AUSTRALIAN NATIONAL BOTANIC GARDENS

Seed persistence in soil-seed banks of sub-Alpine Bogs and Fens

Alive with diversity

FINAL REPORT TO THE AUSTRALIAN ALPS LIAISON COMMITTEE JUNE 2017

Lydia K. Guja and Heather M. Brindley

Australian Government
Director of National Parks

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PROJECT BACKGROUND

Alpine *Sphagnum* bogs and associated fens (ASBAF) are a Nationally EPBC-listed endangered wetland community in the Australian Alps. Climate change is predicted to be the single greatest threat to the biodiversity values of the Australian Alps catchments, especially ASBAFs. More frequent and hotter fires, the drying out of important *Sphagnum* bogs and wetlands, and overall ‘up-mountain’ movement of vegetation communities are likely to occur by 2050.

The detailed knowledge required to appropriately conserve, manage, and restore endangered ASBAF communities in the face of rapid climate change is lacking. Current restoration techniques for bog and fen communities rely on slowing water flow and minimising erosion through damming, and shading to encourage *Sphagnum* regrowth. Although successful to date, these practices rely on existing soil propagules for plant re-establishment and their effectiveness will likely become less viable under climate change. Shorter disturbance regimes, such as changes to fire frequency and intensity, may limit the ability of plants to reach reproductive maturity and replenish the soil seed bank between disturbance events.

To improve the conservation, management, restoration and resilience of endangered ASBAF plant communities we require a clear, scientifically-based understanding of key processes that underpin re-establishment of these communities. One of the most critical processes requiring research is re-generation of bog and fen species from seed, and in particular the persistence of seeds in soil seed-banks. Understanding how long soil seed banks persist will ensure that post-disturbance restoration is not relying on diminished seed supply. Accordingly, the National Seed Bank (NSB) at the Australian National Botanic Gardens (ANBG) is conducting projects focussed on expanding *ex situ* conservation seed collections that will be used to provide the knowledge required to collect and use seed to supplement current restoration techniques.

The scientific project reported here, supported by the Australian Alps Liaison Committee (AALC) (reference A13-14/CC1), aims to promote understanding of the persistence of seed in endangered ASBAF communities. This has Alps-wide implications and will assist in efforts to mitigate biodiversity loss due to climate change. Research outcomes may also inform the development of operational, on-ground, management actions.

Cover images

Left: Seed samples being placed in field plots to be periodically retrieved.

Centre: Seed collectors making conservation collections at Snowy Flat, ACT.

Right: Data logger recording the temperature and moisture experienced by seeds buried in an alpine *Sphagnum* bog.

Outcomes

Specific outcomes for agency staff, land managers and other stakeholders working in the Australian Alps include:

- Improving the management and restoration of an endangered ecological community through an understanding of seed persistence and germination patterns in soil seed banks
- Identifying priority species for *in situ* management and conservation by understanding limits to their persistence in the soil and how this affects species' resilience
- Reducing the risk of erosion, and colonisation by invasive species, by understanding whether soil seed banks are sufficient to generate good plant cover after disturbance events
- Continuing collection and *ex situ* storage of seed of ASBAF species. Seed collections appropriately sourced, geo-referenced and genetically diverse, stored at the NSB for conservation, research and restoration

Please see **Appendix 1** for more detailed information.

Aims

This scientific research project aimed to understand:

1) Seed persistence in the soil for ASBAF species
2) Germination patterns of seeds in Endangered ASBAF communities

Results of the research will have Alps-wide implications and will assist in efforts to mitigate biodiversity loss due to climate change.

Relevance to AALC Strategic Plan 2012-2015

This project addresses **KRA1 Climate Change and Adaptation** by providing a contemporary approach to planning, responding and adapting to climate change. Increasing our knowledge of seed persistence and germination ecology in ASBAFs will assist mitigation of climate change impacts through appropriate *ex situ* conservation, better informed restoration, and adaptive management.

The research will assist with the development of landscape-wide approaches to managing endangered ASBAF ecological systems and processes, addressing **KRA2 Ecological Systems and Processes**. Increasing the knowledge and awareness of processes causing recruitment vulnerability in endangered ASBAF communities will improve *in situ* conservation, restoration techniques and management methods.

This novel approach will provide information that can assist mitigation of the effects of climate change, improve resilience through optimised restoration methods, and further develop restoration practices, thereby addressing **KRA3 Water and Catchments**. Improved restoration that considers seed availability will help to reduce erosion, improve hydrologic processes, and enhance the health of aquatic environments.

Please see **Appendix 1** for more detailed information.

Relevance to AALC Strategic Plan 2016-2018

The overall theme of the strategic plan 2016-2018 is **Connectivity through Catchment Health.** The strategic plan recognises that the underlying issue for the greater Australian Alps landscape is **Catchment Health**. The strategic plan also recognises that a healthy **Alps Biodiversity** is connected to healthy **Alps People** and that both hang off a healthy **Alps Catchment**.

This research project on seed persistence in sub-alpine bogs and fens extends understanding of the ecology and regeneration processes of healthy peatlands at catchment sources to inform management activities that maintain Alps biodiversity.

Please see **Appendix 1** for more detailed information.

REPORT ON ACTIVITIES

This report summarises activities undertaken over the life of this project (financial year 2013-14 to 2016-17). Outcomes and/or planned activities were delivered as expected, along with some additional events. Activities included research, *ex situ* conservation and communication of results.

Research activities

The data required to build an understanding of seed biology to inform management and restoration practices were collected from a seed burial experiment that started in June 2013 and was concluded in December 2015. Seed collecting was undertaken prior to the experiment and data analysis and interpretation have been undertaken since the conclusion of the experiment and up to the time this report was produced.

Methods

- Control samples (germination tests of fresh seeds that were not buried in the field) indicated that the seeds used in the 3 year experiment were viable (**Table 1**).
- Ten field plots were established in 2013 at Ginini Flats, Australian Capital Territory (ACT). Freshly collected seeds of 13 ASBAF species (*Baeckea gunniana*, *Baeckea utilis*, *Carex iynx*, *Epacris celata*, *Epacris paludosa*, *Epilobium gunnianum*, *Isolepis crassiuscula*, *Melaleuca pityoides*, *Olearia algida*, *Oxylobium ellipticum*, *Ozothamnus cupressoides*, *Ranunculus lappaceus*, and *Richea continentis*) were buried (3 months after the average collection date) in a randomised plot design. The species represent a mixture of germination strategies, dormancy types, life forms and plant families.
- In total 39,000 seeds were buried. These seeds were retrieved periodically to monitor seed germination and survival in the field. Retrieval of seeds occurred 6, 9, 12, 15, 24 and 30 months after dispersal (i.e. 3, 6, 9, 12, 21 and 27 months after burial).
- All of the retrieved, potentially viable and ungerminated seeds were tested in laboratory germination tests. Each of the 6, 9, 12, 15, 24 and 30 month seed batches underwent 140 days of germination trials, followed by cut-testing to determine seed fill and estimate the viability of non-germinated seeds.
- A second ‘control’ ran alongside the final 30 month seed batch. The second ‘control’ used seeds that had been stored *ex situ* (15 °C and 15% relative humidity). This tested for any underlying changes in seed dormancy or germination that were due to seed age rather than burial.
- All experimental seeds and equipment were removed from the field plots on 7 October 2015 (27 months after plots were established) and plots were restored to pre-burial conditions.

Table 1. *Germination test results for fresh seed prior to establishment of the burial experiment. Germination results are shown with and without treatments (S = Moist stratification from warm to cold to warm temperature, PY = Physical scarification with sandpaper, No = No treatment).*

Family	**Species**	**Authority**	**Accession**	**Treatment**	**Germination (%) ± se**	
					Without treatment	**With treatment**
Myrtaceae	*Baeckea gunniana*	Schauer	CANB 866373	S	32.8 ± 0.4	87.9 ± 1.2
Myrtaceae	*Baeckea utilis*	F.Muell.	CANB 866372	S	74.2 ± 0.9	71.6 ± 0.9
Cyperaceae	*Carex iynx*	Nelmes	CANB 865438	S	65.5 ± 1.5	19.4 ± 0.2
Ericaceae	*Epacris celata*	Crowden	CANB 866367	S	39.2 ± 0.0	37.7 ± 2.0
Ericaceae	*Epacris paludosa*	R.Br.	CANB 813615	S	14.4 ± 0.8	35.8 ± 0.6
Onagraceae	*Epilobium gunnianum*	Hausskn.	CANB 866342	No	95.2 ± 0.9	NA
Cyperaceae	*Isolepis crassiuscula*	Hook.f.	CANB 813612	S	51.3 ± 1.4	39.0 ± 1.8
Myrtaceae	*Melaleuca pityoides*	(F.Muell.) Craven	CANB 813602	No	89.3 ± 0.0	NA
Asteraceae	*Olearia algida*	N.A.Wakef.	CANB 813614	S	53.3 ± 1.8	72.3 ± 1.0
Fabaceae	*Oxylobium ellipticum*	(Vent.) R.Br.	CANB 865436	PY	0.0 ± 0.0	56.8 ± 0.0
Asteraceae	*Ozothamnus cupressoides*	Puttock & D.J.Ohlsen	CANB 866370	S	21.8 ± 1.1	12.5 ± 0.5
Ranunculaceae	*Ranunculus lappaceus*	Sm.	CANB 813613	S	36.2 ± 0.7	56.9 ± 0.6
Ericaceae	*Richea continentis*	B.L.Burtt	CANB 865439	S	5.6 ± 1.8	39.2 ± 1.3

se = standard error
CANB = Australian National Herbarium

- Environmental factors were also measured to determine whether they may be associated with any observed variation in germination. Measurements were made for pH from each of 10 field plots, and temperature and moisture from 4 plots (analysis of these data are ongoing and not included in this report).
- Data handling and analysis involved collating, cleaning, plotting and checking the data. Data were presented graphically to allow initial interpretation of the results while statistical analyses are completed.
- Trials were undertaken to grow some of the laboratory seedlings at the ANBG nursery in preparation for the establishment of a small ASBAF garden. Seedling survival was low upon transfer from laboratory tests to soil. Furthermore, the seedlings that survived were very slow growing. Therefore, direct sowing of seed and propagation via cuttings were also trialled to ensure plants were available for a display garden.

Results and discussion

Seed persistence

- The data indicate that seeds of 12 of the 13 tested plant species can persist and survive in the soil without germinating for at least one growing season (one species, *Melaleuca pityoides*, had very low seed viability which prevented analysis of results). Therefore, we can now confirm that a persistent (not transient) soil seed bank is formed in ASBAF communities which contain these species.
- Very few seeds germinated or died while buried in the field (see **Figure 1** for an example). Therefore, germination from the soil seed bank likely occurs in response to cues such as light and/or certain temperatures which may only occur in the soil after disturbance events.
- Seed germination 30 months after dispersal (and after 27 months burial) was often equal to or greater than germination of fresh seeds, indicating that further maturation, ripening or dormancy alleviation may occur while seeds are buried in the soil seed bank.
- It appears that only one species, *Isolepis crassiuscula,* may be losing viability after 27 months of burial (once the seed is 30 months old) (**Figure 2**).

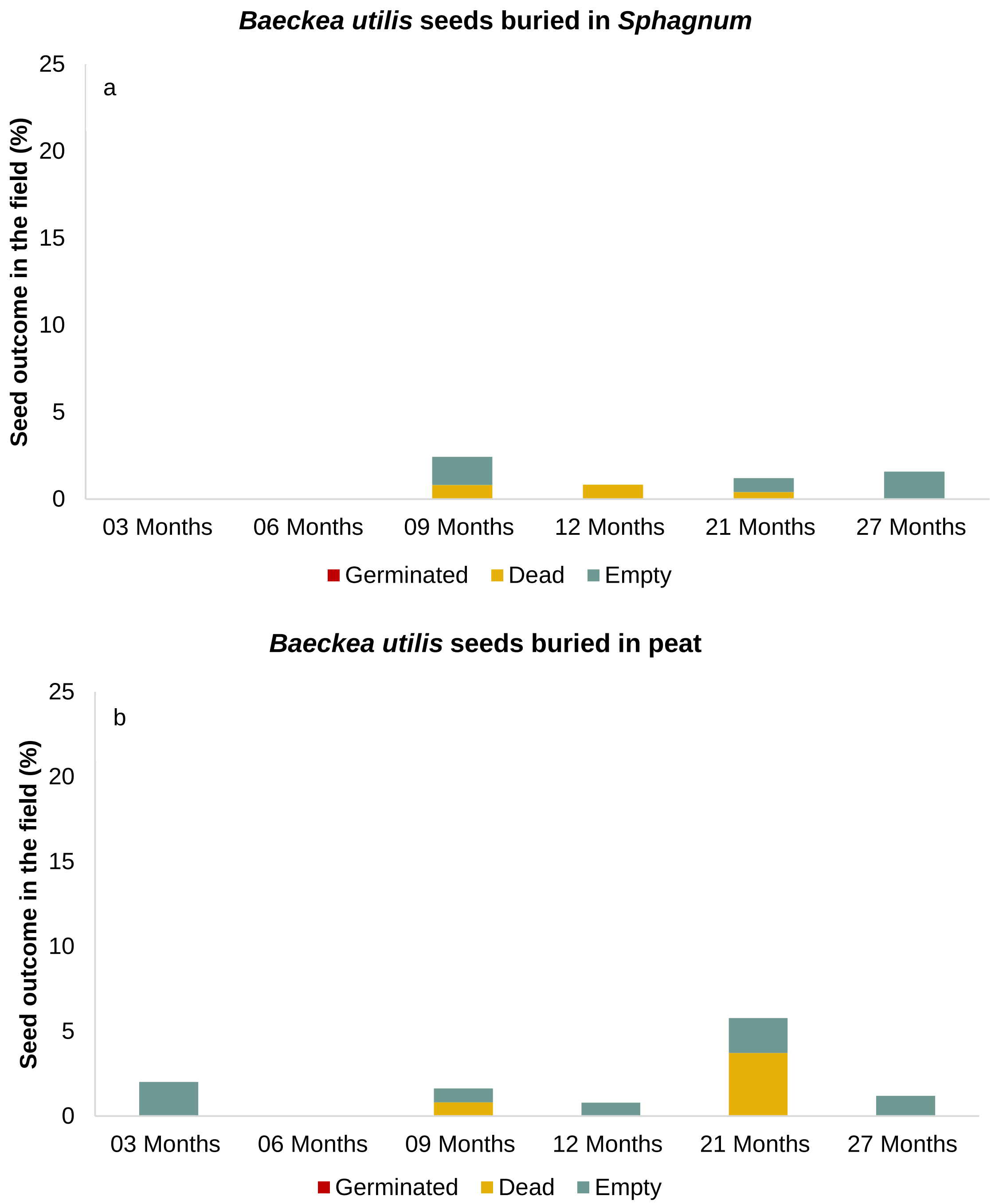

Figure 1. *Seed outcomes for* Baeckea utilis *seeds which were assessed as germinated (red), dead (yellow) or empty (blue) after 3, 6, 9, 12, 21 or 27 months buried in the field in a)* Sphagnum *moss and b) waterlogged peat. All seeds not clearly assignable to one of the above outcome categories were potentially viable and used in laboratory germination experiments. Note no seeds of this species germinated in the field.*

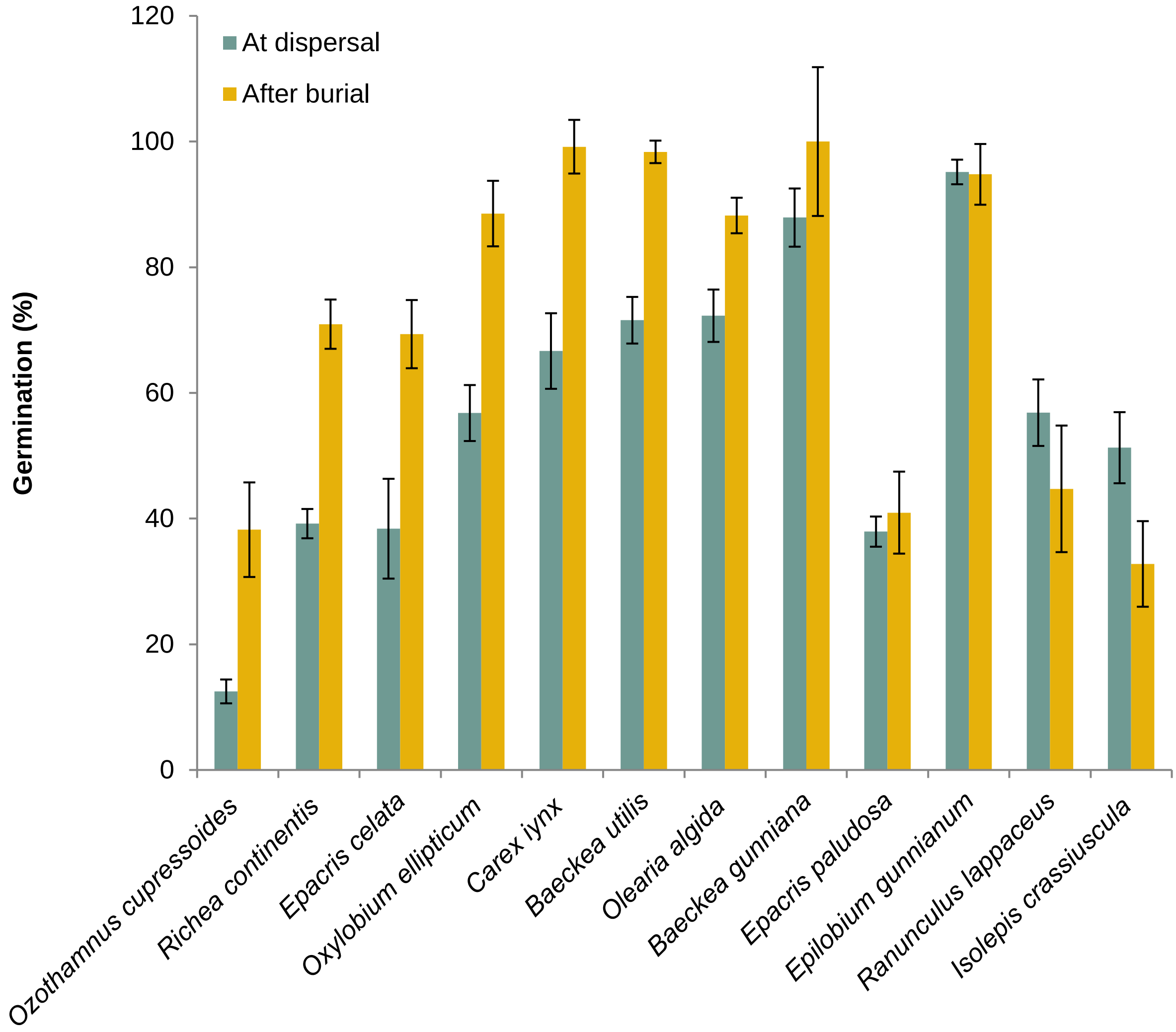

Figure 2. *Seed survival (measured by laboratory germination) as an indicator of survival and persistence in the soil. In this figure the highest laboratory germination of seed is displayed and the error bars represent standard error. Seed that was screened soon after dispersal (blue) is shown alongside a comparable germination treatment and the results achieved after 27 months burial plus lab treatment (yellow). Species are ordered by the size and direction of change in germination between dispersal and 27 months burial. Most species' seed germination after burial was higher than or equal to germination at dispersal (higher = left to right* O. cupressoides *to* O. algida*, and equal* = B. gunniana *to* R. lappaceus)*. Only* Isolepis crassiuscula *showed signs of viability loss (decline in germination).* Melaleuca pityoides *was omitted due to low seed fill which compromised the results for that species.*

Seasonal dormancy/germination cycling

- Most of the species (11/13) produce seeds that are dormant at dispersal.
- For two species interesting patterns of seasonal dormancy and germination cycling are evident. The proportion of seeds that germinate varies significantly between seasons. Species with dormancy cycling are most responsive to germination triggers during Spring (e.g. *Baeckea utilis* **Figure 3**). Therefore, recruitment will occur if soil seed banks are exposed to germination triggers such as light during spring. Conversely, even if germination triggers are present, the seeds have an extremely low germination capacity between Summer and Autumn.

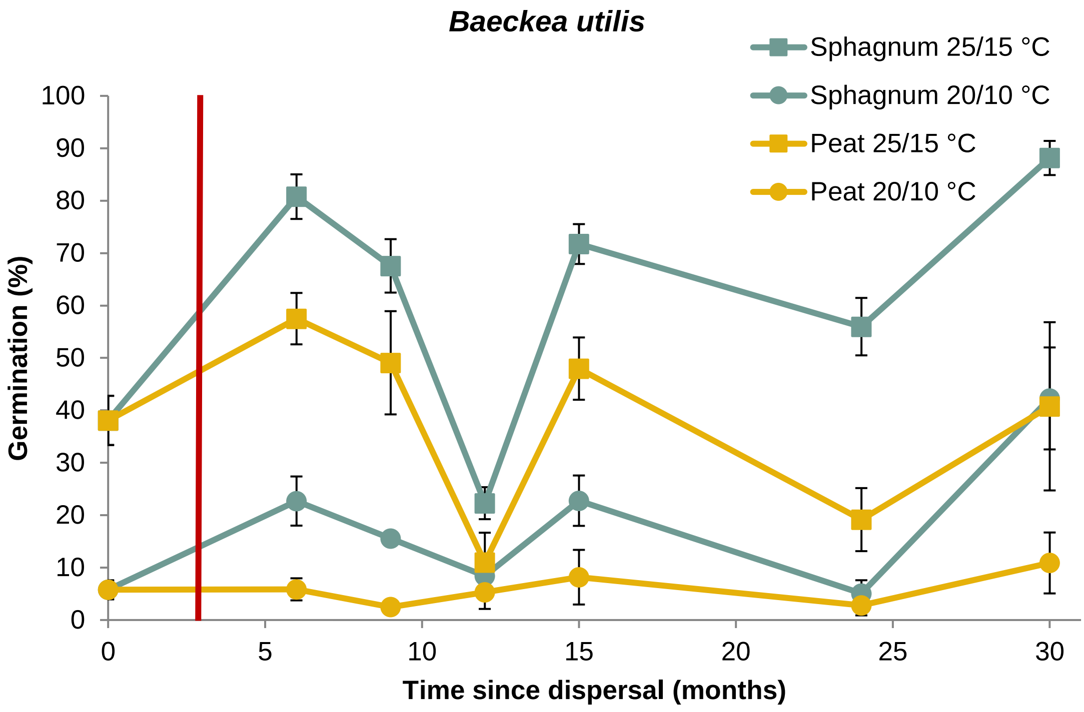

Figure 3*. Variation in germination (dormancy cycling) of* Baeckea utilis *seed from the time of dispersal (0 months) to 30 months after dispersal. Seeds were buried 3 months after dispersal (red vertical line) in* Sphagnum *(blue) or peat (yellow). Seed samples were regularly retrieved from the burial plots and the non-germinated viable seeds underwent laboratory tests to generate the data presented here. All germination tests were conducted under identical laboratory conditions and tested the effect of two different temperature regimes on germination. Seeds were exposed to a daily regime of 12 hours at 25 °C in light and 12 hours at 15 °C in dark (square symbols); or, a regime of 12 hours at 20 °C in light and 12 hours at 10 °C in dark (circular symbols).*

Effect of substrate

- Since no seeds germinated, and very few died in the field, seed survival in the two adjoining substrates (waterlogged peat versus *Sphagnum* moss) was equivalent. However, in some species, the proportion of seeds responsive to germination cues differed markedly between substrates (e.g. *Baeckea utilis* **Figure 3**).
- These results mean that, for some species (e.g. *Baeckea utilis* **Figure 3**), when exposed to cues such as light and warm temperatures (as they were in the laboratory) seeds that have been buried in *Sphagnum* moss (blue) are more likely than those that had been buried in peat (yellow) to germinate and exit the soil seed bank. The germination of other species (e.g. *Carex iynx* **Figure 4**) did not differ as much with substrate.

Germination speed

- For some species the time buried made much more of a difference to germination speed than the substrate. In **Figure 4**, the time to first germination in the lab (y axis, days) of *Carex iynx* seed was reduced from approximately 120 days for fresh seed to approximately 20-30 days for seeds 15 months or longer after dispersal. This demonstrates that duration buried in the peatland increased germination speed.

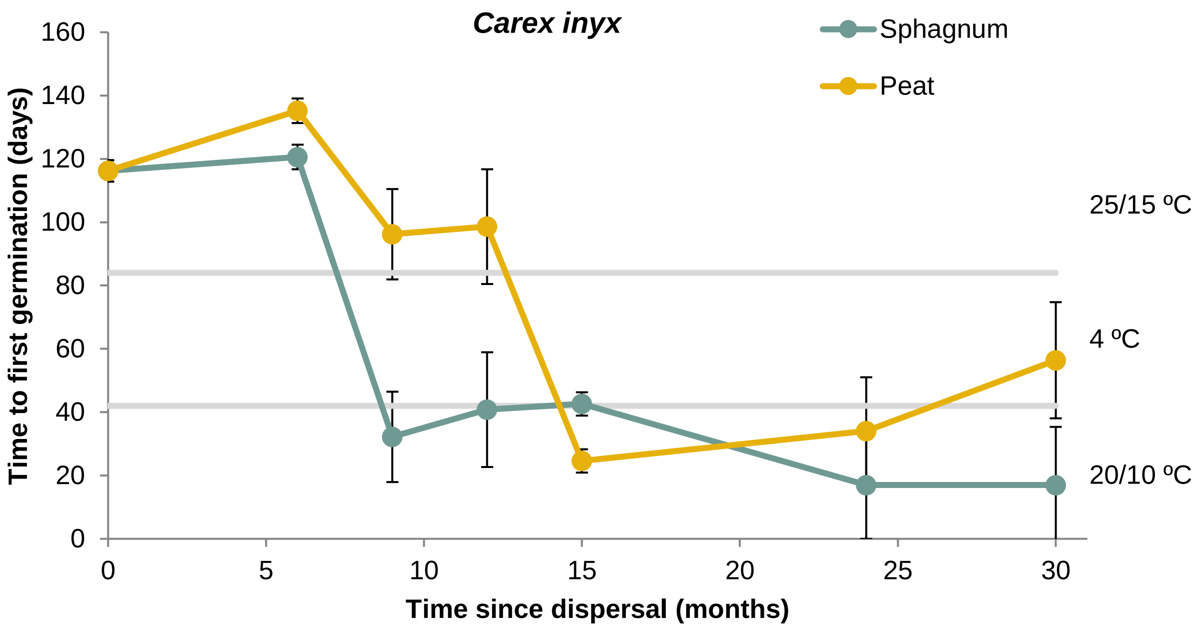

Figure 4. *Variation in germination speed of* Carex iynx *seeds from the time of dispersal (0 months) to 30 months after dispersal. Seed was buried 3 months after dispersal, in* Sphagnum *(blue) or peat (yellow). Seed samples were regularly retrieved from the burial plots and the non-germinated viable seeds underwent laboratory tests to generate the data presented here. All seeds were exposed to 20/10 ºC (12/12 hours) and light/dark (12/12 hours) for 42 days, followed by 4 ºC in constant light (24 hours) for 42 days, and finally 25/15 ºC (12/12 hours) and light/dark (12/12 hours) for 42 days. Temperature changes are indicated by grey horizontal lines.*

Summary

When our research began it was unclear whether soil seed banks formed in alpine bog and fen communities, and little was known about the seed ecology of key plant species. We discovered that:

A viable soil seed bank is likely to be present and capable of germinating

- Thirty months after dispersal only one of the 13 species, *Isolepis crassiuscula*, demonstrated a decrease in viability (decline in germination). Seed viability for one of the 13 species, *Melaleuca pityoides*, was too low to assess persistence.
- Seeds of 12/13 tested plant species can form a persistent soil seed bank.
- The soil seed bank of 11/13 species can survive for at least 30 months after dispersal.

Many seeds are dormant at dispersal

- Most (11/13) of the species studied had seeds that were dormant at dispersal and required a pre-treatment to germinate (**Table 2**). In general, mimicking seasonal temperature changes was an effective method for improving germination i.e. moving moist seeds through warm>cold>warm temperatures.
- Germination of the two non-dormant species was fast, while for the rest of the species it took between 18 and 186 days for germination to finish (**Table 2**).

Table 2. *Summary of the seed and germination traits of the study species.*

Name and seed traits						Germination in the best treatment and/or substrate, after 56 to 140 days of lab incubation (viability adjusted)			Germination result and/or rate typically increases after a moist warm>cold>warm treatment?	Comments
Photo key	Species	Approx. dimensions (mm)	Weight of 1000 seeds (g)	Dormant at dispersal?	Viability at dispersal (%)	Fresh unburied seeds (%)	Seeds buried for 27 months (%)	Time to 50% of max. germination for seeds buried for 27 months (days (temp. regime °C))		**Method:** After burial, seeds were incubated in a laboratory in light/dark (12/12 hours) with a temperature regime of either 20/10 °C (12/12 hours) or 25/15 °C (12/12 hours). For warm>cold>warm treatments, seeds were kept moist and placed in one of the warm regimes for 6 weeks, then a cold regime (constant 5 °C) with 24 hour light for 6 weeks, and then into one of the warm regimes for 8 weeks.
1	***Baeckea gunniana***	0.92 x 0.53	0.072	Yes	98	88	100	17 (25/15 °C)	Yes	Seasonal dormancy changes. Germination is tightly tuned to environmental conditions.
2	***Baeckea utilis***	0.90 x 0.58	0.068	Yes	73	72	98	12 (25/15 °C)	Yes	Seasonal dormancy changes. Germination is tightly tuned to environmental conditions.
3	***Carex iynx***	3.8 x 1.8	2.030	Yes	95	67	99	13 (25/15 °C)	Variable	Ripening and/or maturation occur after the seeds are dispersed, but germination also seems to depend on other processes or cues the seeds receive in the soil. The time to first germination grew shorter the longer seeds were buried.
4	***Epacris celata***	0.52 x 0.33	0.022	Yes	82	38	69	26 (25/15 °C)	Yes	Most seeds were viable and germinated best with burial and a moist warm>cold>warm treatment.
5	***Epacris paludosa***	0.54 x 0.40	0.028	Yes	99	38	41	26 (25/15 °C)	Yes	Most seeds were viable but did not germinate >50% under the conditions tested.
6	***Epilobium gunnianum***	1.2 x 0.43	0.068	No	99	95	95	4.5 (20/10 °C)	Not tested	These seeds are not dormant. They germinate under a broad range of temperatures in light.
7	***Isolepis crassiuscula***	1.6 x 0.97	0.142	Yes	93	51	33	17 (25/15 °C)	Variable	Most seeds were initially viable but did not germinate >50% under the conditions tested. Germination declined over time and the seeds may be starting to degrade by 27 months.
8	***Melaleuca pityoides***	1.2 x 0.35	0.040	No	13	--	--	4-19 (20/10 °C)	Not tested	Also known as *Callistemon pityoides* . Viable seeds are non-dormant and germinate under a range of temperatures and in light. However, only very few seeds (5%) were viable and could germinate. Most seeds appeared intact externally but contained no embryo or endosperm. Allow for this when sowing seed.
9	***Olearia algida***	1.6 x 0.50 *	0.159	Yes	96	72	88	9 (20/10 °C)	Yes	Ripening and/or maturation occur after the seeds are dispersed, but germination also seems to depend on other processes or cues the seeds receive in the soil.
10	***Oxylobium ellipticum***	2.4 x 1.7	3.170	Yes	74	57	89	16 (20/10 °C)	Not tested	These seeds are physically dormant. Once the hard seed coat is broken by scarification (e.g. with sand paper), the seeds germinate under a broad range of temperatures in light. Young seedlings were very prone to mould and fungi.
11	***Ozothamnus cupressoides***	1.3 x 0.48 *	0.159	Yes	90	13	38	25 (25/15 °C)	Variable	Most seeds were viable but did not germinate >50% under the conditions tested.
12	***Ranunculus lappaceus***	3.9 x 2.1	2.730	Yes	99	57	45	93 (20/10 °C)	Variable	Ripening and/or maturation occur after the seeds are dispersed, but germination also seems to depend on other processes or cues the seeds receive in the soil.
13	***Richea continentis***	0.90 x 0.42	0.066	Yes	100	39	71	32 (20/10 °C)	Yes	Most seeds were viable and germinated best with burial and a moist warm>cold>warm treatment.

* seed only, appendanges excluded -- data not available due to low number of viable seeds

Four germination strategies were observed

- The ability of seeds to germinate varied by species and can be summarised using four conceptual models (**Figure 5**).

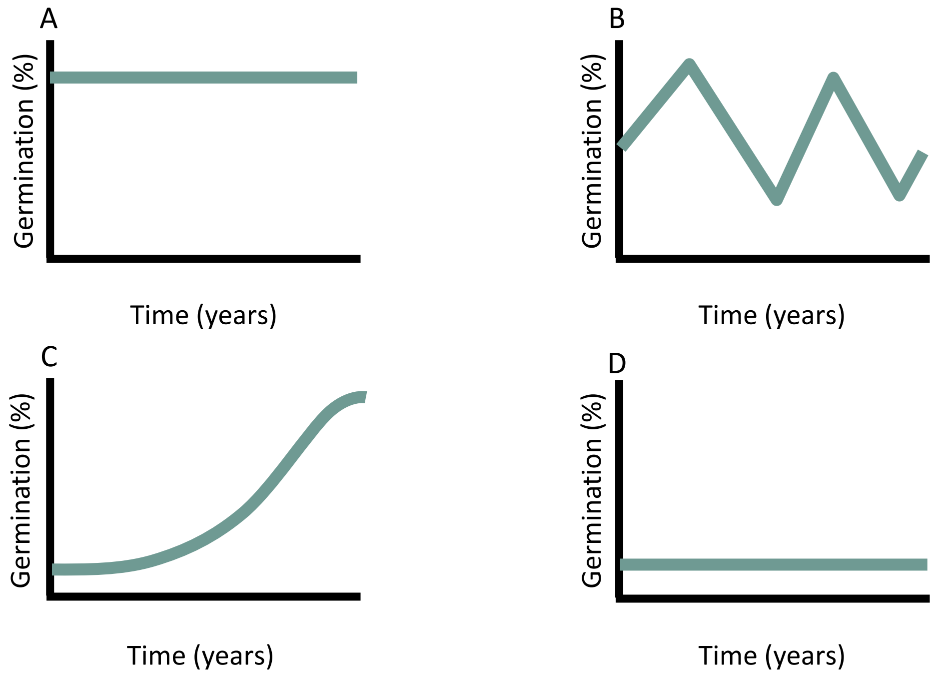

Figure 5. *Conceptual models describing seed germination and dormancy cycling patterns observed among the 13 study species. The responsiveness of buried seeds to germination cues changes with time and this will likely affect community composition relative to disturbance timing, and can have evolutionary and land management implications.*

Model A. Seeds germinate well any time the environmental conditions are appropriate e.g. *Epilobium gunnianum, Melaleuca pityoides* and *Oxylobium ellipticum* (once physical dormancy is alleviated).

Model B. Seeds show seasonal dormancy cycling. Germination is very tightly tuned to the environment and the probability of seeds germinating changes each season e.g. *Baeckea gunniana* and *B. utilis*.

Model C. Ripening and/or maturation occur after the seeds are dispersed, but germination also seems to be affected by some unidentified processes or cues the seeds receive in the soil e.g. *Carex iynx, Olearia algida*, and *Ranunculus lappaceus*. Germination of these species may increase after a moist warm>cold>warm treatment but results can be variable (**Table 2**).

Model D. Fresh seeds are viable but do not germinate well (<50%) under the experimental conditions trialled in this study e.g. *Epacris celata, E. paludosa, Isolepis crassiuscula, Ozothamnus cupressoides*. Burial in the field followed by a moist warm>cold>warm cycle in the lab was somewhat successful for germinating the key ASBAF species *Richea continentis* (**Table 2**) and future research may reveal why burial promotes germination by identifying the processes or cues the seeds receive in the soil.

Ex situ conservation activities

Seed banking

Conservation seed collections were made from bogs and fens across the Australian Alps during the project and are being/have been processed and incorporated into the long term, *ex situ*, conservation collection of the National Seed Bank at the ANBG in Canberra.

- Overall, 165 collections (accessions) were made from ASBAF communities across the Australian Alps (ACT, New South Wales (NSW), and Tasmania) in the summers of 2013-14, 2014-15, and 2015-16 under permit from each jurisdiction as required.
- 13 collections from the ACT were used in the seed burial experiment (**Table 1**).
- As collections are cleaned, equilibrated to 15% relative humidity, and germination tests are completed the collections will be placed in long term storage at -20 °C in the NSB. This work is progressing with the assistance of volunteers and as resources become available.
- Two main datasets were collected for this project, seed collection data and the experimental data.
    - Seed collection data including details of herbarium vouchers, collection date, locality and habitat can be accessed online via the Australian National Herbarium Specimen Information Register (ANHSIR) https://www.anbg.gov.au/cgi-bin/anhsir. The amount of seed available per accession can be retrieved via an advanced search of the Australian Seed Bank Partnership website http://asbp.ala.org.au/. Please contact the authors for assistance in interrogating these databases.
    - Experimental data detailing the proportion of seeds that were viable or germinated over time will be prepared for publication in scientific journals. The data may be included in publications or may otherwise be supplied by the authors upon request.
- To understand the representativeness of ASBAF species in the NSB's *ex situ* seed collections a student project was undertaken. A summer student from the Commonwealth Scientific and Industrial Research Organization (CSIRO) was supervised by the NSB Seed Conservation Biologist from December 2014 to February 2015. The student investigated how well the NSB's collections represent ASBAF communities. They found that of the 211 species known to occur (frequently and occasionally) in ASBAFs, 54% were conserved in the NSB's collection at that time. The remaining 46% of species will be targeted for collecting efforts in the future, as opportunities arise.

Display garden

Living plants were grown for display in a bog and fen garden at the ANBG. This garden also contributes to the *ex situ* conservation of bog and fen plants. Preparations for the display garden included:

- Trial display bowls were initially established for an art exhibition at the ANBG Visitor Centre in April 2015 (**Figure 6a**). The design of the permanent display garden was built on the success of these trial bowls, including a compilation of multiple bowls and a central reservoir to cool the soil during summer heat waves.
- Different propagation methods were trialled to produce plants for the display garden, including direct sowing of seed, transplant of seedlings from laboratory experiments to tube stock, and propagation via cuttings (**Figure 6b**).
- The display garden was installed in a highly visited area at the entrance to the Visitor Centre at the ANBG, at the starting point for guided tours (**Figure 6c**).
- An interpretative sign and visitor information leaflet have been produced to describe the significance of the alpine bog and fen wetland community (**Figure 7**).

***Figure 6 a)** Trial bog and fen display garden and interpretative sign at the ANBG Visitor Centre in April 2015. This garden was initially displayed as part of a Craft ACT Artist-In-Residence exhibition **b)** plants ready for planting in the final display garden **c)** installation of the final garden. All images L Guja © Director of National Parks.*

Communication of results

During the course of the project results have been discussed with various stakeholders via informal communication as well as more formally to land managers, other scientists and the general public.

Land managers

- Presentation titled '*The role of botanic gardens and seed banks in ex situ conservation, restoration & research: Examples from the Australian Alps*' at a 'Plant Conservation and Management for the Australian Alps' symposium in Canberra, June 2014. Proceedings available at http://www.anbg.gov.au/gardens/living/seedbank/2014-alpine-symposium-report.pdf.

- Presentation titled '*Seed persistence in soil seed banks of sub-alpine bogs and fens*' at a symposium hosted by Australian Alps National Parks, Thredbo, November 2015.

- An ANBG Fact Sheet about seed germination of Alpine Bog and Fen Plants that is in preparation to summarise the research findings for land managers. The fact sheet will be made available at http://www.anbg.gov.au/gardens/living/seedbank/ in 2017.

Research scientists

- Presentation titled '*Ecological drivers of seed germination in endangered Australian bog & fen communities*' for peatland conservation specialists in December 2013 at the 'International Mire Conservation' Symposium held at the Fenner School of the Australian National University (ANU), Canberra.

- A bulletin article published for international peatland conservationists and interested public in January 2015 and available at http://www.imcg.net/pages/publications/bulletin.php.

- Presentation titled '*Comparative longevity & persistence of Australian alpine seed*' at an international conference 'Seed Longevity', hosted by the International Society for Seed Science, in Wernigerode, Germany, July 2015 (approximately 160 delegates from 40 countries).

- A poster (**Appendix 3**) presented at the National Seed Science Forum, hosted by the Australian Seed Bank Partnership and Australian Grains Genebank, in Sydney, Australia, March 2016 (145 delegates from 9 countries).

- A presentation titled '*Seed dormancy and germination ecology in endangered alpine peatlands: informing conservation and management*' delivered to ecologists in a 'Seed Ecology' symposium at the Ecological Society of Australia conference, Fremantle, Perth, WA, Australia, December 2016 (approximately 60 attendees).

- Two scientific papers are in draft preparation for submission to peer reviewed journals.

General public

- A Friends of ANBG ‘Thursday Talk’ titled ‘*Seed biology and conservation of endangered plants at the National Seed Bank*’ delivered in April 2014 (approx. 50 attendees).
- Participation in a Craft ACT Artist-In-Residence program focussing on peatland conservation. Two parallel exhibitions of the artists’ final work (at Craft ACT) and research process (at ANBG) were held from April to May 2015. A small ASBAF garden was established at the ANBG Visitor’s Centre as part of the exhibition (**Figure 6a**). These exhibitions communicated the significance of ASBAFs and the need for their conservation to the public.
- Presentation titled ‘*Native seed conservation and research*’ for the Friends of ANBG Plant Science Group in March 2017 (31 attendees).
- New display garden near the ANBG Visitor Centre where the general public can learn about the significance of ASBAFs and their conservation. The display garden will soon be launched on the ANBG’s Facebook page when the interpretative sign and leaflets are released.
- Regular updates on the project and development of the display garden to all ANBG staff during monthly ‘Staff Awareness’ meetings 2013-2017.

Figure 7. *New alpine bog and fen display garden and interpretative sign, opened in 2016 at the Australian National Botanic Gardens, Canberra.*

Key research findings and interpretation

The key findings regarding seed persistence, dormancy and germination of each species are summarised in a fact sheet (**Appendix 2**). Some of the implications for Alps agencies, land managers and other stakeholders to consider when making land management decisions or prioritising peatland restoration include:

- If the studied plant species are present as mature standing vegetation that is reproducing then their seeds can form a persistent soil seed bank.
- Seeds will be capable of germinating after a disturbance event and in the presence of germination cues such as warmer soil and increased light.
- Species exhibit different germination strategies, therefore, will germinate at different times or in response to different environmental factors.
    - Soil seed banks of species in group A and B may be most resilient to disturbance. Germination and a new cohort of seedlings can be expected after disturbance, therefore, these species are less likely to require restoration planting if mature plants were present recently or are still present nearby.
    - Species in group C may be slow to germinate after disturbance. If soil seed banks are young (<1-2 years), or seedlings are not present after the first year, restoration intervention could be considered. Be aware that if fresh seed is used germination may be low and sites should be monitored for germination for several seasons/years.
    - The seeds of species in groups B and C often (but not consistently) germinate better than fresh seeds if they are exposed to moist warm>cold>warm conditions. Other alpine research shows that species with these germination strategies may be most affected by climate warming if the duration or temperature of chilling becomes insufficient to alleviate dormancy.
    - Species in group D may be very slow and difficult to germinate in the field or in production. Because their germination cues are not yet well understood these species are likely difficult to produce from seed for restoration.
- While propagating plants for the display garden we noted these species are slow to grow. While cuttings or division may be the best option/s for obtaining suitably sized plants in a shorter timeframe, genetic diversity should be considered if this method is used.

ACKNOWLEDGEMENTS

Thanks to ANBG staff and seed bank volunteers who assisted with seed collection, processing and counting replicates of seeds. In particular, volunteers John Fitz Gerald, Annette Harry, and Doreen Wilson prepared samples for deployment to the field plots. Liam Banyer, Bruce Clarke, Brook Clinton, Pete Feilen, Suzie Fletcher, Geoff Hope, Fanny Karouta-Manasse, Ben Keany, Sue Lawatsch, Tom North, Amelia Stevens and Mark Wallace provided technical assistance with field work, germination testing and assessment of seed survival, on occasion, during the course of this project. Assistance in species determination and seed collecting was provided by taxonomic staff from the Centre for Australian National Biodiversity Research (CANBR). Phil Hurle and Julie Percival are thanked for design and horticultural work for the display garden, and Kate Adams and Ben Harvey for assistance with interpretative material.

ACT park rangers and conservation staff who assisted with seed collection and experiment set-up are also thanked; in particular Emma Cook, Brandon Galpin, Luke Johnston and Ben Stevenson. The Bush Blitz team, CANBR and James Wood of the Tasmanian Seed Conservation Centre are thanked for making seed collection in Tasmania possible.

Contact

Lydia Guja

Research Scientist and Manager, National Seed Bank

Biodiversity Science Section, Australian National Botanic Gardens and

Centre for Australian National Biodiversity Research, CSIRO

Address: Clunies Ross St, Acton ACT 2601

Postal address: GPO Box 1777, Canberra ACT 2601

Phone: (02) 6250 9471

Email: Lydia.Guja@environment.gov.au

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APPENDIX 1

Project outcomes

AALC Strategic Plan 2016-2018		AALC Strategic Plan 2012-2015	This Project (A13-14/CC1)		
Core Values	Priority Issues and Objectives	Key Result Areas (KRA)	Desired outcome	Approach to achieve outcome	Result/Output
Resilient Natural Landscapes	Invasive species Landscape-wide Catchment and Ecological Issues Climate Change and Adaptation	KRA1 Climate Change and Adaptation KRA2 Ecological Systems and Processes KRA3 Water and Catchments KRA4 Invasive Species Management KRA5 Fire Management	Improving the management and restoration of an endangered ecological community	Understand seed persistence in soil seed banks Understand germination patterns in soil seed banks	All study species can form persistent soil seed banks Species' dormancy and germination summarised in 4 conceptual models This project report and Fact Sheet for Land Managers (Appendix 2) (see 'Communication of results' section)
			Identifying priority species for *in situ* management and conservation	Understand limits to the persistence of seeds of ASBAF species in the soil Understand the implications of persistence limits for species' resilience	

AALC Strategic Plan 2016-2018		AALC Strategic Plan 2012-2015	This Project (A13-14/CC1)		
Core Values	Priority Issues and Objectives	Key Result Areas (KRA)	Desired outcome	Approach to achieve outcome	Result/Output
			Reduce risk of erosion, and colonisation by invasive species after disturbance	Understand whether soil seed banks are sufficient to generate good plant cover after disturbance events	Two scientific papers (in preparation) (See ‘Communication of results’ section)
			Collection and *ex situ* storage of seed of ASBAF species for conservation, research and restoration	Seed collections appropriately sourced, geo-referenced and genetically diverse, stored in the NSB at the ANBG	(see ‘Seed banking’ section)

AALC Strategic Plan 2016-2018		AALC Strategic Plan 2012-2015	This Project (A13-14/CC1)		
Core Values	Priority Issues and Objectives	Key Result Areas	Desired outcome	Approach to achieve outcome	Result/Output
Living Cultural Landscapes	Aboriginal Peoples Involvement	KRA8 Indigenous People's Engagement KRA9 Cultural Heritage	Acknowledge Traditional Owners and other relevant Heritage.	Relevant permits for collections	Approved permits for accessions (see 'Seed banking' section)
Connecting People to the Australian Alps National Parks	Aboriginal Peoples Involvement (as above) Communications	KRA6 Visitor Experiences KRA7 Stakeholder Engagement and Communication	Informed and engaged people	Provide input into the development of visitor experiences Communicate project findings through relevant publications Communicate project findings at interested forums	ANBG Bog and Fen Display Garden (see 'Display garden' section) Fact Sheet for Land Managers (Appendix 2) (see 'Communication of results' section) Other communication activities (see 'Communication of results' section)

AALC Strategic Plan 2016-2018		AALC Strategic Plan 2012-2015	This Project (A13-14/CC1)		
Core Values	Priority Issues and Objectives	Key Result Areas	Desired outcome	Approach to achieve outcome	Result/Output
Skilled and well-connected agency staff	Communications (as above) Knowledge Management	KRA10 Program Management KRA11 Program Promotion and Information	Progress Reporting	Standard Reports	Interim and Final Reports
			Accession information of ASBAF seed collections available for further research	Databases (ANHSIR website and the Australian Seed Bank Partnership website)	Collection data available (see ‘Seed banking’ section) Germplasm available on request to the NSB.
			Original data available for further research	Document nature and location of data, contact person, data analysis environment etc.	Retrievable data (on approved request) (see ‘Contact’ section)
			Capture ANBG Nursery experience of propagation methods for ASBAF species	Document propagation methods trialled.	Forms part of Land Managers Information Leaflet (Appendix 2). (see ‘Communication of results’ section)

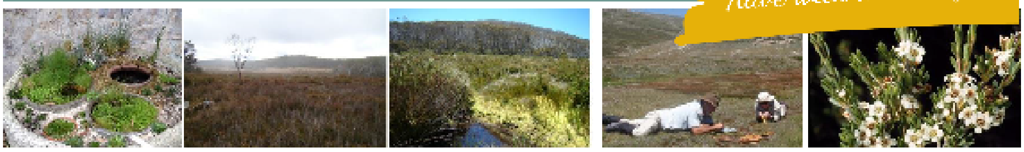
APPENDIX 2

Project summary fact sheet

National Seed Bank Fact Sheet

AUSTRALIAN NATIONAL BOTANIC GARDENS

Alive with discovery



SEED GERMINATION OF SUB-ALPINE BOG AND FEN PLANTS

Alpine *Sphagnum* bogs and associated fens occur in alpine and subalpine parts of Australia and are federally listed as endangered ecological communities. These peatlands provide many benefits such as water filtration at catchment sources. They also provide breeding habitat for endangered species such as Corroboree frogs. Peatlands are small, fragmented and can be damaged easily. Increasing fire frequency has been of particular concern for land managers. The methods used to successfully restore peatlands after fire rely on unknown stocks of plant material surviving in the peat after fire. When our research began it was unclear whether soil seed banks formed in these communities, or how they might affect regeneration of the plant community.

We investigated 13 bog and fen plant species to find out whether their seeds can form soil seed banks, and how their germination might be affected by burial. We found that the seeds of most of these species were still viable after burial for multiple growing seasons and could germinate under particular conditions detailed on the next page. Land Managers can use these findings to inform restoration practices for key plant species in degraded alpine peatlands.

Seed persistence

We found that a persistent soil seed bank (2+ years) can form in peatland plant communities. Laboratory germination tests showed that after 27 months burial, seed germination was often equal to or greater than germination of dry-stored or fresh seed. Only one species[7] began to decline in viability.

Very few seeds died or germinated while buried. This indicates that germination from the soil seed bank is most likely to occur after disturbance events, when seeds are exposed to certain environmental cues.

Seed dormancy

The fresh seeds of two species[6,8] germinated well within days of starting laboratory tests. The seeds of the remaining study species were dormant at dispersal i.e. they need exposure to various ecological processes before germination can occur. We found that a period of moist warm>cold>warm treatment (mimicking progression through autumn>winter>spring) increased germination in most of the dormant species. The hard seed coats of *O. ellipticum*[10] had to be scarified to allow water into the seed and germination.

For some species[1,2] the proportion of seeds that germinated in the laboratory after burial varied significantly among seasons. For these species, seedling recruitment will most likely occur if soil seed banks are exposed to germination cues during Spring. In Summer these species will have a low germination capacity, even if the same cues are present.

Growing bog and fen plants

We established an Alpine Bogs and Fens display garden in Canberra. Our plants showed typical alpine behaviour – small, slow-growing, and may spend years establishing roots before enlarging above ground.

Some of our plants were grown from seeds germinated in the laboratory. Many of these died upon transfer to soil (tube stock), presumably due to root damage. Survival rates might be better if seeds are sown directly into tube stock. If time is limited, dividing an existing clump, or taking cuttings, may propagate these species faster than growing from seed. Genetic diversity should be considered if this method is used for restoration.

Temperature and moisture variation are a challenge for the display garden because Canberra (mean January max temp 30 °C, mean annual rainfall 637 mm) is hotter and dryer than the Australian Alps (16 °C and 1430 mm). We grew young plants in a sheltered glasshouse and then gradually acclimatised them to conditions in their final location. The display garden is protected from the direct afternoon sun. On hot days we use large pieces of ice to cool the soil and water.

Produced by National Seed Bank, Biodiversity Science Section
www.anbg.gov.au/gardens/living/seedbank

Centre for Australian National Biodiversity Research
Australian Government
Director of National Parks

Summary of species and key findings

1 2 3 4 5 6 7 8 9 10 11 12 13

Name and seed traits						Germination in the best treatment and/or substrate, after 56 to 140 days of lab incubation (viability adjusted)			Germination result and/or rate typically increases after a moist warm>cold>warm treatment?	Comments
Photo key	Species	Approx. dimensions (mm)	Weight of 1000 seeds (g)	Dormant at dispersal?	Viability at dispersal (%)	Fresh unburied seeds (%)	Seeds buried for 27 months (%)	Time to 50% of max. germination for seeds buried for 27 months (days (temp. regime °C))		**Method:** After burial, seeds were incubated in a laboratory in light/dark (12/12 hours) with a temperature regime of either 20/10 °C (12/12 hours) or 25/15 °C (12/12 hours). For warm>cold>warm treatments, seeds were kept moist and placed in one of the warm regimes for 6 weeks, then a cold regime (constant 5 °C) with 24 hour light for 6 weeks, and then into one of the warm regimes for 8 weeks.
1	*Baeckea gunniana*	0.92 x 0.53	0.072	Yes	98	88	100	17 (25/15 °C)	Yes	Seasonal dormancy changes. Germination is tightly tuned to environmental conditions.
2	*Baeckea utilis*	0.90 x 0.58	0.068	Yes	73	72	98	12 (25/15 °C)	Yes	Seasonal dormancy changes. Germination is tightly tuned to environmental conditions.
3	*Carex iynx*	3.8 x 1.8	2.030	Yes	95	67	99	13 (25/15 °C)	Variable	Ripening and/or maturation occur after the seeds are dispersed, but germination also seems to depend on other processes or cues the seeds receive in the soil. The time to first germination grew shorter the longer seeds were buried.
4	*Epacris celata*	0.52 x 0.33	0.022	Yes	82	38	69	26 (25/15 °C)	Yes	Most seeds were viable and germinated best with burial and a moist warm>cold>warm treatment.
5	*Epacris paludosa*	0.54 x 0.40	0.028	Yes	99	38	41	26 (25/15 °C)	Yes	Most seeds were viable but did not germinate >50% under the conditions tested.
6	*Epilobium gunnianum*	1.2 x 0.43	0.068	No	99	95	95	4.5 (20/10 °C)	Not tested	These seeds are not dormant. They germinate under a broad range of temperatures in light.
7	*Isolepis crassiuscula*	1.6 x 0.97	0.142	Yes	93	51	33	17 (25/15 °C)	Variable	Most seeds were initially viable but did not germinate >50% under the conditions tested. Germination declined over time and the seeds may be starting to degrade by 27 months.
8	*Melaleuca pityoides*	1.2 x 0.35	0.040	No	13	–	–	4-19 (20/10 °C)	Not tested	Also known as *Callistemon pityoides*. Viable seeds are non-dormant and germinate under a range of temperatures and in light. However, only very few seeds (5%) were viable and could germinate. Most seeds appeared intact externally but contained no embryo or endosperm. Allow for this when sowing seed.
9	*Olearia algida*	1.6 x 0.50 *	0.159	Yes	96	72	88	9 (20/10 °C)	Yes	Ripening and/or maturation occur after the seeds are dispersed, but germination also seems to depend on other processes or cues the seeds receive in the soil.
10	*Oxylobium ellipticum*	2.4 x 1.7	3.170	Yes	74	57	89	16 (20/10 °C)	Not tested	These seeds are physically dormant. Once the hard seed coat is broken by scarification (e.g. with sand paper), the seeds germinate under a broad range of temperatures in light. Young seedlings were very prone to mould and fungi.
11	*Ozothamnus cupressoides*	1.3 x 0.48 *	0.159	Yes	90	13	38	25 (25/15 °C)	Variable	Most seeds were viable but did not germinate >50% under the conditions tested.
12	*Ranunculus lappaceus*	3.9 x 2.1	2.730	Yes	99	57	45	93 (20/10 °C)	Variable	Ripening and/or maturation occur after the seeds are dispersed, but germination also seems to depend on other processes or cues the seeds receive in the soil.
13	*Richea continentis*	0.90 x 0.42	0.066	Yes	100	39	71	32 (20/10 °C)	Yes	Most seeds were viable and germinated best with burial and a moist warm>cold>warm treatment.

* seed only, appendanges excluded – data not available due to low number of viable seeds

This research project was supported by the Australian Alps national parks Co-operative Management Program. The ACT Government assisted with seed collection and establishment of plots in Namadgi National Park.

APPENDIX 3

Poster presented at National Seed Science Forum, NSW, March 2016

Seed persistence in endangered alpine bog and fen peatlands

AUSTRALIAN NATIONAL BOTANIC GARDENS

Centre for Australian National Biodiversity Research

CSIRO

Alive with discovery

Lydia Guja[1,2] and Heather Brindley[2]

[1] Centre for Australian National Biodiversity Research, CSIRO National Research Collections Australia, Clunies Ross Street, Acton ACT, Australia, 2601, Lydia.Guja@environment.gov.au

[2] National Seed Bank, Australian National Botanic Gardens, Clunies Ross Street, Acton ACT, Australia, 2601, Heather.Brindley@environment.gov.au

INTRODUCTION

Alpine regions of the world are under significant pressure from warming climates. Australia's alpine region is restricted in distribution and elevation, limiting options for range shifts (Hughes 2003). In particular, Australian alpine peatlands are endangered because they are threatened by numerous processes and have an inherently fragmented distribution and small size (Commonwealth of Australia 2009). Many peatlands have experienced recent fire events which triggered restoration and research efforts (Hope *et al.* 2005; McDougall 2007). Although restoration techniques such as damming water flow to halt peat erosion have been successful (Hope *et al.* 2005), the techniques rely entirely on the soil seed and rhizome bank for regeneration of the plant community. However, the soil seed banks of these endangered communities were undefined. Our research aimed to:

1) determine whether persistent soil seed banks form in alpine bog and fen peatlands

2) investigate the effect of substrate on seed persistence

3) understand seasonal changes in dormancy and germination.

METHODS

To address the aims we designed a seed burial experiment with a randomised block design. Wild seed was harvested during autumn 2012 (the natural time of dispersal). Fresh seed of 13 species was screened for germination (**Table 1**), and buried in late autumn in two substrates (live *Sphagnum* moss or water logged peat) in a sub-alpine peatland in the ACT. Buried samples were retrieved 6 (spring), 9 (summer), 12 (autumn), 15 (winter), 24 (autumn) and 30 (spring) months after dispersal and immediately germinated under laboratory conditions; with and without dormancy-alleviation treatments.

RESULTS AND DISCUSSION

We discovered that:

- Seeds of all 13 species formed a persistent soil seed bank and survived at least one growing season (15 months after dispersal) (**Figure 1**).
- The probability of a seed exiting the soil seed bank via germination appears to be dependent on both substrate and dormancy cycling. Although substrate did not affect seed survival, it influenced the proportion of seeds that were responsive to germination cues (**Figure 2**) and substrate may therefore affect long-term persistence (supporting Long *et al.* 2015).
- Seasonal dormancy cycling (Baskin and Baskin 2014) was exhibited by some species (**Figure 2**) and this altered the proportion of seeds that were responsive to germination cues throughout the year. This is the first record of dormancy cycling in many of the study species.

Understanding soil seed bank dynamics in alpine bog and fen peatlands has improved our ecological knowledge of the plant community and has provided information that can be utilised by restoration and land management practitioners working to conserve the endangered community.

Table 1. Family, species, authority and accession numbers used in the seed burial experiment. The germination treatments applied to each species are specified and total, viability-adjusted germination of fresh seed after 140 days is presented.

Family	Species	Authority	Accession	Treatment	Germination (%) ± se: Minus treatment	Germination (%) ± se: Plus treatment
Myrtaceae	*Baeckea gunniana* ^	Schauer	CANB 866373	CS	32.8 ± 0.4	87.9 ± 1.2
Myrtaceae	*Baeckea utilis* ^	F.Muell.	CANB 866372	CS	74.2 ± 0.9	71.6 ± 0.9
Myrtaceae	*Callistemon pityoides* ^	F.Muell.	CANB 813602	No	89.3 ± 0.0	NA
Cyperaceae	*Carex lynx*	Nelmes	CANB 865438	CS	65.5 ± 1.5	19.4 ± 0.2
Onagraceae	*Epilobium gunnianum* ^	Hausskn.	CANB 866342	No	95.2 ± 0.9	NA
Ericaceae	*Epacris paludosa* ^	R.Br.	CANB 813615	CS	14.4 ± 0.8	35.8 ± 0.6
Ericaceae	*Epacris* sp. ^	Cav.	CANB 866367	CS	39.2 ± 0.0	37.7 ± 2.0
Cyperaceae	*Isolepis crassiuscula*	Hook.f.	CANB 813612	CS	51.3 ± 1.4	39.0 ± 1.8
Asteraceae	*Olearia algida*	N.A.Wakef.	CANB 813614	CS	53.3 ± 1.8	72.3 ± 1.0
Fabaceae	*Oxylobium ellipticum*	(Vent.) R.Br.	CANB 865436	PY	0.0 ± 0.0	56.8 ± 0.0
Asteraceae	*Ozothamnus cupressoides*	Puttock & D.J.Ohlsen	CANB 866370	CS	21.8 ± 1.1	12.5 ± 0.5
Ranunculaceae	*Ranunculus lappaceus* ^	Sm.	CANB 813613	CS	36.2 ± 0.7	56.9 ± 0.6
Ericaceae	*Richea continentis*	B.L.Burtt	CANB 865439	CS	5.6 ± 1.8	39.2 ± 1.3

se = standard error
^ = Field identification only. Still to be confirmed.
CANB = Australian National Herbarium
CS = Cold stratification
PY = Physical scarification with sandpaper
No = No treatment

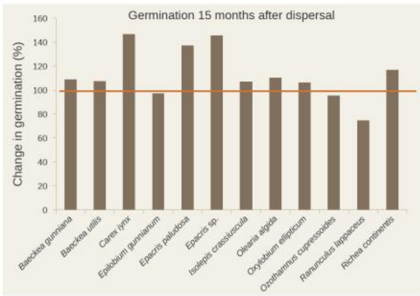


Figure 1. Germination (%) of buried seed tested 15 months after the time of dispersal. Germination is presented relative to the highest final germination of fresh seed (i.e. 100% from **Table 1**). *Callistemon pytiodies* were omitted from the figure because seed viability was too low.

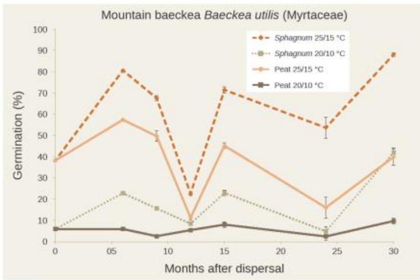


Figure 2. Germination (%) of Mountain baeckea seeds after burial in *Sphagnum* or peat. After retrieval seeds were germinated in the laboratory for 42 days at either alternating 25/15 °C 12/12 h light/dark or alternating 20/10 °C 12/12 h light/dark.

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Further information

NATIONAL SEED BANK
http://www.anbg.gov.au/gardens/living/seedbank/

RESEARCH
https://www.anbg.gov.au/cpbr/staff/guja-lydia.html

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- Australian Alps National Parks for research funding.
- Tom North, Fanny Karouta-Manasse, Mark Wallace, many 'Seedy Volunteers', and Park rangers for assistance with seed collection, processing and germination testing.

APPENDIX 4

Project management

Project management and delivery have been undertaken by the principal investigator, Lydia Guja. Project delivery has also been supported by in-kind and project-funded technical assistance from ANBG staff and volunteers, in particular Heather Brindley who is a co-investigator for this project.

Financial expenditure

Over three years funding, of $46,000 (of the original proposal seeking $53,538) was awarded to carry out this three year project. Funds contributed to some of the wages and salary and goods and services costs associated with delivering the project outcomes. Expenditure as at March 2017 is summarised below. Many volunteer and staff resources were also contributed in-kind to deliver this project.

	Predicted expenditure ($)*	Actual expenditure ($)
Wages and salary	40,400	34,907.87
Goods and services	5,600	11,227.58
Total	**46,000**	**46,135.45**

**Predicted based on the original funding proposal, adjusted to reflect the proportion of requested funds awarded.*

Variations

Some of the deliverables originally proposed were varied slightly during the course of this project. Continuous review of experimental results informed a re-evaluation of the design that altered the times samples were retrieved from burial. The retrieval times were changed from 18 and 24 months to 21 and 27 months. This extension of the planned burial duration allowed the retrievals to coincide with the next autumn (predicted low germination) and spring (predicted peak germination). Considering those altered timelines an extension was granted by the Australian Alps national parks Program Manager from June 2016 to March 2017 for delivery of remaining project actions, generally those associated with dissemination of the results. The extension did not require additional funding.

Given the multiple constraints on land managers' time and travel it was decided to replace the proposed collaborative field day for agency staff and land managers with dissemination of a fact sheet communicating the key results in a land management context (**Appendix 2**). This form of communication can be delivered to a much larger audience.

Two journal articles are in preparation but were not submitted at the time this report was prepared. However, work toward publication of the manuscripts will continue beyond the term of the project.