viability = 90%
High levels of germination were observed in all incubators. These seeds fall into the category nondormant.

Eutaxia microphylla EBSKAN131

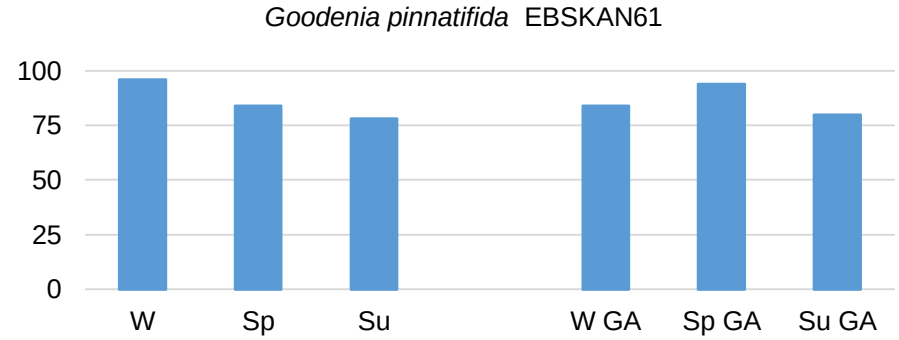


Viability = 72%
Eutaxia microphylla seeds have physical dormancy which was broken when the seed coat was nicked. High levels of germination were achieved in all the incubators after seed nicking.



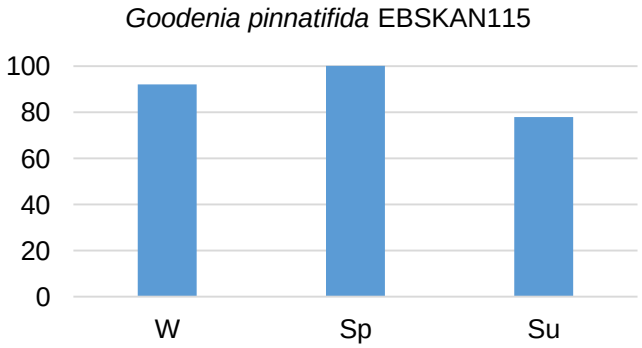
Viability = 40%

Germination increased after the application GA indicating that some of these seeds may have physiological dormancy.



Viability = 80 %

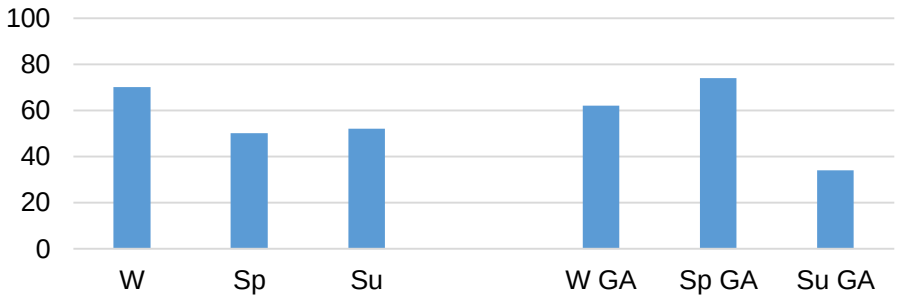
High levels of germination were achieved in all incubators with or without GA. These seeds fall into the category of nondormant.



Viability = 94%

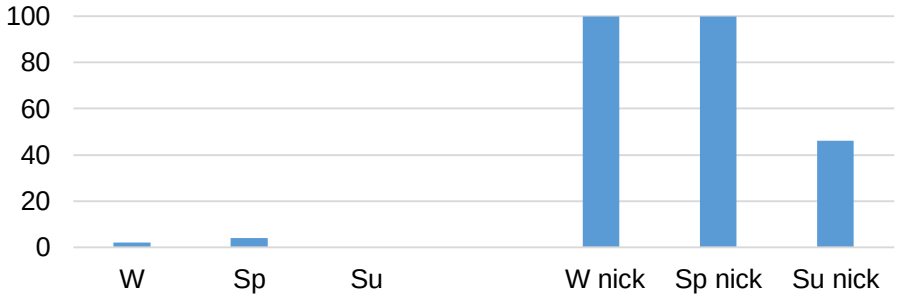
High levels of germination were achieved in all incubators without treatment. These seeds fall into the category of nondormant.

Helichrysum leucopsideum EBSKAN128



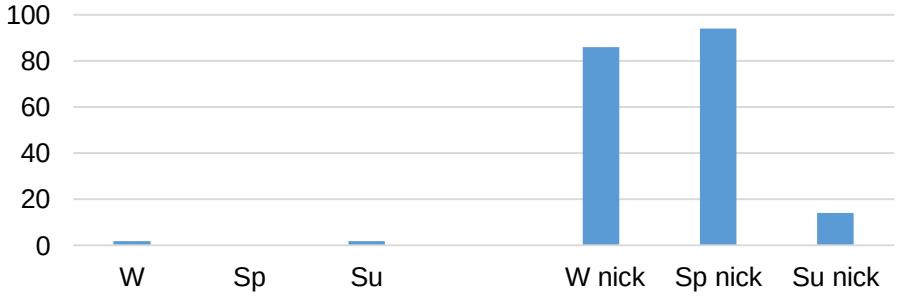
Viability = 56%
High levels of germination were achieved in all incubators without GA, considering the initial viability. These seeds fall into the category of nondormant.

Hardenbergia violacea EBSKAN126

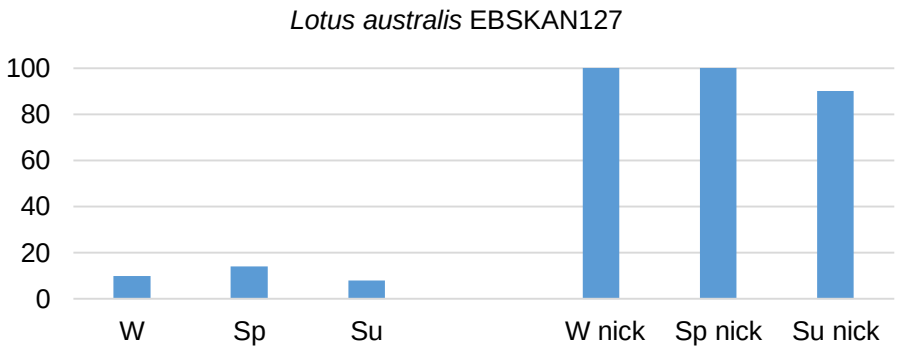


Viability = 96%
Hardenbergia violacea seeds have physical dormancy which was broken when the seed coat was nicked. High levels of germination were achieved in winter and spring/autumn incubators after seed nicking.

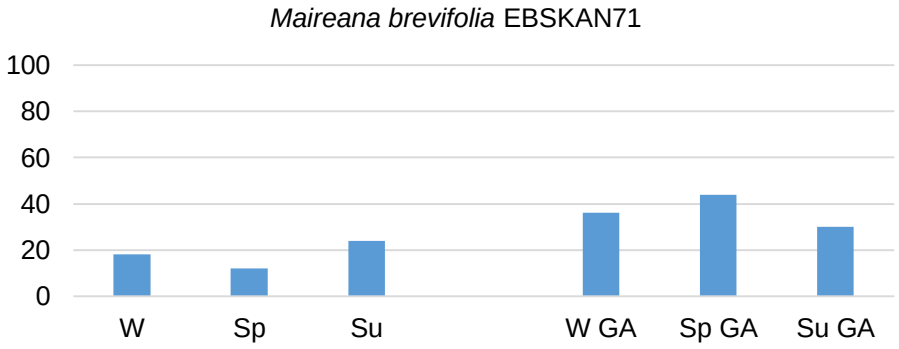
Kennedia prostrata EBSKAN122



Viability = 100%
Kennedia prostrata seeds have physical dormancy which was broken when the seed coat was nicked. High levels of germination were achieved in winter and spring/autumn incubators after seed nicking.

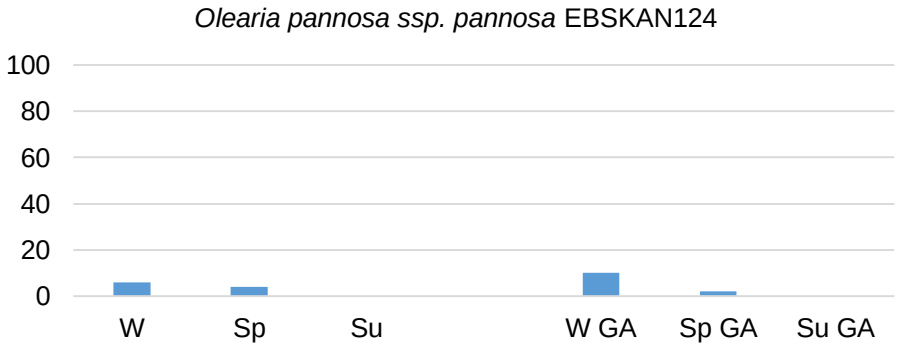


Lotus australis seeds have physical dormancy which was broken when the seed coat was nicked. High levels of germination were achieved in all incubators after seed nicking.



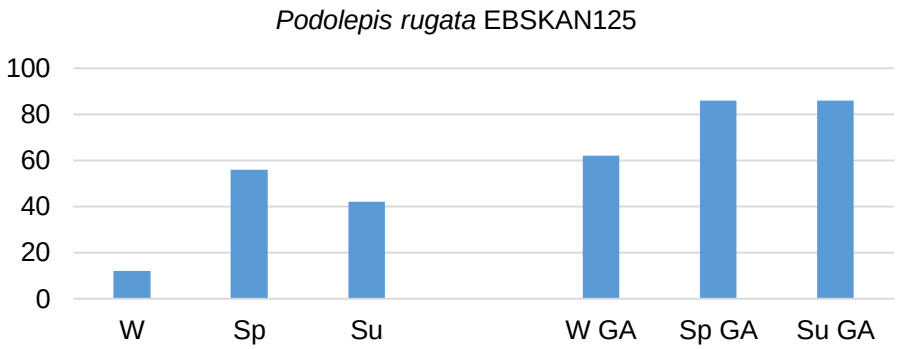
Viability = 82%

Low levels of germination were observed without GA treatment. Germination increased after the application GA indicating that these seeds may have physiological dormancy.



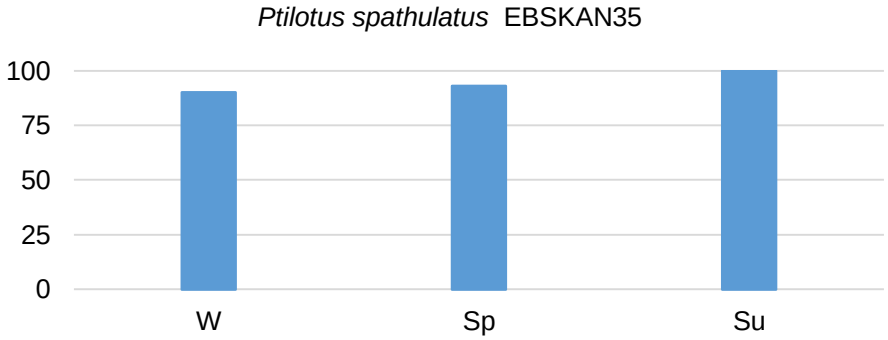
Viability = 24%

The main problem with these seeds is low viability, however, it is possible to distinguish viable and nonviable seed by sight. Extra care should be taken during seed collection to collect mature, viable seed. Low viability should be taken into account when preparing seed mixes.



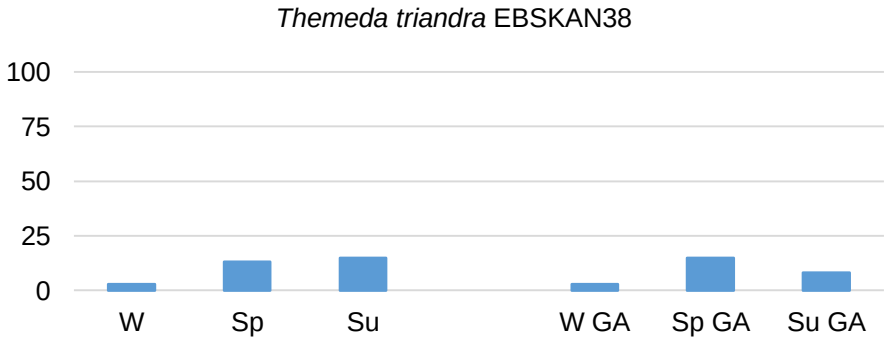
Viability = 62%

Germination increased after the application GA indicating that some of these seeds may have physiological dormancy.



Viability = 100%

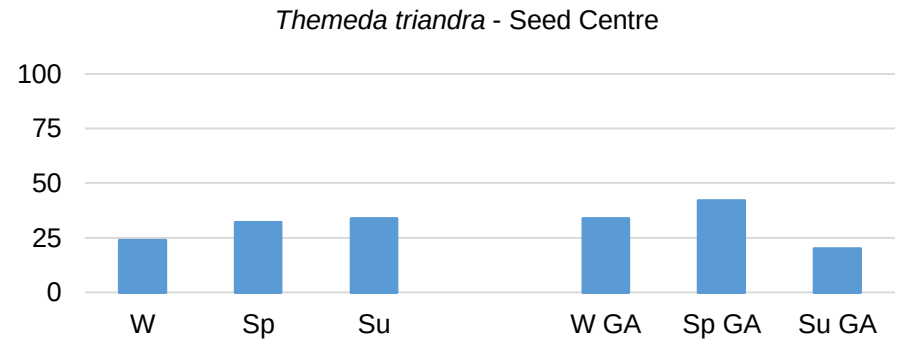
High levels of germination were achieved in all incubators without GA. These seeds fall into the category of nondormant and should germinate well with sufficient moisture.



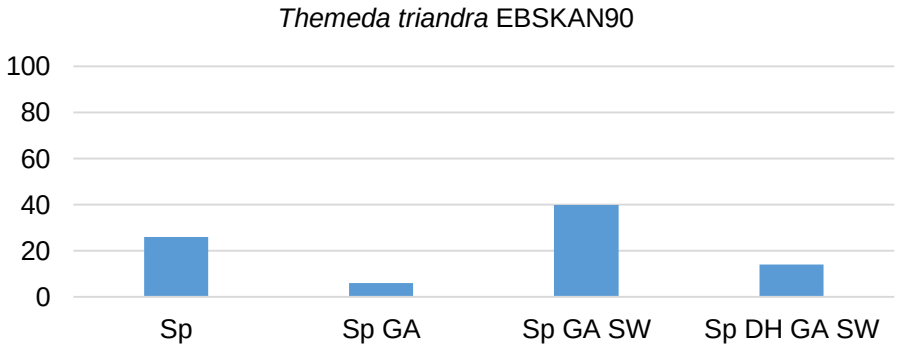
Viability = 18%

Relatively high levels of germination were achieved in the spring/autumn and summer incubators without GA, considering the viability of the seed lot.

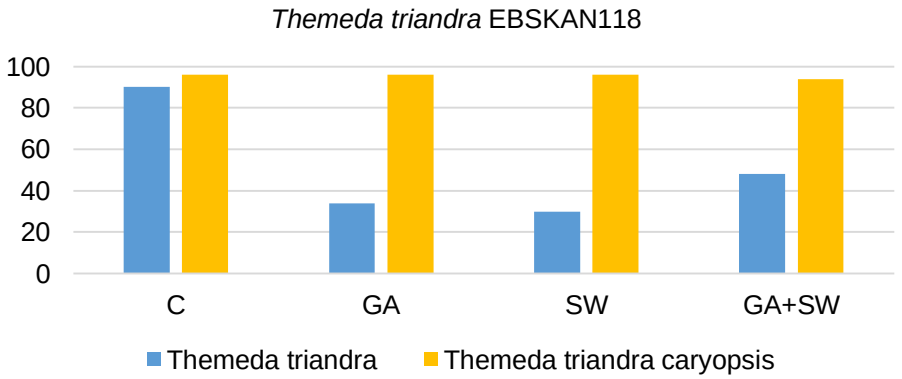
The main problem with these seeds is low viability, however, it is possible to distinguish viable and nonviable seed by sight. Extra care should be taken during seed collection to collect mature, viable seed. Low viability should be taken into account when preparing seed mixes.



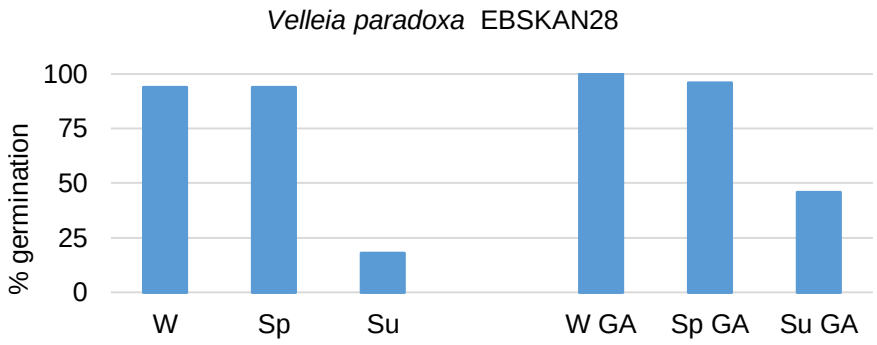
Viability = 66%
Relatively high levels of germination were achieved in the spring/autumn and summer incubators with or without GA, considering the viability of the seed lot.



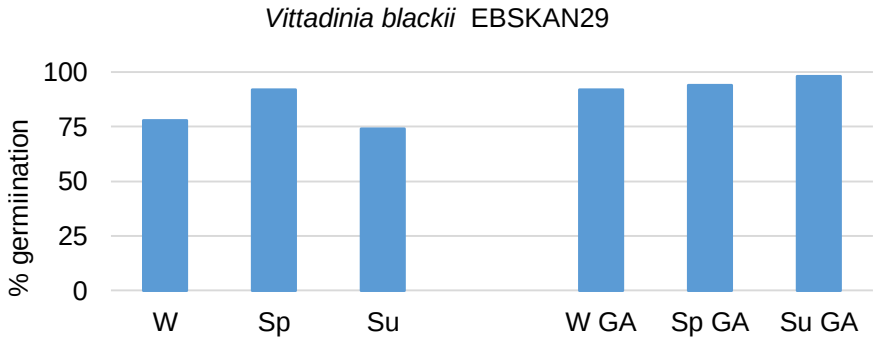
Viability = 28%
Relatively high levels of germination were achieved in the spring/autumn and summer incubators without GA or smoke water, considering the viability of the seed lot



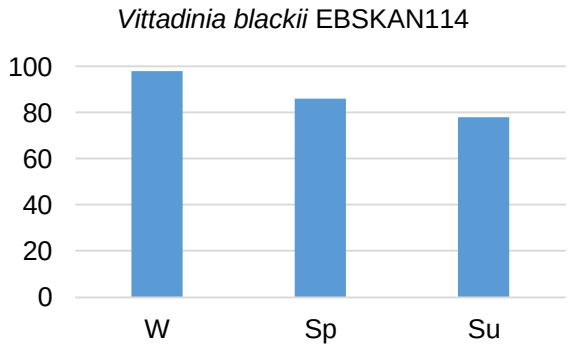
Viability = 100%
Only viable seeds were used for this experiment. Rapid germination of excised caryopses was observed for all treatments. The control (C) treatment had high germination levels from caryopses and from seeds within the lemma indicating that the seeds were nondormant. Treatment with GA and SW appeared to be inhibitory for the nonexcised seeds.



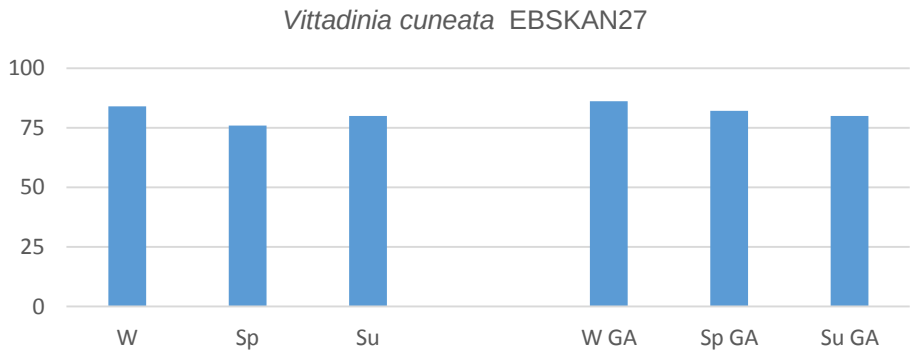
Viability = 95%
High levels of germination were achieved in the spring/autumn and winter incubators without GA. These seeds should germinate well in cooler seasons, given adequate moisture.



Viability = 98%
High levels of germination were achieved in all incubators with or without GA. These seeds fall into the category of nondormant.

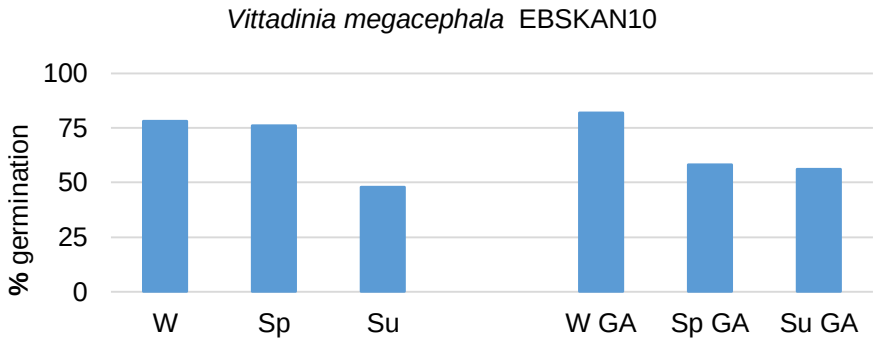


Viability = 86%
High levels of germination were achieved in all incubators. These seeds fall into the category of nondormant.



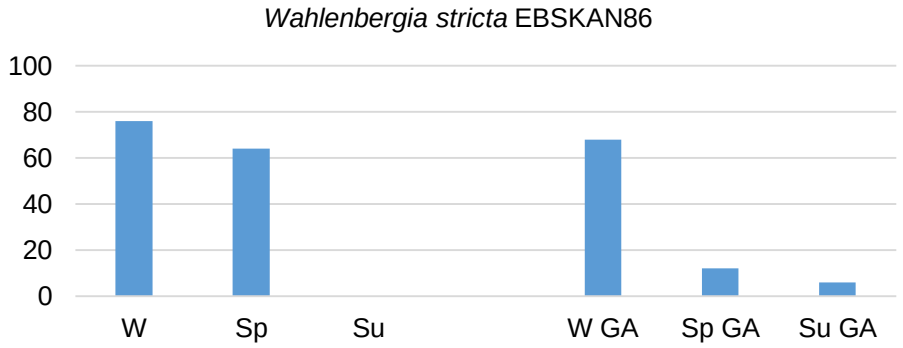
Viability = 88%

High levels of germination were achieved in all incubators. These seeds fall into the category of nondormant.



Viability = 75%

High levels of germination were achieved in the spring/autumn and winter incubators without GA. These seeds should germinate well in cooler seasons with adequate moisture. A high portion of the seeds would also germinate in summer given sufficient moisture.



Viability = 90%

High germination levels without GA shows that the seeds are nondormant in winter and spring/autumn conditions. Low levels of germination in the summer incubator show that seeds did not germinate at higher temperatures.

Appendix 4. Images of Seedlings

Acacia paradoxa (cotyledons) 	*Acacia paradoxa* (phyllodes) 	*Acacia pycnantha* (cotyledons) 
Acacia pycnantha (phyllodes) 	*Allocasuarina verticilliata* 	*Anthosachne scabra* 

Aristida behriana

Arthropodium strictum

Atriplex semibaccata

Austrodanthonia sp

Austrostipa blackii

Austrostipa elegantissima

Austrostipa nodosa

Bursaria spinosa

Callitris gracilis

Chloris truncata

Chrysocephalum apiculatum

Clematis microphylla

Convolvulus remotus

Cullen australasicum

Dodonaea viscosa

Einadia nutans

Enchylaena tomentosa

Eucalyptus calycogona

Eucalyptus odorata 	*Eucalyptus phenax ssp. phenax* 	*Eucalyptus socialis* 
Eutaxia microphylla 	*Goodenia pinnatifida* 	*Hardenbergia violacea* 
Helichrysum leucopsideum 	*Kennedia prostrata* 	*Lotus australis* 

Podolepis rugata

Ptilotus spathulatus

Themeda triandra

Velleia paradoxa

Vittadinia blackii

Vittadinia cuneata

Vitadinia megacephala

Wahlenbergia stricta