Germination of Central Australian Plant Seed After Long-term Storage©

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A seed trial was conducted on 31 central Australian plant species to test for germinability and explore dormancy and storage requirements. The seed had been collected up to 35 years ago and has been stored in uncontrolled conditions. Fresh seed of 24 of these species were also tested as a comparison and a variety of dormancy-breaking treatments was used on both "fresh" and "old" seed. Of the 31 species tested, 16 germinated, 13 being leguminous. The remaining 15 species mainly included annual and Poaceae species. Of these, four species did not germinate whether fresh or old. Seed that did not germinate was tested for viability, the result of which suggests further germination trials are required.

INTRODUCTION

Central Australia is a land of extremes, but despite its harsh and rugged landscape, it is rich and diverse in life. Both flora and fauna have adapted to this environment, being able to cope and thrive in its droughts and ephemeral rains.

Plants have a variety of adaptations including seed dormancy to ensure their seeds only germinate when conditions are perfect. Little is known about how long the seed of central Australian species can sit in the soil and still remain germinable. Similarly there is little known about how long this seed can remain in storage and what their storage requirements may be.

This trial aimed to discover how long the seed of a variety of species remain germinable and the effect of age on dormancy. It also provided preliminary information on the storage requirements for seed of central Australian species. The seed had been collected all over central Australia between the 1960s and the 1980s and now forms part of the reference collection in the Alice Springs Desert Park seed store.

At the onset, it was completely unknown how well any of the seeds would respond. Some species had not been used in germination tests before so little information was available. This meant that investigation into dormancies and germination methods was required as part of this study in order to apply appropriate treatments.

METHODS

Species Used in Trial. This seed trial was conducted over a 2-month period using 31 species representing 14 families and 26 genera, and had a wide range of collection years from 1966 to 1988.

Fresh Seed. For the purpose of this seed trial the term "fresh seed" is used in reference to seed either collected straight from the parent plant or the youngest collection of seed of the same species or genus in the Alice Springs Desert Park seed store. The age of the seed ranged from less than 1 year to 8 years but the average age of fresh seed tested was 2 years (Table 1).

Twenty-four species were chosen to correspond with the same species used in the Reference Collection seed trial. The treatment requirements used for older seeds were applied to fresh seed to compare their germinability.

Seed Storage. Storage of the "old" seed in the past had been in paper exposed to varying temperatures and pests. Recently the undamaged seed has been cleaned, weighed, placed in foil packets, and stored at 4°C

Germination. Fifty seeds of each species per treatment were placed in petri dishes lined with filter paper and moistened with distilled water. The petri dishes were sealed with Parafilm™ to ensure moisture was not lost. Each test ran for 10 days and germination was checked daily.

Temperature Regimes. All of the seed was trialed in the germination cabinet at a temperature regime of 20 to 35°C. Alternative temperature regimes trialed to stimulate germination of Poaceae and annual species included: uncontrolled summer temperatures in a tunnel house (15 to 61°C); a constant temperature of 17°C in the seed store, and a winter temperature regime in the germination cabinet (10 to 20°C).

Pre-treatment of Seed for Germination. A range of treatments were used depending on the species and their suspected dormancies. If the dormancy of a species was unknown, no treatment was applied. If the germination of a particular species was highly successful, no other treatments were applied. If germination was poor or did not occur at all another treatment was tested. This process was continued until a satisfactory result was achieved. If no germination occurred following several treatments, samples of that species were sent to Kings Park and Botanic Garden for viability analysis.

Treatments Used for Coat-imposed Dormancy. All seeds were soaked in water for 24 h following treatment to ensure the seeds were fully imbibed.

Hot Water. Used to treat seeds that were known to have hard seed coats and permeability barriers. Water was boiled and poured into the jar containing the seed.

Chipping. Small clippers were used to cut a very small corner of testa off the cotyledon end of the seed, allowing penetration of water.

Scarification. Seeds were rubbed with sandpaper to remove a portion of testa without damaging any part of the inner seed.

Baking. Seeds were placed in the oven at 105°C for 10 h and allowed to imbibe.

Boiling. Seeds were placed in boiling water for 1 min and left to soak in cooler water.

Seed Coat Removal. Seeds were chipped and left to imbibe in water. The seed coats were carefully removed using a sharp scalpel and placed in the petri dish.

Smoke Water. Seeds were soaked in a solution of smoke water and distilled water or boiled water (1 : 10) if heat treatment was also required.

Fire Over Punnet. Seeds were placed in a punnet containing seed raising mix and covered with sand. A fire was lit directly on top of the punnet for 1 min, then drenched with water.

Cold Stratification. Seeds were placed in between wet filter paper and placed in the fridge at 15°C. Due to time restraints, seeds could only be stratified for 3 days.

Gibberellic Acid (Ga_3). Seeds were placed in a solution of 500 ppm GA_3 with distilled water and left to imbibe for 24 h.

Bleach. Seeds prone to fungal attack were dipped in a bleach solution, then rinsed thoroughly in distilled water and treated accordingly.

RESULTS

The highest germination results achieved for "old" and "fresh" seed for each species tested is given in Table 2. It should be noted that no untreated species responded with above 10% germination.

DISCUSSION

As the seed tested in this trial had been subject to uncontrolled conditions for most of its storage life, it was unknown whether any of it would be viable. The Germplasm Conservation Guidelines produced by the Australian Network for Plant Conservation (1997) recommends that for medium-term storage (5 to 25 years) -18°C is the ideal. However, given the naturally varied and sometimes extreme environmental conditions that occur in central Australia and the nature of the plants adapted to survive here, their requirements for storage may not be as exacting.

Although little research has been done to determine their storage requirements, some generalisations can be made. A study by Ewart (1908) in Cavanagh (1987) showed that many leguminous species stored for long periods in uncontrolled conditions were still capable of germination, where few other families had the same ability. From this we could assume that seeds of leguminous families such as Mimosaceae, Fabaceae, and Caesalpiniaceae can remain viable and germinable for extended periods of time. Poaceae species may only remain viable for up to 5 or 6 years in laboratory storage (Whalley, 1987). The results of this study confirm these findings with 13 species that showed some germination being leguminous and the species not germinating being grasses or annuals.

However, some leguminous species, such as *Dichrostachys spicata,* still required further investigation into their specific dormancy-breaking treatments following relatively poor initial germination results. *Dichrostachys spicata* seeds that were chipped and soaked in both boiled and warm water produced poor germination results. When seeds had imbibed, it was observed that a mucilaginous (gel-like) substance would swell between the outer coat and the seed inside. According to Baskin and Baskin (1998) this mucigel expands in the presence of water, trapping it between the tissues of the seed. This forms an oxygen barrier and prevents the exchange of gasses necessary for germination. Once the entire seed coat and mucigel has been removed, germination of this species increased dramatically.

While most of the species achieved higher germination from fresh seed, this was not the case for *Convolvulus erubescens*. After a long period of storage it was able to germinate (70%) without treatment, but when fresh seed was tested without treatment, no germination occurred. Assuming this was associated with their particularly hard seed coat, the fresh seeds were chipped and achieved 100% germination. This would suggest that coat-imposed dormancy in this species breaks down over a long time, with some seed still being affected by it after 12 years.

Many seeds suffered from severe fungal attack despite the use of sterilized equipment. According to Baskin and Baskin (1998) fungal attack is only an indication of dead or inferior seeds. Seeds that suffered fungal attack and did not germinate after several treatments were tested for viability. Results show that most species tested were in fact still viable, indicating that further germination trials are needed.

Only one grass species, *Aristida holathera*, germinated (46%) without treatment when fresh, whereas none of the older seed germinated at all. Some of the annual species such as *Brachyscome ciliaris, Schoenia cassiniana,* and *Ixiochlamys filicifolia* germinated at a cold temperature regime, where older seed showed no response. This would suggest that older seed of these species has lost viability. However, it was also found that the annual species took longer to germinate and may have needed 20 days rather than 10 to show the full germination result. This may be linked to their greater response to cooler conditions and again needs further investigation.

A trial of baking in the tunnel house and germinating at low temperatures still needs be tried for the winter annuals, as does placing summer germinating Poaceae seeds in cold conditions before germinating at hot temperatures.

CONCLUSION

This trial was conducted in order to establish the germinability of the seed of numerous arid zone plants collected up to 35 years ago. In doing so, this study aimed to discover more about the effect of age on dormancy and germination, and consider the storage requirements of seed of central Australian species.

This study has shown that several leguminous plants such as *Acacia* and *Senna* species germinate readily after long periods of uncontrolled storage. This indicates that these species may not need the strict storage conditions often stipulated for medium- and long-term seed storage. In addition, it has been demonstrated that while several species of central Australian seed were found to germinate after long-term storage, pretreatments to overcome coat-imposed dormancy are still important for their germination. In comparison to the leguminous species, most annual and Poaceae species tested were found to not germinate readily. When more information on the long-term viability of such seed has been gathered it will be possible to determine whether there is a need to investigate their seed dormancy further.

In conducting this study it has become obvious that little is known about the storage life of these arid-zone species under different conditions or the best seed storage conditions required. Despite the fact that 70% of Australia is classified as arid or semi-arid, knowledge on desert seed dormancy and germination behaviour is very limited. As a part of the research necessary, there is a great need for the testing of other germination treatments with an emphasis on the effect of soil temperatures and wet and dry cycles. With an increased interest in central Australian plants it is essential that more research into the dormancy, germination, and storage conditions of seed be conducted for the future of seed banks, conservation, and the horticultural industry alike.

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Table 1. List of species and age (years) of seed used in trial.

Species	Reference collection	Fresh seed trial
Acacia aneura *L	35	1
Acacia cuthbertsonii *L	31	NA
Acacia melleliodora *L	23	3
Aeschynomene indica *L	23	5
Aristida holathera *P	31	7
Brachyscome ciliaris *A	26	3
Convolvulus erubescens	13	Fresh
Crotalaria cunninghamii *L	23	1
Dichrostachys spicata *L	29	NA
Enneapogon cylindricus *P	31	1
Eriachne pulchella *P	31	6
Eucalyptus gamophylla	18	8
Gastrolobium grandiflorum *L	29	1
Hakea macrocarpa	34	NA
Isotropis atropurpurea *L	23	NA
Ixiochlamys spp. * A	23	Fresh
Petalostylis cassioides *L	29	2
Radyera ferragei	24	Fresh
Rhodanthe charsleyae *A	23	NA
Ricinocarpos glora-medii	21	2
Schoenia cassiniana *A	28	1
Sclerolaena cornishiana	23	2
Senna helmsii (syn. *S. artemisioides* subsp. *Helmsii*) *L	22	Fresh

Senna sturtii (syn. *S. artemisioides* subsp. *sturtii*) *L	31	Fresh
Senna pleurocarpa *L	32	Fresh
Solanum sturtianum	32	3
Trachymene glaucifolia *A	28	3
Trachymene villosa *A	30	NA
Trichodesma zeylanicum	13	Fresh
Triodia briziodes *P	30	5
Vigna lanceolata *L	18	NA

*L= leguminous, A= annual, P= Poaceae.

Table 2. Highest percent germination results achieved for reference collection and fresh seed trials.

Species	Successful treatment	Reference collection (%)	Fresh seed(%)
Acacia aneura	Boil 1 min	80	92
Acacia cuthbertsonii	Bleach + chip	8	NA
Acacia melleiodora	Chip + Hot H_2O	76	38
Aeschynomene indica	Chip + H_2O @ 40°C	74	100
Aristida holathera	No treatment	0**	46
Brachyscome ciliaris	Winter temp regime	0**	4
Crotalaria cunninghamii	Chip + Hot H_2O	54	60
Convolvulus erubescens	H_2O 24 hours	70	4
Dichrostachys spicata	Coats removed	36	NA
Enneapogon cylindricus	No treatment	0**	0
Eriachne mucronata	No treatment	0**	0
Eucalyptus gamophylla	No treatment	4	68
Gastrolobium grandiflorum	Boil 1 min	90	86
Hakea macrocarpa	Hot smoke H_2O	0	NA
Ixiochlamys spp.	Winter temp regime	0**	24
Petalostylis cassioides	Chip + H_2O @ 40°C	42	100
Radyera ferragei	Hot H_2O	0	18
Rhodanthe charsleyae	Winter temp regime	0**	NA
Ricinocarpos gloria-medii	No treatment	0	0
Schoenia cassiniana	Winter temp regime	0*	6
Sclerolaena cornishiana	No treatment	0**	32

Senna sturtii (syn. *S. artemisioides* subsp. *sturtii*)	Chip + Hot H_2O	74	82
Senna helmsii (syn. *S. artemisioides* subsp. *helmsii*)	Chip + Hot H_2O	68	72
Senna pleurocarpa	Chip + H_2O @ 40°C	94	32
Solanum sturtianum	Fire over punnet	0	0
Trachymene glaucifolia	No treatment	0**	0
Trachymene villosa	No treatment	0**	0
Trichodesma zeylanicum	No treatment	10	14
Triodia spp.	No treatment	0**	0
Vigna lanceolata	Hot H_2O	90	NA

*Viability tested (cut test method).
**Above 80% viable.

Fifty Years of Change in the Nursery Industry©

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On 13 January1956 I started work. I was employed by the Master Gardener, Mr. Paul Sorensen, of Sorensen's Nursery in Leura as an apprentice for 5 years on a handshake and stayed for 14 years. During this time I did the Horticultural Certificate course at Ultimo in Sydney, now the Ryde School of Horticulture. I had no inkling then of the roller coaster ride I would have through horticulture up to the present day.

When I started my career, propagation was mainly hardwood cuttings under constant misting, using brass nozzles, outside under the shade of some high canopy trees. When the misting was left on in the winter there were times of the mist freezing and some quite spectacular displays created. The cuttings were stuck in community terracotta pots using a sand and peat mixture as the medium. Results were never very encouraging.

At this stage cold frames were built which consisted of a frame base of 16 ft long and 8 ft wide with a height of 18 inches. This was constructed of hardwood timber. The frame was covered with 8 ft × 4 ft-sash windows glazed with horticultural glass. In an effort to control the internal temperature, the frames were closed at night and opened to various levels during the day depending upon the weather. The unique thing about these frames was that they had a sloping side on one long side of the frame. The angle was 30° with the sheeting material being galvanised iron painted with silver paint for heat reflection. These frames were positioned in place, side facing true north, and they were sited in place with a compass.

Propagation was still hardwood cuttings, e.g., *Philadelphus, Deutzia, Kolkwitzia,* and *Berberis*. *Wisteria* cuttings were often bundled and held bottom end up until callus formed. These were then planted with often a good strike rate being achieved.