AUSTRALIAN NATIONAL
BOTANIC GARDENS

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



Alive with diversity

PROGRESS REPORT TO AALC
MARCH 2016

Australian Government
Director of National Parks

PROJECT BACKGROUND

Alpine *Sphagnum* bogs and associated fens (ASBAF) are a Nationally-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 the practices will likely become less viable under climate change. Shorter disturbance regimes, such as changes to fire frequency and intensity, will 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 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 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 in the National Recovery Plan for ASBAFs.

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 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 National Seed Bank (Australian National Botanic Gardens) for conservation, research and restoration.

AIMS

This scientific research project aims to understand:

1) Seed persistence in the soil

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 manage 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.

The project will foster innovation and best practice in the areas of stakeholder engagement, communication of conservation and research to the general public, and education.

This project also addresses future focus areas **catchment health** and **biodiversity** for the **future Strategic Plan.**

REPORT ON ACTIVITIES

The majority of activities and expected outcomes of this project have been delivered as planned in the first half of 2015-2016. This three-year project is on track to deliver the majority of outcomes by the end of the 2015-2016 financial year.

Research activities

During year 3 (2015-16) we have continued to collect the data required to build an understanding of bog and fen seed biology. Performance measures for year three are final data collection, statistical analysis of data, and compilation and dissemination of results.

Research activities undertaken since July 2015 include:

- Monitoring of the ten field plots that were established in 2013 at Ginini Flats, Australian Capital Territory (ACT)
- Removal of all experimental seed and equipment from the field plots on 7 October 2015 (27 months after plots were established) and restoration of plots to pre-burial conditions
- Detailed measurements of environmental factors for use in statistical analysis. Measurements were made for pH from each of 10 field plots, and temperature and moisture from 4 plots. The environmental factors may be associated with observed variation in germination
- Completion of control germination tests and laboratory testing of 3, 6, 9, 12, 21 and 27 month batches of buried seed. After retrieval each batch undergoes 140 days of germination trials, followed by cut-testing to determine seed fill and estimate the viability of non-germinated seeds. The 27 month batch is currently undergoing cut-testing and is on track for completion by June 2016
- A repeat of the control tests using *ex situ* stored seed. This was undertaken to monitor any baseline changes in germination that may have occurred (e.g. due to dry after-ripening) during the 27 month period. The test will be completed on 24 March 2016 and cut tests will be completed by June 2016
- Data entry and processing. All data for the 3, 6, 9, 12, 15 and 21 month seed batches have been recorded electronically, collated, cleaned, plotted and checked. Although some of the 21 month batch still requires cut-testing, the collation and cleaning of germination data are complete
- Statistical analysis. Most data are now prepared and appropriately formatted for statistical analysis using generalised linear models, to be conducted in mid 2016
- Preliminary data analysis. Data have been presented graphically to allow initial investigation and interpretation of the results prior to statistical analysis

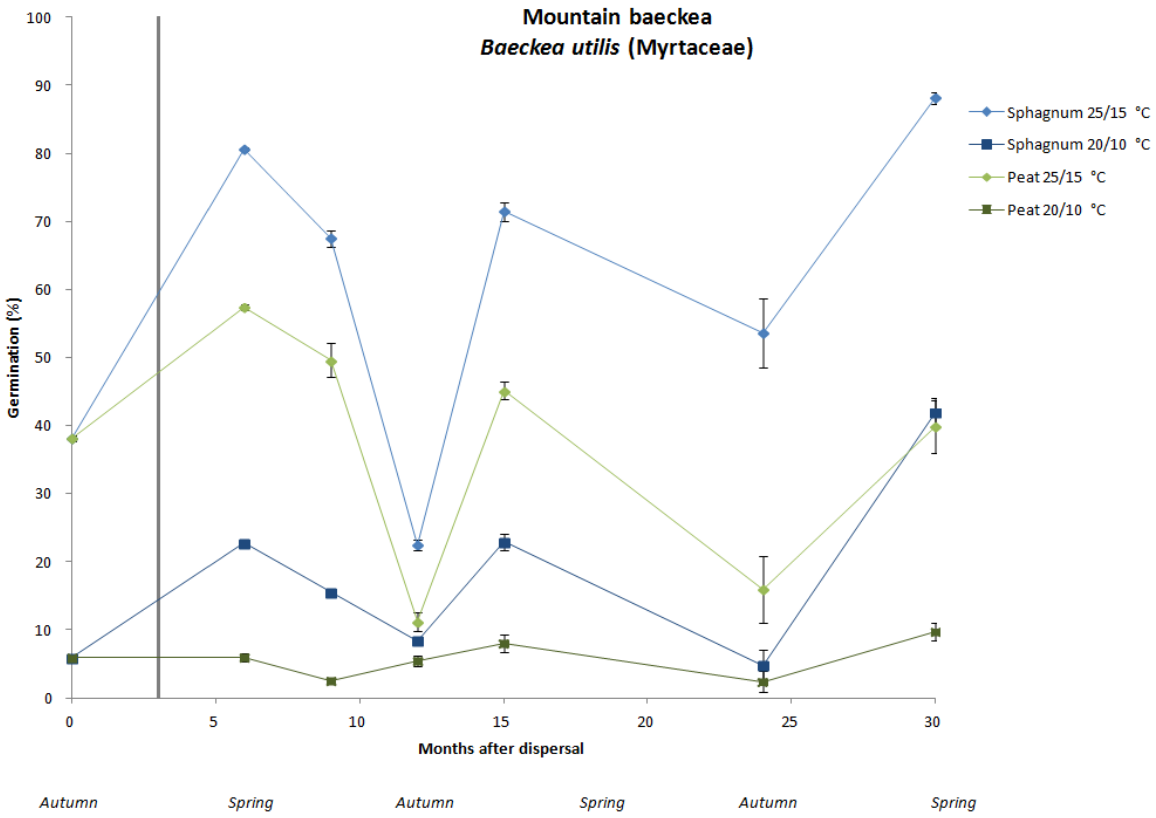

***Figure 1**. Variation in germination (dormancy cycling) of* Baeckea utilis *seed from the time of dispersal (0 months) to 30 months after dispersal. Seed was buried 3 months after dispersal (grey vertical line) in* Sphagnum *(blue) or peat (green-brown). Seed samples were regularly retrieved from the burial plots and non-germinated seeds were examined in 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. A daily regime of 12 hours at 25 °C in light and 12 hours at 15 °C in dark (diamond symbols); or, a regime of 12 hours at 20 °C in light and 12 hours at 10 °C in dark (square symbols).*

Results and discussion

- The data suggest seeds persist and survive in the soil without germinating for at least one growing season. Therefore, a persistent (not transient) soil seed bank is formed. It appears that some species are beginning to deteriorate and lose viability after 27 months of burial, a result requiring closer analysis
- Interesting patterns of seasonal dormancy and germination cycling are emerging for many of the species. The proportion of seeds that germinate varies significantly between seasons. The majority of dormancy cycling species are responsive to germination triggers during spring (e.g. *Baeckea utilis* **Figure 1**). 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 during autumn.
- The ability of species to germinate (dormancy alleviation and responsiveness to germination cues) over time can be summarised in four conceptual models (**Figure 2**)

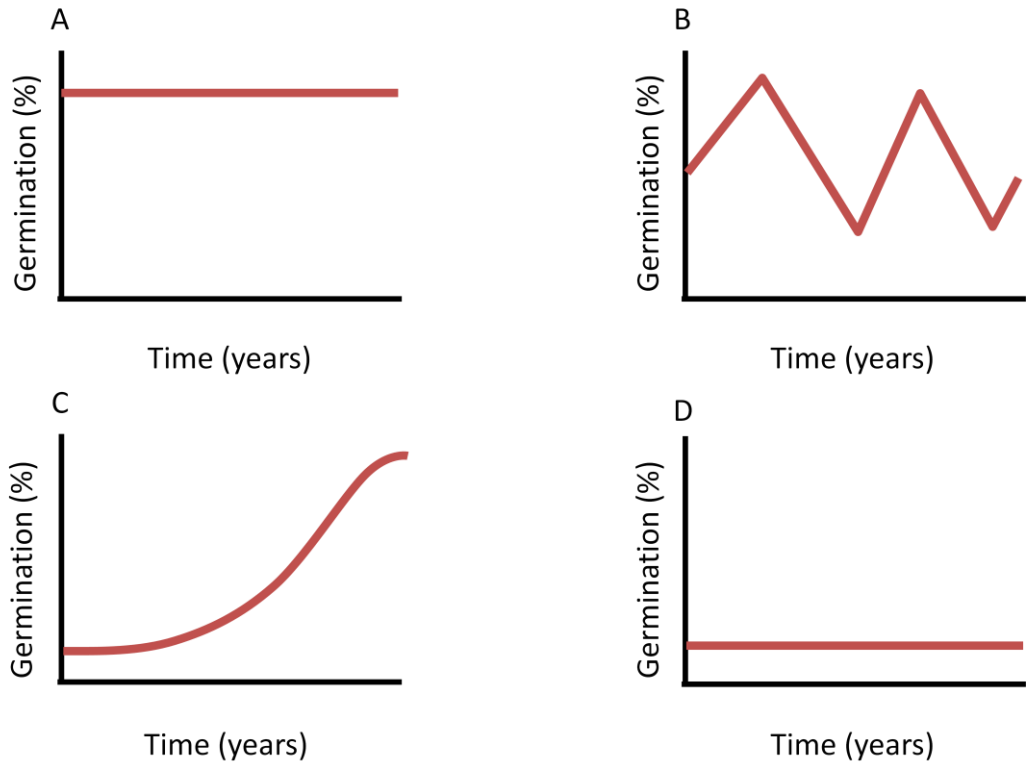

Figure 2. *Four conceptual models describing seed germination and dormancy cycling patterns observed among the 13 study species. The responsiveness of buried seeds to germination cues changes with time and this will likely have different evolutionary and land management implications.*

Ex situ conservation activities

Conservation seed collections (made in summer 2015-16) from local sub alpine and montane bogs and fens are being processed and incorporated into the long term, *ex situ*, conservation collection of the National Seed Bank. Living plants are also being grown for display in the ANBG for a future bog and fen display garden, as an alternative method of *ex situ* conservation of bog and fen plants.

Seed banking

Seed collection activities since July 2015 include:

- Targeted collecting activities which focused only on priority species identified as gaps by a research student. In December 2014 54% of 211 species known to occur (frequently and occasionally) in ASBAFs were conserved in the NSB's collection. The CSIRO student (Kathryn McGlip, supervised by the principal investigator Lydia Guja) identified missing and/or underrepresented species and prioritised those for future collection
- Collection of 40 seed accessions from ASBAF species
- Processing of seed collections. Once collections are cleaned, dried to 15% relative humidity, and germination tests have been completed they will be placed in long term storage at -20 °C in the National Seed Bank.

Display garden

Preparations for the display garden undertaken since July 2015 include:

- Trial display bowls established for an art exhibition in the previous reporting period have been very successful and attracted considerable interest from visitors to the ANBG, and therefore remained on display at the ANBG Visitor's Centre for the duration of the reporting period
- Propagation and concept planning are underway in preparation for the establishment of a small ASBAF garden in the ANBG during 2015-16. The design will extend on the trial bowls and includes building a large new central bowl with a reservoir to cool soil during summer heat waves
- Direct sowing of seed and propagation via cuttings are in progress to provide material for the display garden

Communication of results

Since July 2015 results of this project have been presented:

- To the general public viewing plants in display bowls at the ANBG Visitor's Centre and raising awareness about the significance of ASBAFs and their conservation
- As an oral presentation 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)
- As an oral presentation at a symposium hosted by Australian Alps National Parks, Thredbo, November 2015
- As a poster (**Appendix 1**) 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)
- Various stakeholders via informal communication

Other communications in preparation include:

- Draft scientific paper
- Interpretive material to accompany the bog and fen display garden at ANBG

Project management

The project is currently on track to deliver most outcomes within the allotted timeframe.

Project management has been undertaken by the principal investigator. Project delivery has been undertaken by the principal investigator with in-kind and project-funded technical assistance from ANBG volunteers and staff. During the reporting period a casual technician was contracted to assist with data management and laboratory work during peak periods. Further assistance in species determination and seed collecting has been provided by taxonomic staff of the Centre for Australian National Biodiversity Research. Local Government staff and park rangers have assisted with seed collection in-kind.

REQUESTED VARIATION

Continuous review of experimental results informed a revaluation of the design that altered the times samples were retrieved. The retrieval times were changed from 18 and 24 months to 21 and 27 months. This extension of the burial duration allowed the retrievals to coincide with the next autumn (predicted low germination) and spring (predicted peak germination). Consequently, some data analysis and interpretation are behind the original schedule. A proposed recovery action is to recruit another casual staff member to work on data entry and the remaining laboratory work so that more time will be available to the principal investigator for statistical analysis and dissemination of results.

Considering the altered timelines described above we are seeking an extension from June 2016 to December 2016 for delivery of remaining project actions, generally those associated with dissemination of the results. An extension will not require additional funding.
The activities we are seeking to deliver between July and December 2016 are:

- Analysis and presentation of results
- Display garden
- Collaborative field day with agency staff and land managers
- Preparation of a journal article. Publication date is difficult to predict due to the peer review processes
- Final report on activities

ACKNOWLEDGEMENTS

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CONTACT

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APPENDIX 1

Seed persistence in endangered alpine bog and fen peatlands

AUSTRALIAN NATIONAL BOTANIC GARDENS

Centre for Australian National Biodiversity Research

CSIRO

Alive with discovery

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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	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 lyrix*	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.

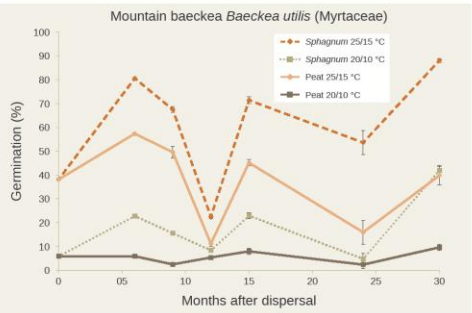

Figure 2. Germination (%) of Mountain baeckea seeds after burial in *Sphagnum* or peat. After retrieval seeds were germinated in the laboratory for 42 days at either alternating 25/15 °C 12/12 h light/dark or alternating 20/10 °C 12/12 h light/dark.

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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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