

# SEED STORAGE BEHAVIOUR AND POPULATION OF JAVAN BETEL NUT (*Pinanga javana* Blume) AN ENDEMIC PALM TREE FROM JAVA

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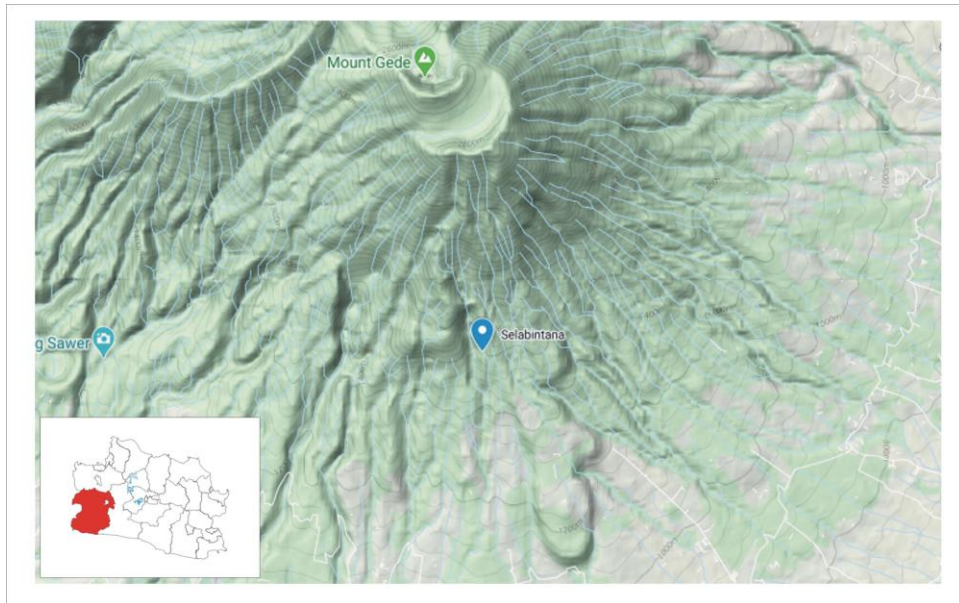
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## INTRODUCTION

Javan betel nut or *Pinanga javana* Blume is an endemic palm from Java and their existence in the wild is endangered based on 1997 IUCN Red List of Threatened Plants. Information of their storage behaviour and its population in the wild are clearly unknown. This research is to predict the desiccation sensitivity and to describe the population of *P. javana* in Selabintana, West Java.

## LOCATION OF FIELD STUDY

*P. javana* population in the wild were assessed in Selabintana forest, West Java, Indonesia with annual rainfall 3,835 mm/year and elevation 1,143-1,416 m asl during April 2019. This study was carried out using transect method along Cibeureum waterfall track i.e., from the forest edge to interior. *P. javana* individu was recorded in fourteen sub-plot of 100 x 10 m<sup>2</sup> based on growth stage, i.e. seedling, juvenile and adult.



Location of *P. javana* population study

## METHODS TO PREDICT DESICCATION SENSITIVITY

1. Seed coat ratio approach (adopted from Daws et al., 2006)

$$P = \frac{e^{3.269-9.974a+2.156b}}{1 + e^{3.269-9.974a+2.156b}}$$

2. 100-seed test for desiccation tolerance (adopted from Pritchard et al., 2004)

## METHODS FOR GERMINATION TEST

Plastic container, agar media 2%, germinator chamber 25°C 12/12h

$$\text{Germination (\%)} = G/X \times 100$$

$$\text{Viability (\%)} = (G + F + A)/X \times 100$$

$$\text{MTG} = \sum (D n) / \sum n$$

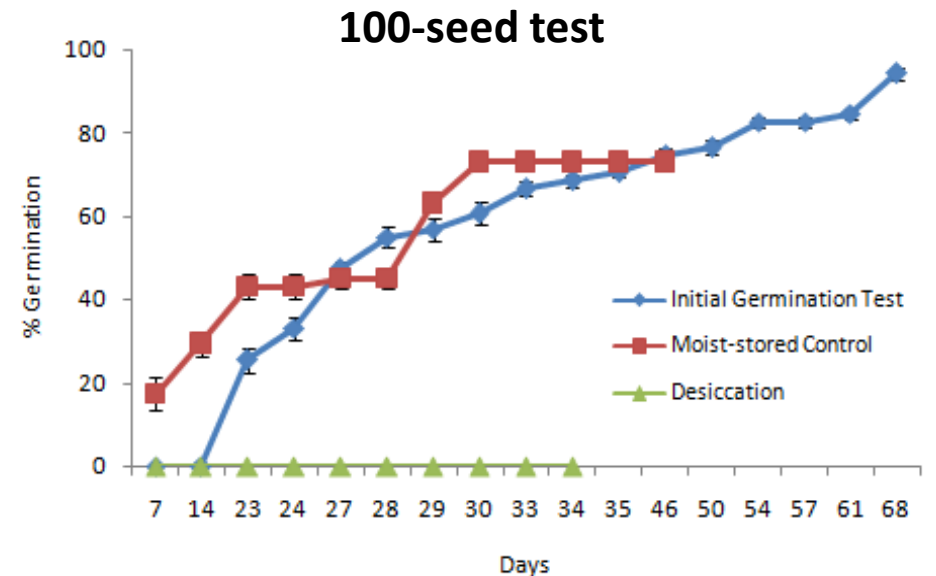
## RESULT

### Desiccation Sensitivity of *P. Javana*

#### Seed coat ratio approach

Embryo mass (g)	Seed coat mass (g)	Seed coat ratio	Seed mass (g)	Probability R/O*
7.23 ± 0.36	1.44 ± 1.03	0.15 ± 0.09	8.67 ± 1.39	0.97 ± 0.02

Probability > 0.5, seed is probably desiccation sensitive/recalcitrant

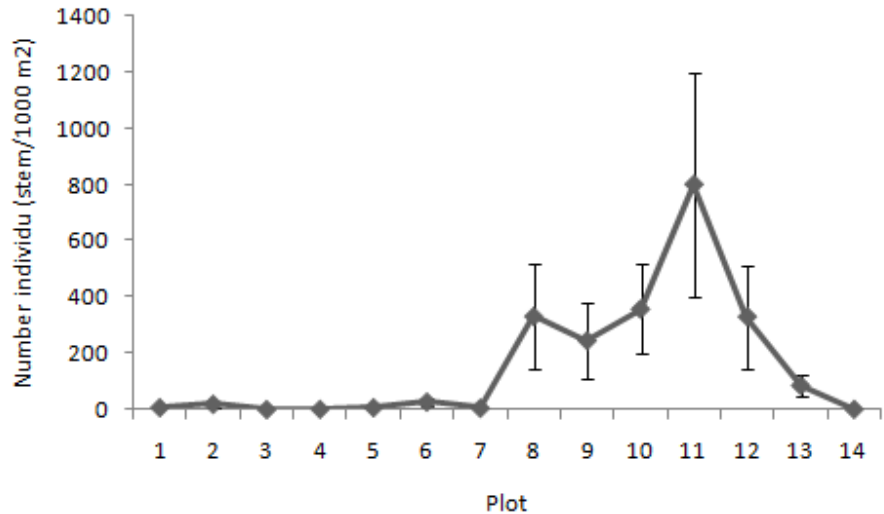


Typical germination progress curves for a desiccation sensitive species

### Seed Characteristics of *P. javana*

Seed dimensions		Seed weight (g)
Length (mm)	Width (mm)	
20.71 ± 1.15	10.74 ± 0.6	1.33 ± 0.35

### Population of *P. javana* in each plot



Javan betel nut was found in all plots. The most of Javan betel nut were concentrated at the last half track which located in the forest interior.

### Germination Test of *P. javana*

	Fresh seed	Moist storage	Desiccation
Seed eRH	99 ± 0	99 ± 0	26 ± 7.07
Seed MC	59.63 ± 3.20	54.15 ± 1.94	6.73 ± 0.23
% Germination	96.16 ± 4,44	98.08 ± 3,85	0 ± 0
% Viability	100 ± 0	100 ± 0	0 ± 0
DAS (days)	23.25 ± 0.5	6.25 ± 0.5	0 ± 0
Mean times to germination (MGT)	34.65 ± 2.53	17.01 ± 11.61	0 ± 0

### Density of *P. javana*

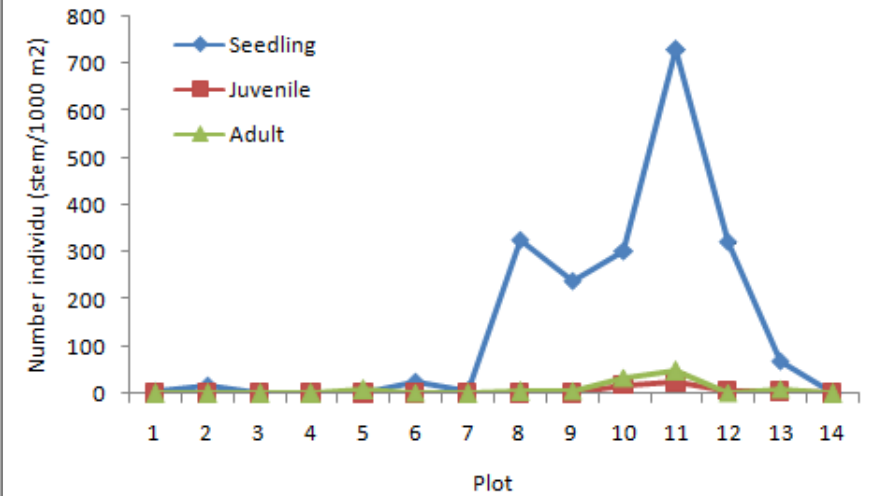
Stage	Seedling	Juvenile	Adult
No. individu	2,053	54	123
Fruiting	-	-	84
Non-fruiting	-	-	39
Density (stem/ha)	14.66	0.39	0.88

The seedlings were clustered in the forest floor along track. The cluster pattern of seedling might be correlated with the disperser agent of Javan betel nut, i.e. common palm civet or *Paradoxurus hermaphrodites* Pallas.

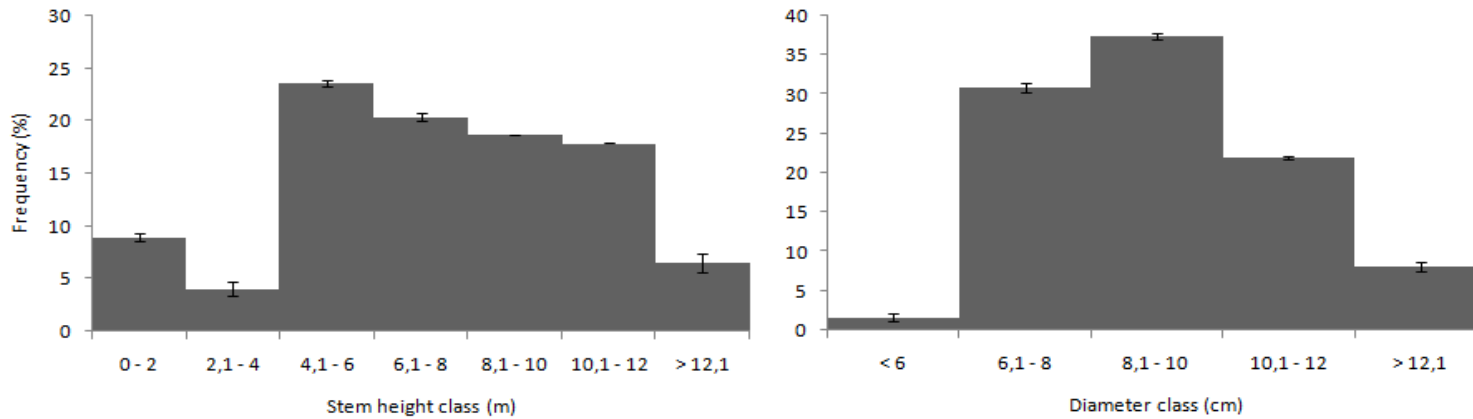
## Developmental stages of *P. javana*: seedling, juvenile and mature



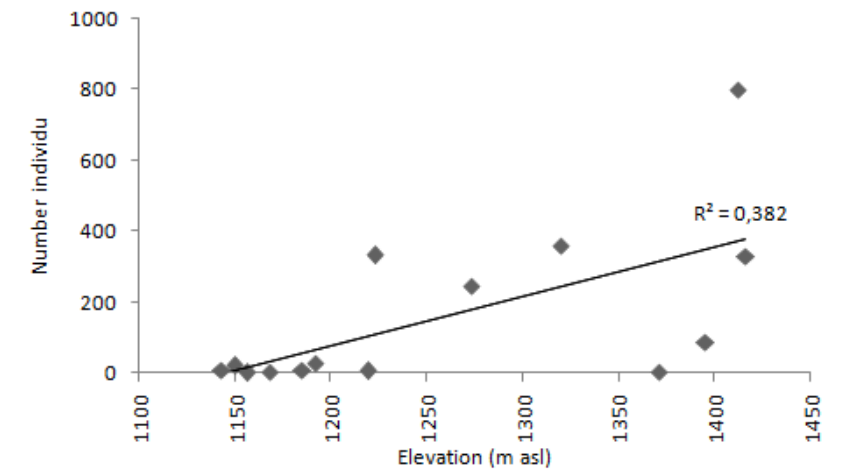
## Number individu of *P. javana* in each stage



## Stem height class and diameter class of *P. javana*



## Regression analysis number individu of *P. javana* along gradient elevation



# Environmental effect on temporal patterns in lentil seed quality development

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**Background.** The Australian Grains Genebank's (AGG) lentil collection contains 5,328\* accessions. It is one of our mandate crops and is widely distributed to research and breeding programmes, globally. Although lentil produces orthodox seeds and are, therefore, expected to survive for a long period of time in genebank storage; little is known about their longevity which makes it difficult to effectively manage.

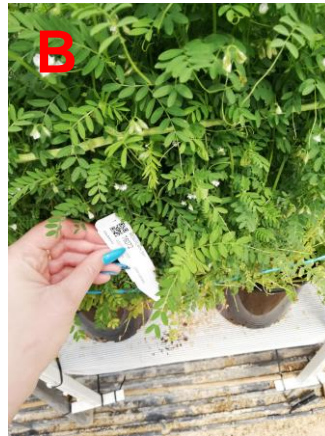
Seed quality is important for long-term conservation of germplasm as it affects storage longevity. Seed quality traits are acquired during seed development and maturation however, the environment affects both the progression through development and end seed quality. Pin-pointing the timing of optimum maturity will allow for timely harvests and high quality seeds with a maximum storage potential.



# How do the subtle environmental differences of the regeneration zones at AGG affect progression through development and subsequent end seed quality?

## Methods.

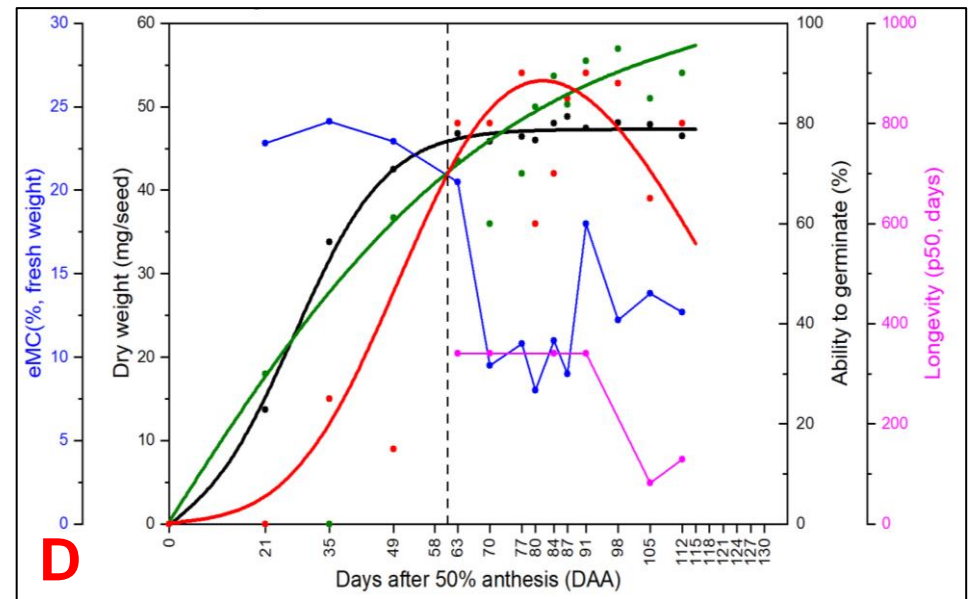
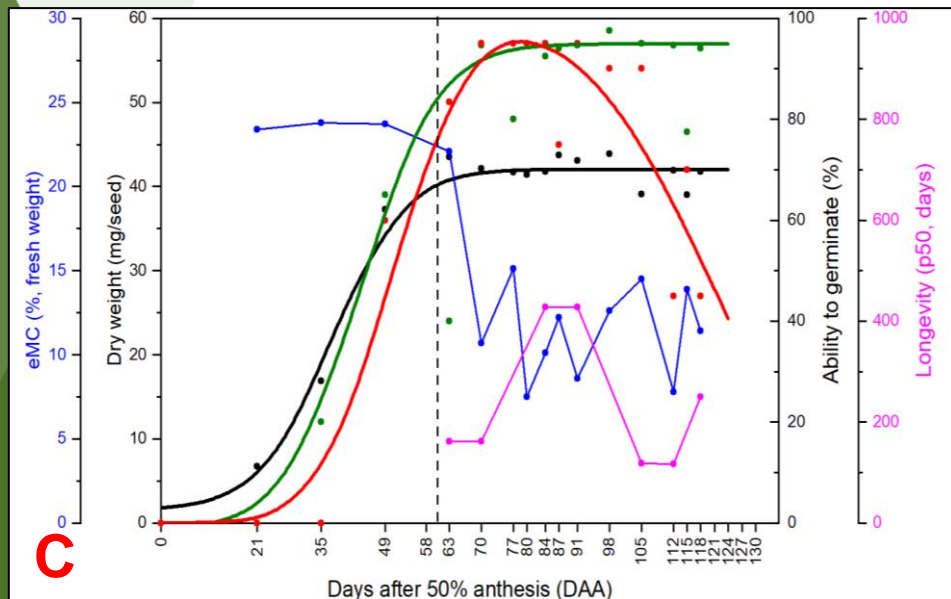
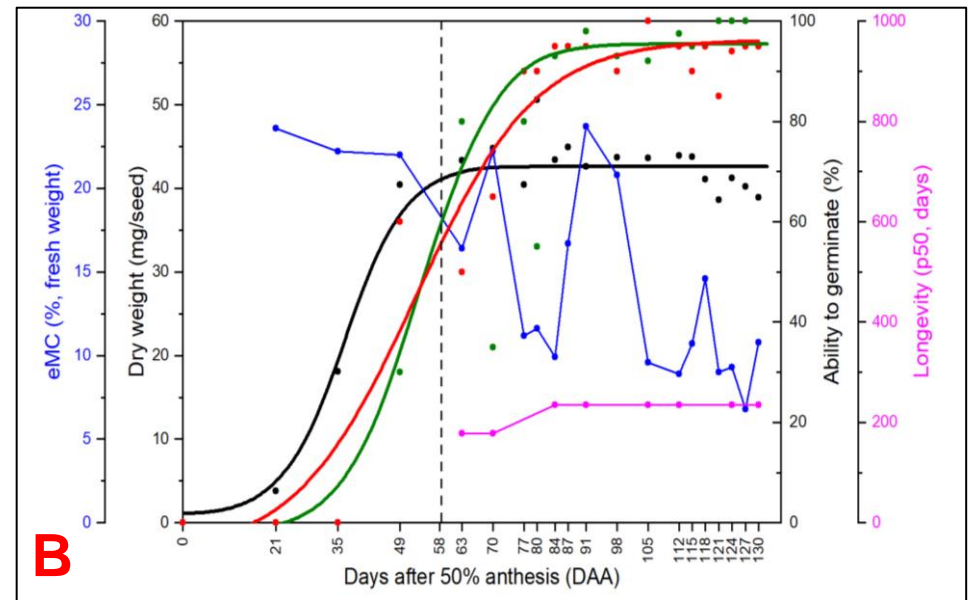
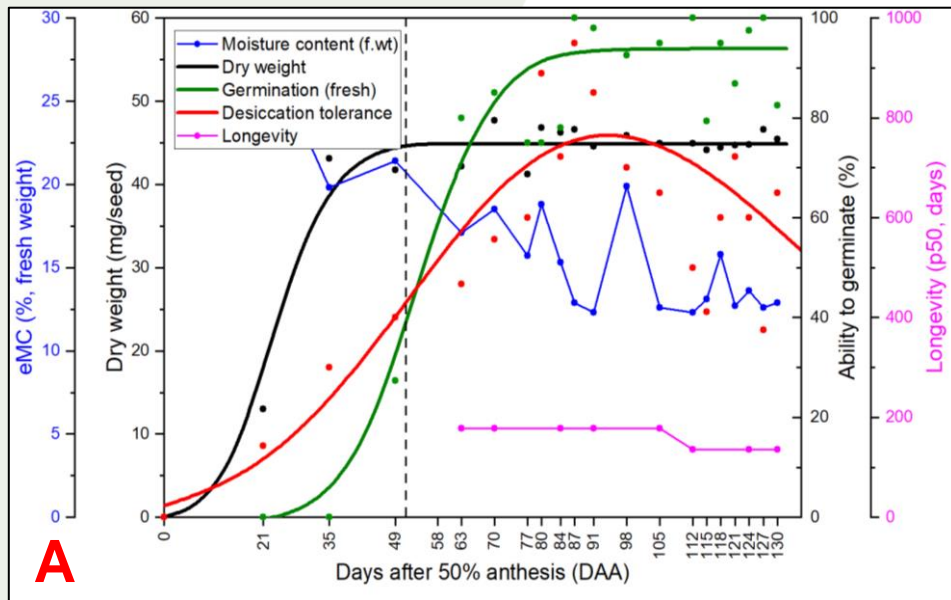
Seeds of two lentil elite breeding lines (*Lens culinaris* Medik.) were grown across four regeneration zones (glasshouse [A], big igloo [B], green igloo [C] and cage [D]) at AGG.



Samples of seeds were harvested from 21 days after 50% flowering until a maximum of 130 days. At each harvest, physiological quality traits, including germinability (fresh and dried seeds) and seed longevity were determined, as well as seed dry weight and moisture content.

## Results.

- Timing of mass maturity (MM), in respect to days after 50% anthesis (DAA) was fairly consistent across accessions and growth environments. Germination was acquired early (21DAA) but reached its maximum after MM. It was during the maturation drying phase that seeds acquired desiccation tolerance to low levels (35-49DAA); as well as maximum longevity.
- Environmental effects on longevity accumulation were apparent; with longevity estimates varying both between accession and accession x growth environment and with significant differences in survival curves ( $P < 0.05$ ) between some, but not all, seed lots harvested at different maturity stages within each accession x growth environment.
- There were several patterns of variation in longevity development: some seedlots were already at maximum longevity at the start of sampling (Fig. 1 A,D) and some showed an increase during development (Fig. 1 B,C). Maximum longevity was maintained for different lengths of time before declining (Fig. 1 A,C,D), with some seeds showing an increase again (Fig. 1 C).



**Figure 1.** The physiological changes which can occur during development, in either accession 76080 or 76072, when grown in the glasshouse (A), big igloo (B), green igloo (C) and cage (D).



## Conclusion.

- Seeds did not show a typical developmental response, rather variation was observed in seed quality development both between and within accessions grown in the different environments.
- The results provide further confirmation that the quality of seeds cannot be accurately predicted post mass-maturity with respect to developmental time (DAA).
- It is not clear whether there is a “maximum longevity” that any developing cohort of seeds can attain, rather the “value” was a direct consequence of the net changes in seed quality (improvement vs. deterioration) governed by the environmental conditions.
- Further research is required but, based on observations thus far, it could be recommended to sow seeds earlier to avoid the onset of rain in the open environments and/or the extreme heat in the big igloo during the late summer months.



# Unlocking the genetic potential of AGG germplasm to improve genebank conservation, management and accelerate genetic gain for crop improvement.

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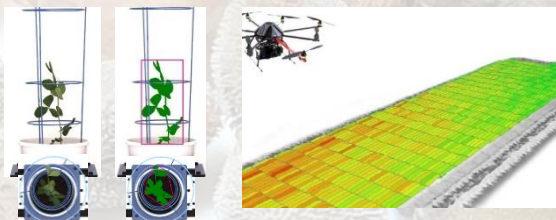
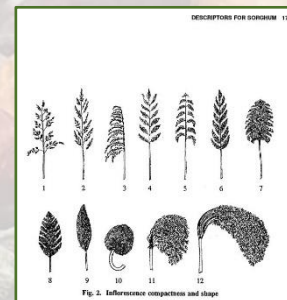
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The **Australian Grains Genebank** (AGG) conserves a large collection of plant genetic resources (c. 165,000 accessions) for cereal, oilseed and legume species of relevance to Australian grain growers. These genetic resources contain vital genetic diversity that has been lost through bottlenecks imposed by domestication and breeding and are used by Australia's plant researchers and breeders for new and novel traits for grain crop improvement.

Genebank passport, characterisation and evaluation data are used by researchers and breeders to select lines for inclusion in their programs. Genebanks also use this data to validate their germplasm and manage the integrity of their collections. Generally, however, there is a lack of detailed characterisation data available for germplasm, which is a major impediment and cost for its utilisation by researchers and breeders.

**Germplasm characterisation** is usually undertaken when lines are grown for seed regeneration to collect trait data based on morphology and phenology. The collection of this trait data is highly labour intensive, some traits are subjective in nature, and they can be influenced by both abiotic and biotic factors during growth, and therefore inconsistencies can occur. These descriptors were largely developed before the advent of molecular/genomic assisted breeding, and as such, plant breeders are not wanting more relevant data to guide their research and breeding programs. Genebanks must move towards high throughput technologies to support agricultural crop breeding programs.



**High throughput phenotyping** has the potential to capture a range of traditional characterisation traits and additional traits of interest to research and breeding programs. RGB and hyperspectral images are computer analysed by algorithms developed on validation data sets, with machine learning able to improve predictions.

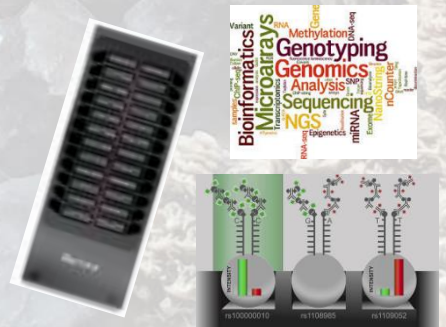
AGG has used UAV and RGB imaging to collect data in field and glasshouse and laboratory environments for growth rates, green biomass production, crop height, spike trait data, and is moving towards days to anthesis, and seed quality.

This data will enable breeders to identify germplasm with phenology and phenotypes of interest to their programs.



**High throughput genotyping** will improve genebank efficiency by identifying duplications, accurately assess genetic diversity and trait identification. These technologies over time will enable the AGG to consolidate its collection through the removal of duplication and refocus resources towards the long-term conservation of germplasm with the widest range of diversity and traits.

AGG is in early stages of implementing a pilot study to genotype germplasm using SNP chips to identify a core diversity set to then optimise both distribution and curation.



**Adoption of high throughput technologies will improve curation efficiency and accuracy of the AGG germplasm collection and provide enriched passport information that will increase breeding efficiencies within Australia and accelerate the production of more resilient grain crop varieties for Australia.**

## Introduction

In a previous study presented at ESA 2019, native species from the Cumberland Plain Woodland in Syd, Aust., responded to **intense and frequent heatwaves** in a manner inconsistent with the hypothesis (Alvarez et al. 2019). Species' seed germination responses were affected by some but not all treatments they were exposed to and there were interactions between some treatments but not all species (Figure).



In that study, seeds were subjected to either an:

- **Intense** heatwave (29/17°C, 39/21°C, 43/25°C, or 60/22°C)
- **Frequent** heatwave (at 60/22 °C for 1 HW, 3 HWs or 5HWs)

To better understand the mechanisms underpinning this variation I present here an examination of four key life-history traits:

- **seed mass**
- **seed dormancy**
- **fire response**
- **plant life form**

**Aim:** To understand the context-specific role of life-history traits on seed germination responses in the Cumberland Plain Woodland.

## Method and Materials

Cumberland Plain Woodland

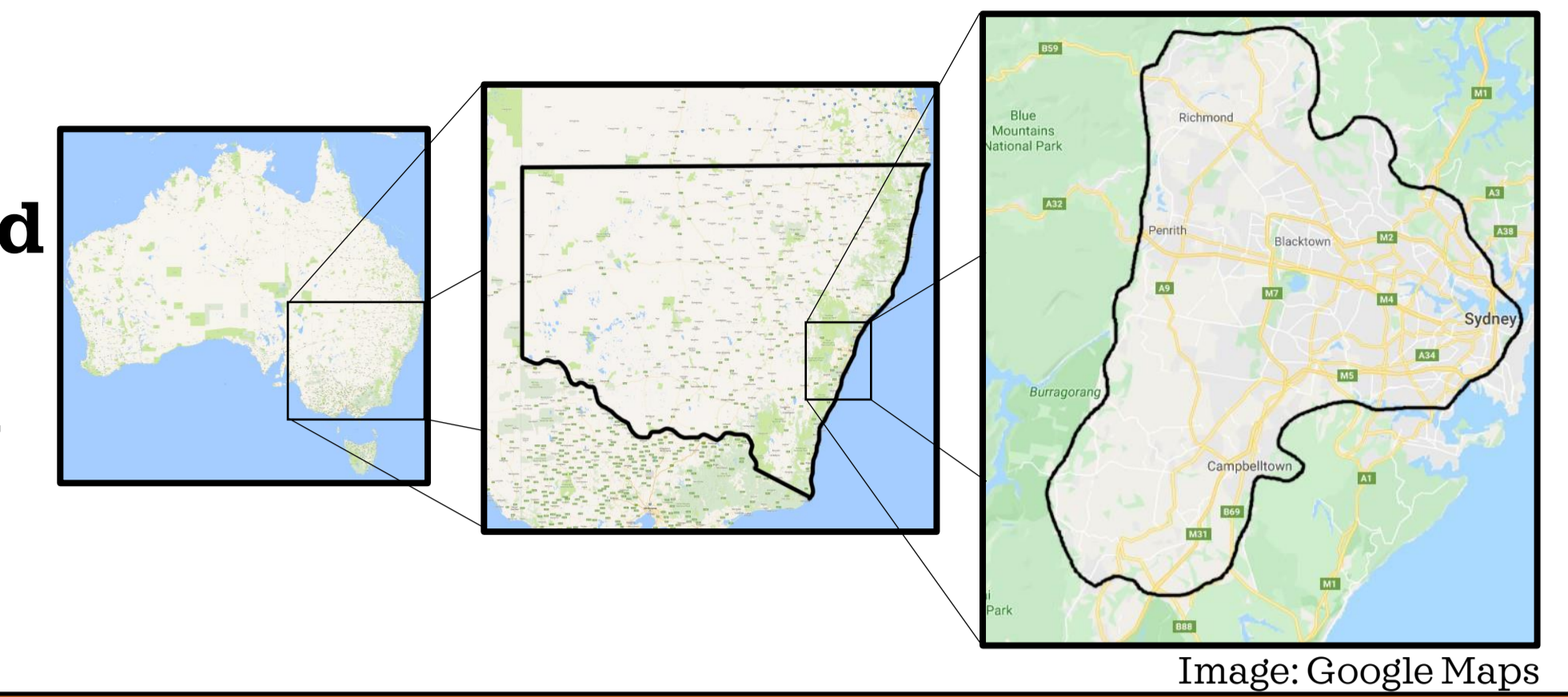
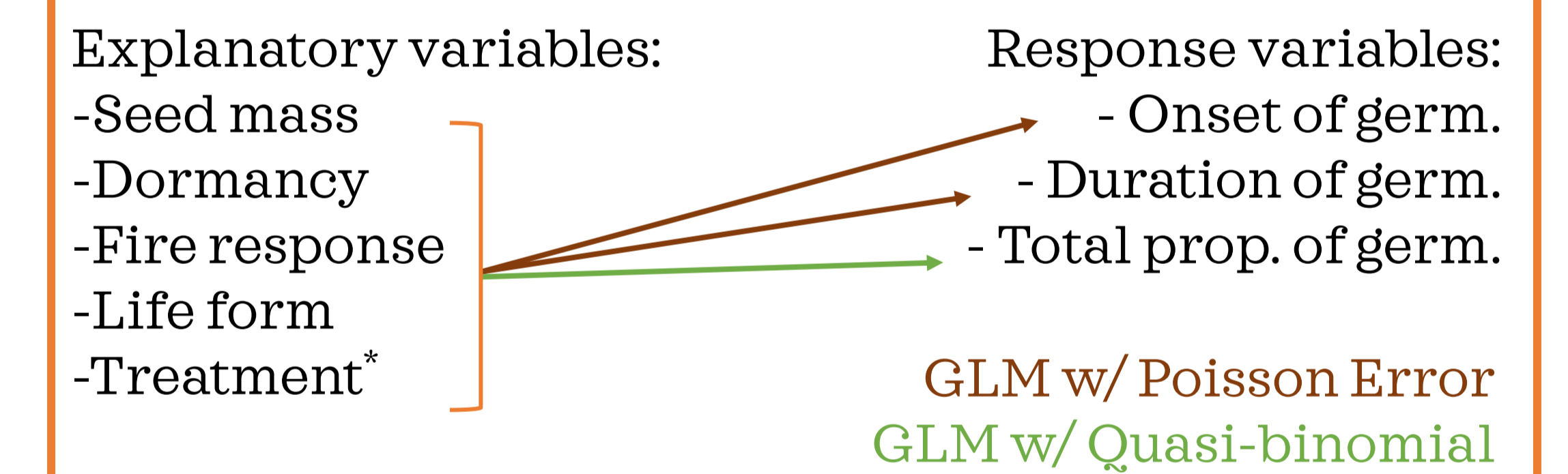


Image: Google Maps

Family (-aceae)	Species	Seed mass (g)	Life form	Fire resp.	Dormancy
Asteraceae	<i>Cassinia aculeata</i>	S	Sh	OS	D
	<i>Calotis lappulacea</i>	S	He	R	D
Chenopodi.	<i>Einadia nutans</i>	S	He	OS	ND
	<i>Indigofera australis</i>	M	Sh	R	D
Fabaceae	<i>Acacia decurrens</i>	L	Tr	OS	D
	<i>Acacia falcata</i>	L	Sh	OS	D
	<i>Hardenbergia violacea</i>	L	Cl	R	D
Lamiaceae	<i>Plectranthus parviflorus</i>	S	He	OS	ND
	<i>Eucalyptus crebra</i>	S	Cl	R	ND
Myrtaceae	<i>Eucalyptus tereticornis</i>	S	Tr	R	ND
	<i>Dichanthium sericeum</i>	M	Gr	R	D
Poaceae	<i>Microlaena stipoides</i>	M	Gr	R	D
	<i>Themeda triandra</i>	M	Gr	R	D
Ranuncul.	<i>Clematis glycinoides</i>	M	Cl	R	D
Sapind.	<i>Dodonaea viscosa</i>	M	Sh	OS	D

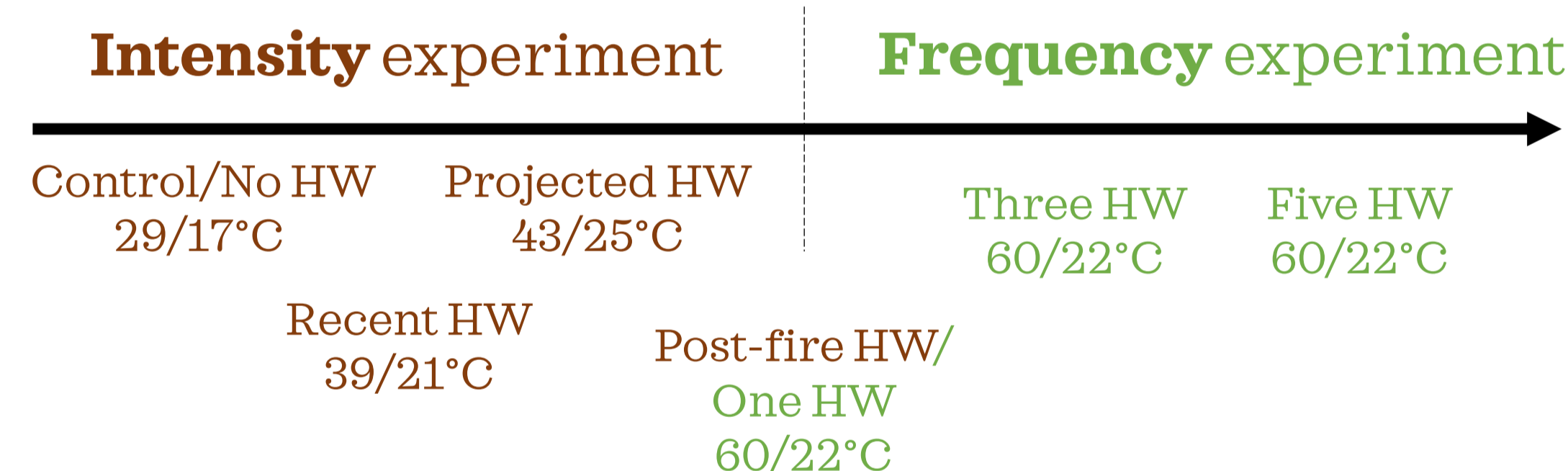
S-Small; M-Medium; L-Large  
Sh-Shrub; He-Herb; Tr-Tree; Cl-Climber; Gr-Grass  
OS-Obligate Seeder; R-Resprouter  
D-Dormant; ND-Non-Dormant



Tested in both Intense and Frequent heatwave treatments (Alvarez 2019)

## Discussion

To better explain the findings of this study, below is a continuum of the temperatures used in the previous study (Alvarez 2019). All temperatures from the experiment have been placed coolest/least frequent to hottest/most frequent.



As the responses of the life-history traits move along the continuum towards the more frequent end, significant relationships changed (see the boxes in Table 1)

**EG** in total seed germination proportion, fire response and seed mass disappeared as they became non-significant whereas dormancy became significant.

Here, I have provided a new conceptual approach for combining the outcomes of the previous experiment (Alvarez 2019) to demonstrate novel interpretations of germination performances across the germination attributes in relation to the life-history traits.

Alvarez, P. 2019, 'Do experimental heatwaves affect seed germination?', poster presented at the ESA conference, Australia, November 2019.

# Relationships between plant life-history traits and seed germination in native plant species of the Cumberland Plain Woodland

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## Results

	Onset of germination		Duration of germination		Germination proportion	
	Intensity	Frequency	Intensity	Frequency	Intensity	Frequency
Life form	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0001
Seed mass	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.002	ns
Dormancy	< 0.0001	< 0.0001	< 0.0001	< 0.0001	ns	0.046
Fire response	ns	ns	ns	0.041	0.007	ns
Treatment	ns	ns	ns	ns	ns	ns

Most  
↓  
Least

	Onset of germination		Duration of germination		Germination proportion	
	Quick	Delayed	Short	Long	Low	High
Life form	Gr	He	Gr	He	Tr	He
	Sh	Tr	Sh	Tr	Tr	Sh
		CL		Cl		Cl
Seed mass	Large	Small	Large	Small	Small <sup>I</sup>	Large <sup>I</sup>
Dormancy	ND	D	ND	D	D <sup>F</sup>	ND <sup>F</sup>
Fire response	ns	ns	R <sup>F</sup>	OS <sup>F</sup>	OS <sup>I</sup>	R <sup>I</sup>

<sup>I</sup> Only significant in the intensity experiment, not the frequency experiment  
<sup>F</sup> Only significant in the frequency experiment, not the intensity experiment



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## Seed collecting of Crop Wild Relatives in Thailand under the Millennium Seed Bank (MSB), Royal Botanic Gardens, Kew

The main genetic diversity of crop wild relatives was initiated to conserve and enhance food security. Seed collecting of Crop Wild Relatives (CWR) for seed bank in Thailand was initiated in 2017, while, Thailand Institute of Scientific and Technological Research (TISTR) was collaborated with Millennium Seed Bank (MSB), under the Royal Botanic Gardens, KEW, United Kingdom to developed the Collecting Thailand's crop wild relatives for ex situ conservation and use project. The objectives were studied the CWR species' distribution areas and seed collecting for seed bank at the MSB. The 3-years project during 2017-2019, which TISTR was collected and sent the collections of CWR seed to MSB about 65 accessions divided to 18 Genera. TISTR were sent CWR seed collections to MSB about 9, 21 and 35 accessions in 2017, 2018 and 2019, respectively. The minimum collections size were 250 seeds, 75% of the collections were larger than 1,000 seeds and 24% reached to the MSB's target size of 10,000 seeds. Although in the last 3 years, the seed bank operations and utilization of crop wild relatives, which operated by TISTR have not able to proceed with concrete. However, TISTR was constructed a seed bank (4 and -20 C°) at Lamtakhong Research Station for seed research and conservation. The main objectives are focus on the conservation of CWR species, edible species and medicinal plants in Thailand. In the future, the utilization of CWR seed collections might be used to pre-breeding program for germplasm and crop improvement.

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**Keywords:** Crop wild relatives, ex-situ Conservation, Seed bank, Thailand

### Introduction

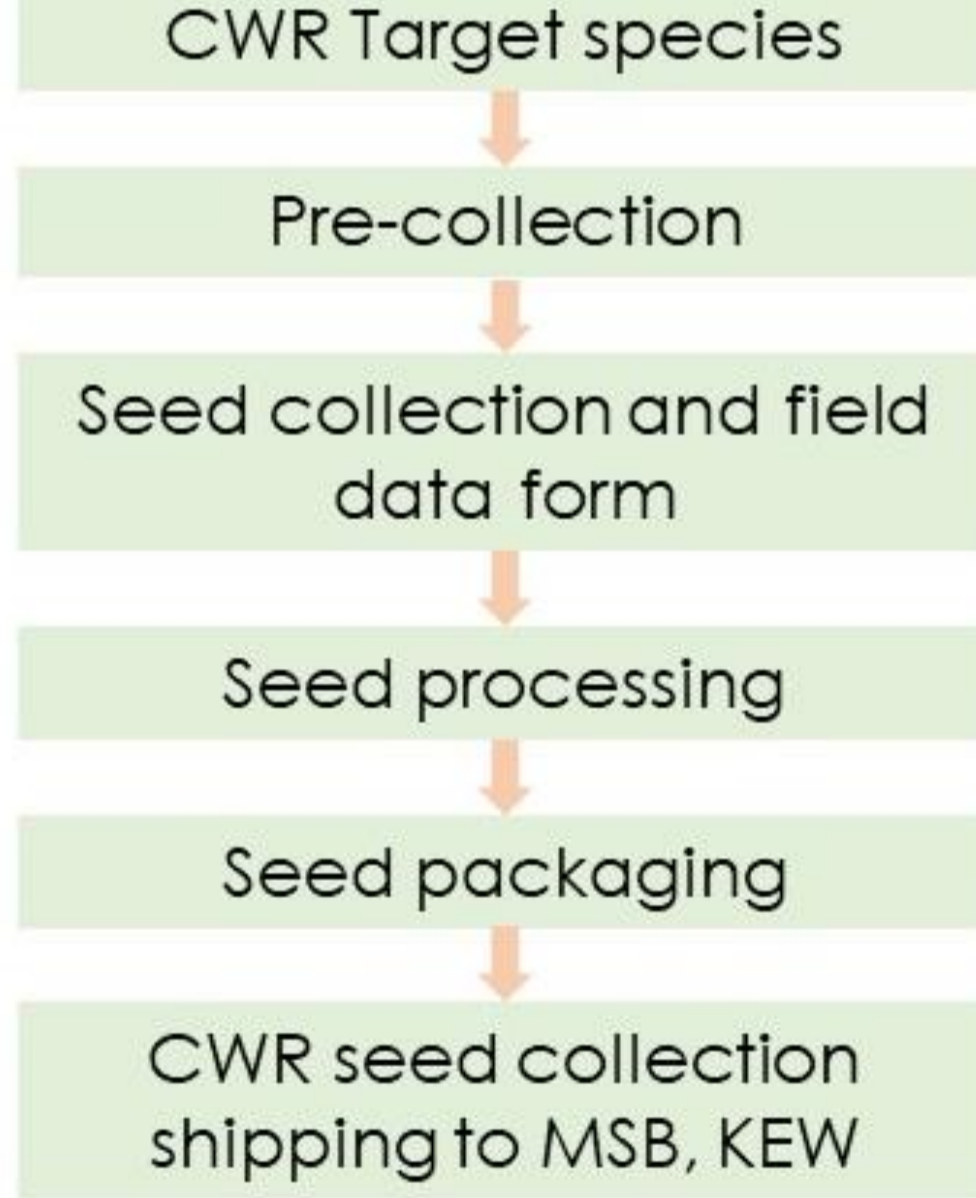
In-situ and ex-situ conservation are the only ways to preserve the genetic diversity which is critically important for our future. Target 9 of the Convention on Biological Diversity states that signatories should seek to conserve 70% of the genetic diversity of crops and wild relatives by 2020. To achieve these goals rapid action is needed now. Therefore, Crop wild relatives plants in Thailand are the most important to conserve.

### Methods

- This project aims to make good quality seed collections from 68 Thai CWR - taxa related to globally important food crops in Thailand.
- This will be achieved through strengthened conservation partnerships between Kew's Millennium Seed Bank and the Thailand Institute of Scientific and Technological Research (TISTR) and other scientific institutions within Thailand.
- The collections will be conserved in country, once the national seed bank is completed, and duplicated at Kew's Millennium Seed Bank, where they will be made available to bona fide crop development institutions for research.

CWR seeds collecting protocols were followed by the field data form and procedure of MSB, KEW.

Field data form for CWR seed collection



### Results

The three years project during 2017-2019, which TISTR was collected and sent the collections of CWR seed to MSB about 65 accessions divided to 18 Genera, including with *Amaranthus*, *Aristolochia*, *Cajanus*, *Coix*, *Digitaria*, *Dioscorea*, *Ensente*, *Ficus*, *Ipomoea*, *Musa*, *Oryza*, *Paspalum*, *Peucedanum*, *Pluchea*, *Saccharum*, *Solanum*, *Sorghum* and *Vigna* around Thailand.

### Acknowledgements

This work was under the Collecting Thailand's crop wild relatives for ex situ conservation and use project that supported by Millennium Seed Bank Partnership, Royal Botanic Gardens, Kew. The authors would like to acknowledge to the staff of Millennium Seed Bank, KEW and Thailand Institute of Scientific and Technological Research (TISTR) who supported and initiated our project to conserve the crop wild relatives diversity in Thailand.

### Conclusions

TISTR was set up a short to medium term seed bank for Crop Wild Relatives, which could be used for agricultural research, food security, and the development of new crops. The Crop Wild Relatives (CWR) project on which TISTR and MSB have collaborated, has enabled TISTR to make valuable seed collections and has given the staff essential training and field and lab experience, enabling them to make high quality collections of CWR seeds that will survive for decades.

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# Challenges of Seed Banking in the Aseasonal Tropics: Experiences from Singapore



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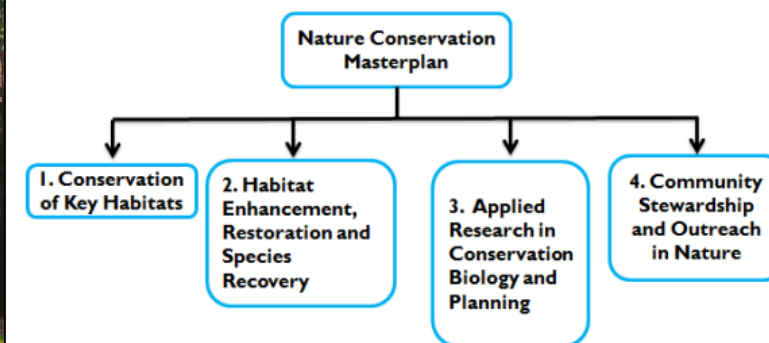
1D Cluny Rd, Singapore Botanic Gardens, Singapore 259600

## Abstract

The Singapore Botanic Gardens Seed Bank was started in July 2019, mindful of the challenges of working in the aseasonal tropics. Tropical species often have low population densities, with a larger proportion of species with recalcitrant seeds. Reproductive phenology in aseasonal tropics tended to sub-annual and supra-annual periodicities, leading to difficulties in monitoring and collection from sporadic small events, while overabundance from masting events leads to exceeding seed storage capacity and wastage. The quality of collection from remnant populations should be monitored, as these populations have experienced severe bottlenecks due to habitat loss and fragmentation. Furthermore, landuse changes, reintroduction of locally extinct species, and non-native conspecifics and horticultural varieties, and cogenerics in living collections, can effect gene flows among wild and cultivated plants, which requires further study on the impact on seed quality. Seed banking complements *in-situ* conservation measures, such as habitat protection and restoration, population augmentation, and species reintroduction. With planning and monitoring, resources allocation could be to optimize for seed banking and minimize conflicts with other conservation measures.

## Purpose of the Seed Bank

Kier et al. (2005) reviewed that Tropical and Subtropical Moist Broadleaf Forest biomes contain ecoregions with the highest plant species richness, and Le Roux et al. (2019) has projected higher extinction rates for such biodiversity hotspots. However, taking reference from a list compiled by Ag Professional (2013), only four out of the 15 largest seed banks are for the storage of wild species seeds. Moreover, none of these large capacity organizations focuses on the conservation of tropical species. The Singapore Botanic Gardens Seed Bank, which opened on 13 July 2019, is making a small, local contribution to this effort. Its expected capacity for 25,000 species, 10,000 seeds per species, is in excess of the total 1,506 species extant native vascular plant species, or about 70% of the total flora as tallied by Chong et al. (2009). Lim et al. (2019) has considered seed banking as an economical alternative method of *ex-situ* conservation, supporting the goals of the Species Recovery Programme, a part of the Nature Conservation Masterplan (National Parks Board, 2015). The excess capacity could be extended to biodiversity conservation for the wider Southeast Asia if managed well.



# Challenges of Seed Banking in the Aseasonal Tropics: Experiences from Singapore

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## Climatic and vegetation characteristics of Singapore

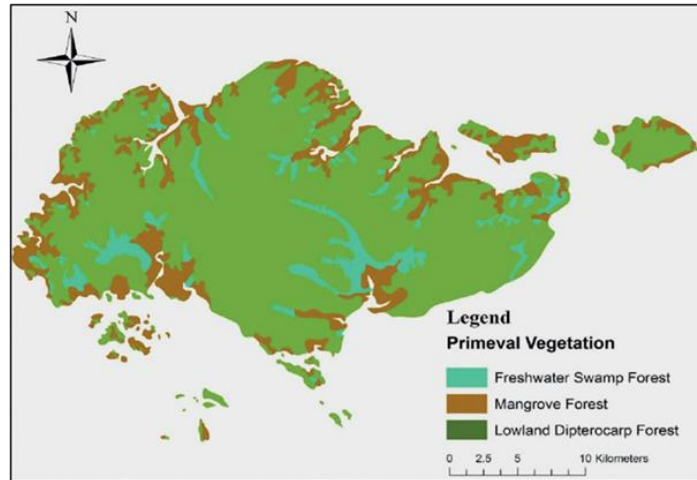


Fig 1. Original vegetation map of Singapore (Corlett, 1991)

Singapore experiences an equatorial climate with little seasonal variations. Meteorological Service Singapore has characterized it as a “typically tropical climate, with abundant rainfall, high and uniform temperatures, and high humidity all year round” with little month-to-month variation (Meteorological Service Singapore, 2020). According to Corlett (1991), 13% of the original vegetation of Singapore was mangrove forest, 5% was freshwater swamp forest, and the remaining was mainly lowland dipterocarp forest (Fig. 1).

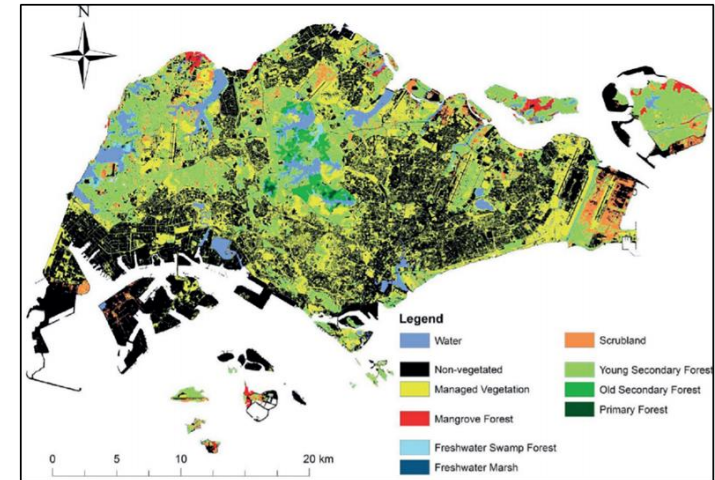


Fig 2. Current vegetation map of Singapore (Yee et al., 2011)

Rapid deforestation occurred after British colonisation in 1819. Much of the primary vegetation was lost by the early 1900s to cultivation of various cash crops (Corlett, 1992). Presently, 50% of the land area of Singapore is vegetated, with half of it managed and the remaining, wild or spontaneous. Of the latter only 200 ha is primary vegetation, in the form of lowland dipterocarp rainforest or freshwater swamp forest remains, accounting for about 0.28 % of the total land area (Yee et al., 2011) (Fig. 2).

# Challenges of Seed Banking in the Aseasonal Tropics: Experiences from Singapore



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## **Challenges Faced and Possible Solutions**

### **1. Recalcitrant Seeds**

Walters et al. (2013) estimated that up to 47% of species in tropical evergreen rainforests have recalcitrant seeds, as compared to the global proportion of 20–25%. Thus, almost half of the species in Singapore can be expected to have recalcitrant seeds. As Walters et al. (2013) has proposed, cryopreservation may be a viable option for the long-term storage of such seeds. However, more work will be needed to tailor the process to work for different species. In the meantime, repeated sampling of species with small, remnant populations and low seed production will be necessary to establish conservation collections of these challenging species.

### **2. Spatial and temporal availability of seeds**

Low population densities and sub-annual and supra-annual fruiting in tropical rainforests (see Corlett 1990) result in the patchy distribution of seeds in space and time. In many cases, disproportionate man-hours will be spent to monitor and collect seeds with low yields, while the overabundance of seeds for masting species could stretch seed collection, processing and storage capacities. The two extremes could be dealt with by pooling resources with other conservation programmes, e.g. conservation collection, to promote timely sharing of information and resources so that seed collection could be maximized and wastage minimized.

### **3. Habitat Fragmentation and Heterogeneity**

The land use history of Singapore has led to severe habitat fragmentation and deforestation, resulting in extreme population dynamics processes experienced by all remnant populations of native species. This could have yet unquantified population genetic consequences. Population genetic tools should be used to monitor the quality of collected seeds and characterize the source populations. This would also provide the basis for future, marker-based sampling to prioritise populations and reproductive events for seed collection and storage.

### **4. Effects from Urban Greening Efforts**

In human-managed landscape, the use of native plants for landscaping has become more prevalent, owing to their adaptation to local conditions. They are often sourced from foreign nurseries, and selected for desirable ornamental and horticultural traits. These non-native conspecifics and cogenics could hybridise with native populations, leading to genetic contamination. While the extent of the problem is not clear, it would be prudent to start monitoring to established some baseline. Given the preponderance of human-managed landscape in Singapore, the effect is likely to be palpable.



# Challenges of Seed Banking in the Aseasonal Tropics: Experiences from Singapore

## Current Operations and Activities of the Seed Bank

### Seed Morphology and Storage Behaviour

Over 290 species of plants from various families have been tested to determine the nature of the seeds and germination characteristics. Over 100 species have also been photographed to document their seed morphology.



### Seed Banking

Seeds which have demonstrated orthodox behaviour are placed under conventional storage. Thus far, about 50 species totalling 694,000 seeds have been banked, of which 13 belong to the genus *Ficus*. Out of these, 11 species are native to Singapore. Seeds from local research institutions have also been banked.



### Testing of Non-orthodox Seeds

Seeds of *Magnolia champaca* are being tested to determine their viability after being subjected to different drying and storage conditions. Similar tests are also being done on *Hanguana neglecta* to determine if the recalcitrant seeds can be optimized for storage over the short to medium term.



### *Ficus* Phenology Surveys

Weekly phenology surveys are conducted for 33 species of *Ficus* in the Singapore Botanic Gardens, of which 14 are native. Synconia are collected regularly to determine the stage of reproduction and the sex of plants of dioecious species. Seed collected during the course of the survey are tested for suitability for seed banking.



# Challenges of Seed Banking in the Aseasonal Tropics: Experiences from Singapore

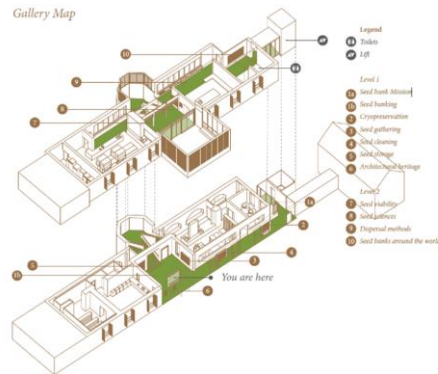


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## Conclusion

Seed banking is not the complete solution for the conservation of all plant species. It is meant to complement other conservation measures such as *in-situ* protection of wild populations and their habitats, and ultimately habitat restoration and species reintroduction. While Schoen and Brown (2001) acknowledged the relevance of seed banks in the *ex-situ* conservation of wild species, they brought attention to the divergence between phenotypic optima of a natural population versus a seed collection or even a conservation collection. The main worry is that a collection might be cut off from biotic interactions and undergo unintended human selection, i.e. domestication. In summary, while seed banking could serve as an insurance against extinction, to reconstitute a species that is ecologically extinct is an untested proposition.



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## Abstract

*Ficus* is a keystone taxon with mutualistic relationships with pollinators and frugivorous vertebrates, thus its conservation is critical for the continued existence of other species. However, it is both a taxon suitable and unsuitable for seed banking. 1. Many *Ficus* species have orthodox seeds, but its agaonide pollinator could not be preserved, as it requires a viable host population for its survival. While growing *Ficus* from banked seed can regenerate a population of previously extinct plant, such a species would be for all practical considerations evolutionarily extinct without its pollinator, thus *in situ* conservation will remain the most reliable means of conservation. 2. Regular refreshing of accessions in the seed bank is advisable to ensure plants grown from the seeds for augmenting populations have not deviated from compatibility with the pollinator. 3. The long distance pollinator dispersal of *Ficus* leads to the expectation of low population differentiation. This is contradicted by work on a number of species of *Ficus*, suggesting it is important to sample seeds from more populations and reproductive events, and characterize them with genetic markers to ensure that enough diversity has been collected. 4. Landscape heterogeneity and habitat fragmentation, by affecting pollinator movement, have implications for *in situ* conservation and seed collection. Reintroduction of plants into suitable habitat outside the main populations is a viable way of increasing connectivity between populations.

## Introduction

The genus *Ficus* (Moraceae) consists of more than 700 species of figs worldwide (Berg and Corner 2005), and is considered a keystone taxon in Southeast Asia and the Neotropics for its aseasonal fruiting that supports a wide range of vertebrate frugivores year-round (Leighton and Leighton 1983; Terborgh 1986; Lambert and Marshall 1991). Each species is pollinated by one specific species of Agaonidae (Chalcidoidea) wasp, and the wasp is in turn dependent on the pollinated ovaries for raising its larvae (Galil and Eisikowitch 1968): two partners in a tightly coevolved mutualism (Weiblen 2001; Weiblen and Bush 2002; Wiebes 1979). The details of the reproductive cycle are as shown in Figure 1.

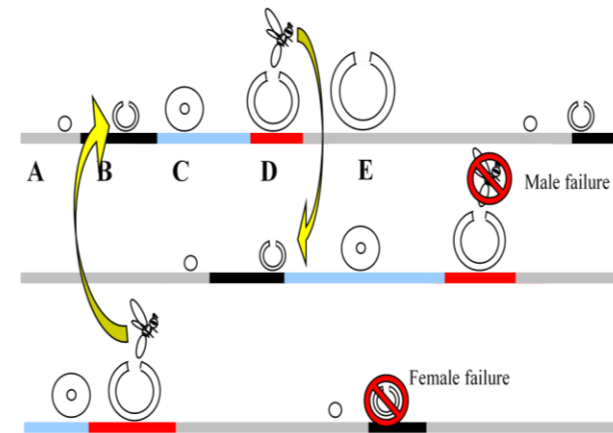


Fig 1. The reproductive phases of *Ficus* follows the stages of Galil and Eisikowitch (1968), from right to left, progressing in time:

1. Vegetative phase (unlabelled): interval between successive flowering.
  2. Prefloral phase A: syconia with immature flowers not attractive to pollinator.
  3. Female phase B: syconia on plants are attractive to pollinator, which will pollinate mature female flowers and lay eggs in some.
  4. Interfloral phase C: the next generation of the wasps and the seeds of the syconia developing.
  5. Male phase D: the male flowers are mature and the wasps emerge and mate before the gravid female wasps leave, carrying the pollen to seek out plants with syconia in the female phase B to reproduce in.
  6. Postfloral phase E: syconia ripen and attracts fruit-eating dispersers.
- Note how any gap in pollinator access to syconia results in reproductive failure of either partner. The male and female failures of the *Ficus* are highlighted. Male failure signifies the failure of the successful pollen transfer and death of the wasp, while female failure often leads to the abscission of unpollinated syconia. For simplicity, a monoecious *Ficus* is considered. In the case of dioecious *Ficus*, there will be a separation of male and female functions.

## **Limitations of Seed Banking in Conservation of *Ficus***

Though *Ficus* seeds are mostly orthodox, making the genus a candidate for long-term seed storage via conventional methods, such surgical conservation apart from all its symbionts is impractical. Its species-specific interdependence with pollinators (Weiblen and Bush 2002), meant that should a *Ficus* species go extinct, the corresponding pollinator would also go extinct, *vice versa* (Wiebes 1979). Harrison (2000) has observed how the disruption of *Ficus* reproduction led to the local extinction of pollinators, while in the case of an introduced species, *Ficus elastica*, the specificity and stability of the mutualism defied any recruitment of pollinators from related *Ficus* species in Singapore for more than seventy years until the “invasion” of the pollinator reestablished seed production (Harrison et al 2017). Thus, unless there is a way to cryogenically preserve the pollinator simultaneously as the seeds are stored, there is no point to regrow a hitherto extinct plants from seeds into a world where the pollinator no longer exists. Such a species would be just a botanical curiosity with limited ecological role and still be evolutionarily extinct. Though, as recent work on orchid seed preservation with mycorrhizal fungi using encapsulation showed that some progress has been made to conserve symbiotic partners for orchids (see Merritt et al. 2013, and Sommerville and Offord, 2014), the concurrent *ex situ* preservation of *Ficus* and its specific pollinator is still an unattained goal. Therefore for *Ficus*, seed storage can not yet be relied upon as the main method for conserving the genus, and *in situ* conservation, complete with the community interactions, remains as the best insurance against extinction.

However, it might still be useful to be able to grow plants from stored seeds while a remnant reproducing population exists. This may be done primarily to augment the gene pool of the population, and secondarily to push the population size safely beyond the Critical Population Size (CPS) *sensu* Bronstein et al. (1990), i.e. the minimum population size of the host required to support the pollinator with overlapping crops of syconia to rule out stochastic events leading to global pollinator extinction. While it may be useful also for establishing new populations should drastic environmental changes shift the habitable range of *Ficus* species and their pollinators, there would still be concern that after many generations, pollinators may have co-evolved with the remnant plants such that they may no longer be optimally adapted to pollinate the regenerated plants. As Schoen and Brown (2001) have hypothesized, seeds produced after numerous generations apart from the biotic and abiotic selective pressures of the natural environment may lead to multilocus adaptations of the plants diverging from the original population optima. This may conceivably apply to but not be limited to traits crucial to the success of the pollinators, e.g. olfactory cues of receptive syconia, the style length the female flowers, and duration of the syconia development. This could only be preempted by continually refreshing accessions in storage.

## Spatial genetic structure and population differentiation

The low population densities of *Ficus* species meant that the pollinators must travel considerable distances (Janzen 1979). Nason et al. (1998) first showed using genetic markers that pollinators may traverse great distances upward of 6 km for monoecious forest species, a generalization upheld by later works (Nazareno and Carvalho 2009; Ahmed et al. 2009). The long distance pollen dispersal of monoecious species has been contrasted with limited dispersal of dioecious species, attributed to the differences in the pollinators' flight heights and dispersal efficiencies (Compton et al. 2000; Harrison and Rasplus 2006). Furthermore, dioecious *Ficus* are typically found in locally dense clusters (Harrison 2000; Wang et al. 2009), making long distance dispersal unnecessary barring exceptional climatic events (Harrison 2000, 2003). Because of this preconception, work on population genetics has focused on dioecious species (e.g. Valdeyron et al. 1985; Chen et al. 2008).

Given the difficulty of recognizing distinct populations, recent studies have focused on spatial genetic structure (SGS) of inland forest dioecious species (e.g. Yu et al. 2010; Zhou and Chen 2010; Dev et al. 2011). Interestingly, monoecious species were overlooked even though the difficulty of delimiting populations is irrelevant, and it would be invaluable for illuminating how aggregation across a heterogeneous landscape with patchy habitats affects the population structure and spatial genetic structure (SGS), despite supposedly unhindered pollinator dispersal. Some recent work on *Ficus superba*, a coastal monoecious species, has shown the species to have significant SGS and population differentiation. Furthermore, insular populations were more similar, while main land populations more distinct, suggesting that dispersal and possibly pollen flow was more restricted over land than sea (unpublished result, refer to Figure 2).

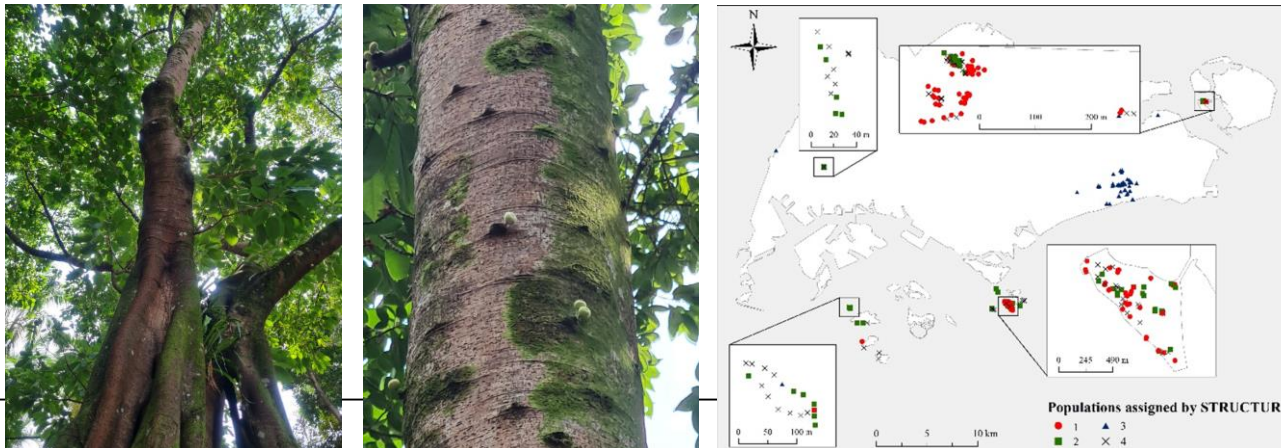


Fig 2. Individuals of *Ficus superba* genotyped using dominant Amplified Fragment Length Polymorphism (AFLP) marker were assigned into four groups by the Bayesian clustering software STRUCTURE 2.3.3. There was significant population genetic structure and SGS found. Isolation by distance (IBD), was higher for the main island populations (Mantle's test correlation 0.735,  $p = 0.001$ ) than the insular and coastal ones (Mantle's test correlation 0.0867,  $p = 0.002$ ).

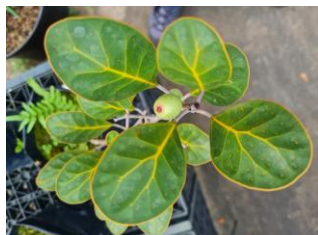
# Challenges of Seed Banking in the Aseasonal Tropics: Experiences from Singapore

## Consideration for Seed Collection

The pollinators of *Ficus* are first passively wind-dispersed then actively flying up-wind to receptive plants (Compton et al. 2000; Harrison and Rasplus 2006). This combined with the randomness of potential pollen donors coming into phase D, coinciding with the receptive plants (phase B), meant that each reproductive event is a sampling of a subset of available pollen-donors. Thus, it might be necessary to collect seeds from different crops over an extended period to include various prevailing wind conditions to obtain seeds fathered by the most diverse set of pollen parents. However, more work is needed to show that how to optimally sample reproductive events and populations using genetic markers to ensure that sufficient seeds are collected to conserve sufficiently representative genetic diversity across populations.

## Landscape Heterogeneity

The greater isolation by distance (IBD) found in main island populations versus the insular and costal populations of *Ficus superba* suggests lower gene flow across the land than sea, and more pollination events within populations than between. Thus, it is hypothesized that the pollinators could be experiencing more impediment over land than sea as the result of greater surface roughness over land, as would be expected for pollinator dispersal aided by the wind. This suggests that connectivity could be enhanced by planting outside the main populations, with newly established plants acting as stepping stones for pollinators. This would lessen the probability of local pollinator extinction through increasing the effective population size of the host, and might have an effect on seed quality.



# Challenges of Seed Banking in the Aseasonal Tropics: Experiences from Singapore



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## Conclusions

While keeping *Ficus* seeds in conventional storage is straightforward, we have discussed why it cannot be the main means of conservation. The main reason is that the species-specific symbiosis with the pollinator is not amendable to conventional storage, with each member of the partnership sustained by interaction with the respective partner, and it can be assumed that should one member of the partnership goes extinct, the other member would also cease to exist in due time. The co-evolution of the partners also meant that seeds stored, numerous generations removed, may no longer be optimally adapted to the pollinator, unless the seed accessions are refreshed on a regular basis.

Furthermore, the collection of seeds with sufficient genetic diversity is not straightforward as it initially seems, as population structure can be significant despite long-distance pollination. This meant that populations and seed collections should be characterized to ensure that sufficient genetic diversity has been captured.

Lastly, given the need to constantly refresh the stored seeds, they should only be used to augment extant populations. With a better understanding of how landscape affects pollinator movement, it would allow us to strategically plant outside existing populations to increase their connectivity. This not only bolsters the resilience of the partners against extinction, but would also have consequences for the population structure and seed quality, which deserve looking into.

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# PREDICTING SEED LIFESPAN FOR THE IMPROVED CURATION OF CONSERVATION SEED BANKS



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## THE PROBLEM

Identifying the storage behaviour and predicting the lifespan of seeds in storage for diverse wild species is key for seed bank management.

In conjunction with seeds that are difficult to germinate, current methodologies of assessing viability are resource intense. We currently have no good method of determining seed longevity in storage.

We aim to develop new technologies to effectively triage and curate seed collections during storage to ensure irreplaceable collections are not lost, and that viable seeds are available for future conservation and restoration.

Western Australia is home to > 12,700 wild, native plant taxa

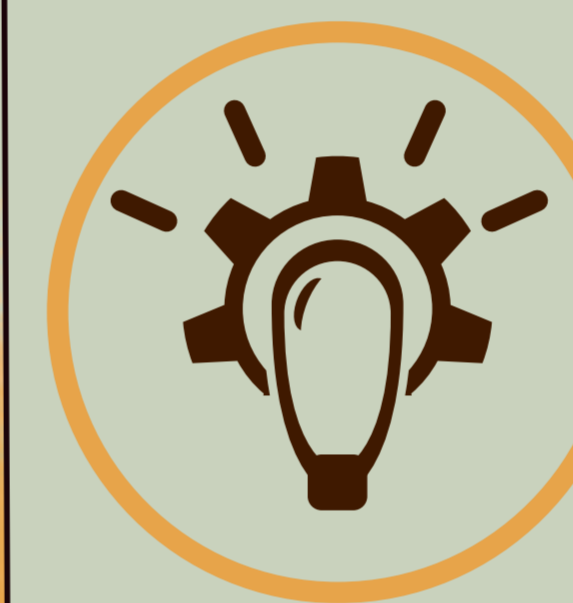
## OUR PROJECT

We will develop novel technologies and data interrogation techniques to predict viability maintenance of seeds of diverse wild species stored in seed banks.

We aim to:

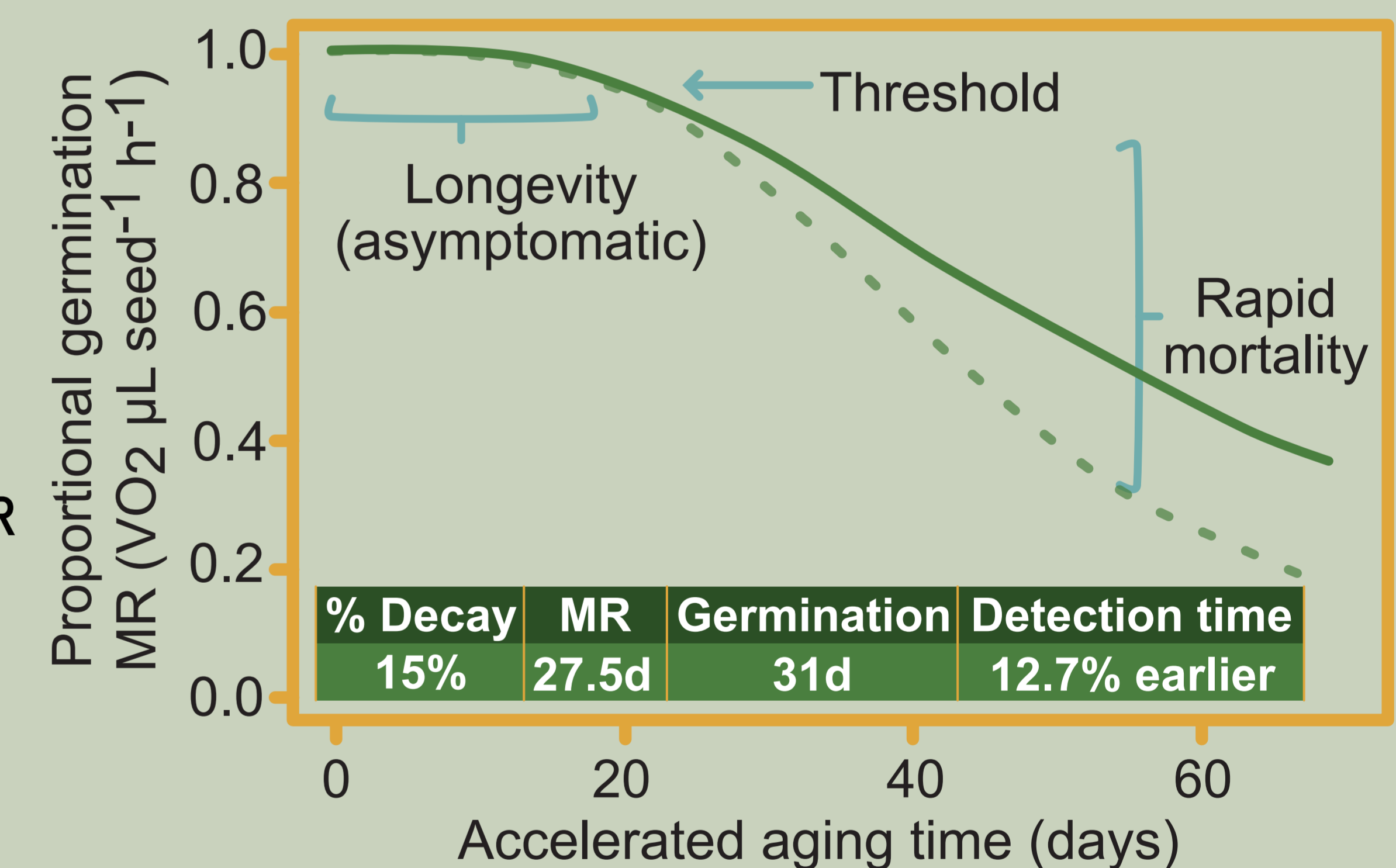


**ONE**  
Identify species or collections of Western Australian species that are performing poorly in storage.



**TWO**  
Investigate seed respirometry and multi-spectral imaging to predict seed viability and longevity.

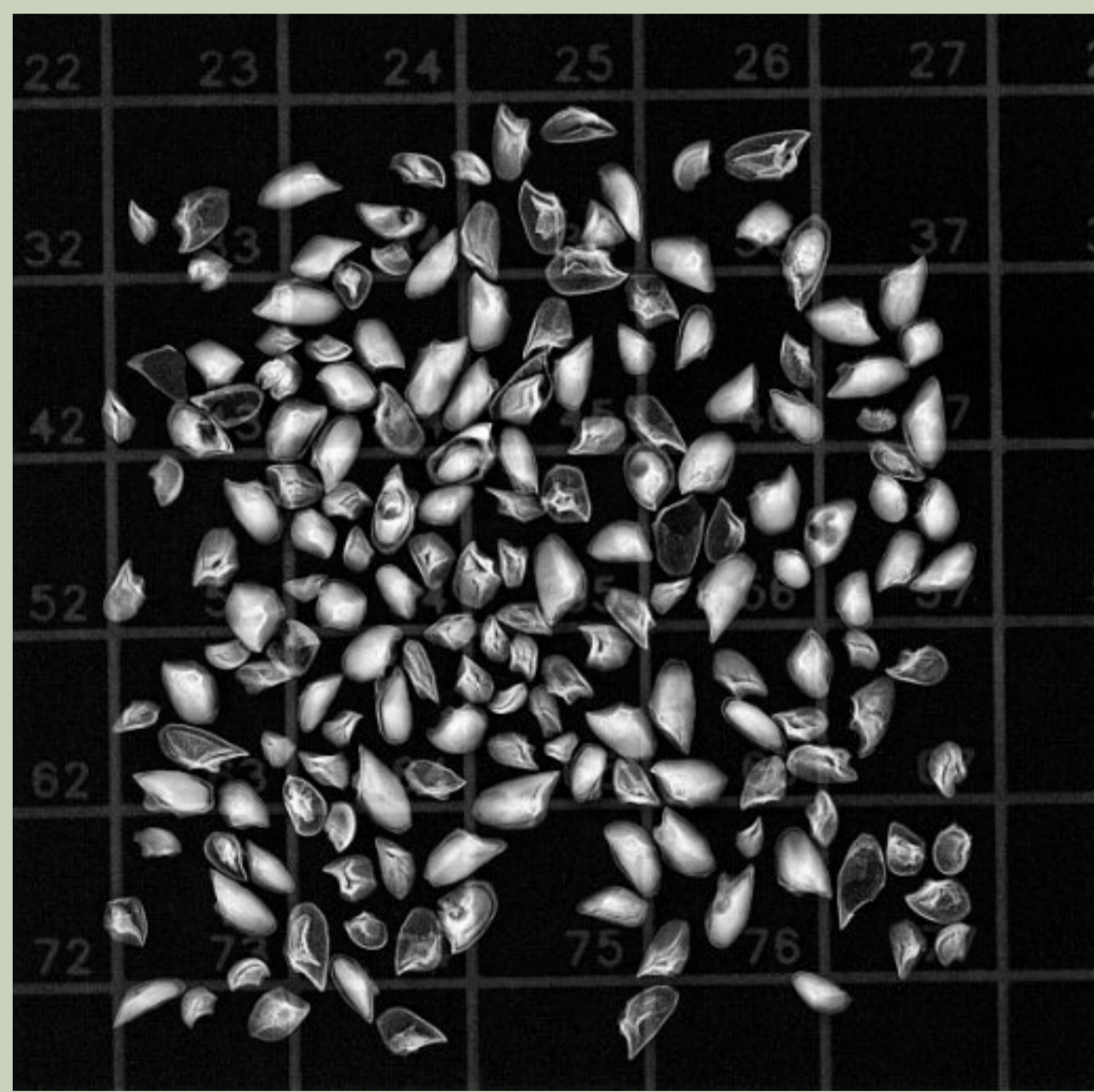
(R) Measurements of metabolic rate (MR) in seeds of oats show promise in the early detection of viability decline. Results obtained more rapidly compared with germination testing (MR = 20h; dashed line, germination test = 14d; solid line)



**THREE**  
Develop alternative storage protocols to improve the storage stability of at-risk species.



**FOUR**  
Develop novel data analysis techniques to more accurately predict the onset of viability decline.



(L) X-rays can only assess seed fill, (R) while cut-tests are destructive. Photos: David Blumer.

## PhD projects available!

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# History of seed collection, storage and use at the Royal Botanic Gardens and Domain Trust

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The knowledge and technology available to support seedbanking has developed considerably in the last 35 years, with common goals provided by the Global Strategy for Plant Conservation and funding through the Millennium Seed Bank Project and other initiatives. Changes at The Australian PlantBank - formerly the NSW SeedBank and now a component of the Australian Institute of Botanical Science - are one example of the parallel changes in purpose and mission to collect wild species.

Our target species have changed over time: initially curating collections for the development of the Australian Botanic Garden, Mount Annan; expanding to include conservation of threatened species; and more recently, addressing the challenges of conserving rainforest seeds and orchids using a variety of *ex situ* methods including cryopreservation. Current projects are supported by a range of partners, including the NSW Government's Saving our Species program<sup>1</sup>, the Australian Seed Bank Partnership<sup>2</sup> and The Ian Potter Foundation<sup>3</sup>.

With an increasing quantity and diversity of seed collections in storage, comes the challenge of curating existing collections so they are available in coming decades. Management action taken now depends on collection quality and quantity, which has been influenced by the seed drying and storage conditions and associated technology available at the time of collection.

This poster describes advances in seed drying, storage conditions, supporting technology and the changing mission, targets and outputs of the *ex situ* conservation program, which is a key focus of the Australian Institute of Botanical Science<sup>4</sup>.

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<sup>1</sup> <https://www.environment.nsw.gov.au/topics/animals-and-plants/threatened-species/saving-our-species-program>

<sup>2</sup> <https://www.seedpartnership.org.au/>

<sup>3</sup> <https://www.ianpotter.org.au/>

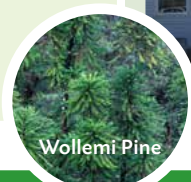
<sup>4</sup> <https://www.rbgsyd.nsw.gov.au/science/australian-institute-of-botanical-science>

# History of seed collection, storage and use at the Royal Botanic Gardens and Domain Trust

- We are storing living collections of an increasing diversity of NSW plant species. Our ex situ (offsite) collections provide an insurance policy against extinction of native plants in the wild.
- We are refining our understanding of the best way to store different species: seed, tissue culture, cryopreservation or plants in the nursery and garden, or a combination of these methods.
- We are active partners in conservation with Australian and global networks responding to threats such as bushfires, plant diseases and climate change.



	<1987	1987-1999	1999	2003-2009
<b>Seed drying</b>	<ul style="list-style-type: none"> <li>• Room conditions</li> </ul>	<ul style="list-style-type: none"> <li>• Heated airing cabinet or air conditioned room</li> </ul>	<ul style="list-style-type: none"> <li>• Seed drying room: 15% RH, 15°C</li> </ul>	<ul style="list-style-type: none"> <li>• Seed drying room: 15% RH, 15°C</li> </ul>
<b>Storage conditions</b>	<ul style="list-style-type: none"> <li>• Room temperature and humidity</li> </ul>	<ul style="list-style-type: none"> <li>• Commercial drinks fridge (5°C)</li> </ul>	<ul style="list-style-type: none"> <li>• -18°C walk-in freezer for long term storage</li> <li>• 4°C walk-in coolroom for short term storage</li> </ul>	<ul style="list-style-type: none"> <li>• -18°C walk-in freezer for long term storage</li> <li>• 4°C walk-in coolroom for short term storage</li> </ul>
<b>Technology</b>	<ul style="list-style-type: none"> <li>• Glass jars</li> </ul>	<ul style="list-style-type: none"> <li>• Vacuum sealed plastic laminated foil envelopes</li> <li>• Tissue culture</li> </ul>	<ul style="list-style-type: none"> <li>• NSW Treasury funding for major upgrade of NSW Seedbank to support conservation of NSW native species, including threatened species.</li> </ul>	<ul style="list-style-type: none"> <li>• Zig-zag aspirator to clean seeds (2004)</li> <li>• Expanded germination testing capacity (2007)</li> </ul>
<b>Mission/targets/outputs</b>	<ul style="list-style-type: none"> <li>• Located at the Royal Botanic Garden Sydney site</li> <li>• Used for botanic garden development and international seed exchange</li> </ul>	<ul style="list-style-type: none"> <li>• Moved to the Australian Botanic Garden Mount Annan to provide living collections for the new botanic garden</li> <li>• Horticultural development and tissue culture of Waratahs and Flannel Flowers</li> <li>• 1994: Wollemi Pine discovered</li> </ul>		<ul style="list-style-type: none"> <li>• Millennium Seed Bank partnership begins, which supports an expanded statewide seed collecting program and increased capacity for seed testing and research. Increased collaboration with Australian conservation seedbanks</li> <li>• Dryland/orthodox species collected eg. Acacia, Eucalypt</li> </ul>



Zig-zag aspirator 2004



Australian PlantBank



Tissue culture



Rainforest fruits



## Australian PlantBank opened

### 2009-2012

### 2013

### 2016 – Present

#### Seed drying



- Seed drying: 15% RH, 15°C

- Seed drying: 15% RH, 15°C

- Expanded capacity and new drying technology, 15% RH, 15°C (2020)

#### Storage conditions



- 4°C walk-in coolroom (fridge)
- -18°C walk-in freezer
- Tissue culture

- 4°C walk-in coolroom (fridge)
- -20°C walk-in freezer (two: one in use, one for expansion)
- Tissue culture

- 4°C walk-in coolroom (fridge)
- -20°C walk-in freezer (two: one in use, one for expansion)
- Tissue culture
- Cryopreservation of sub-samples of incoming seed collections from 2019

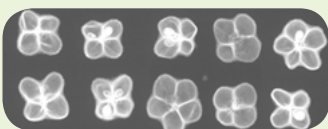
#### Technology



- X-ray machine (2011), Digital microscope (2012)

- Cryostorage facilities 2013

- Differential Scanning Calorimeter 2018
- Bar coding of seed packets for database recognition
- Increased use of DNA technology



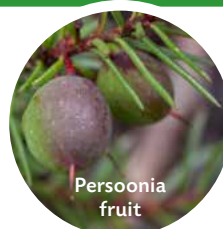
#### Mission/targets/outputs



- Cryostorage research (ultra low temperature -196°C)
- Alpine seed research
- Orchid seed and fungi cryopreserved
- Initial screening of rainforest seeds for desiccation tolerance funded by Allianz and private benefactors 2010
- On-site restoration of local species following African Olive control

- Rainforest Seed Conservation Project funded by the Arcadia Fund and others
- Cryostorage research
- Seed collection projects with Australian Seed Bank Partnership (1000 Species and Global Trees projects)
- Plant disease Myrtle Rust prompts urgent ex situ collection development within plant family Myrtaceae
- Persoonia research

- Conservation focus: Saving our Species program initiated by NSW state government to address threats to plant extinction
- Increased focus on translocation of threatened species
- Rainforest Seed Conservation Project continues, funded by The Ian Potter Foundation (2019)
- PlantBank team win BGCI International Global Seed Conservation Challenge in 2017



Persoonia fruit



Rainforest fruits

## Abstract

Seed morphological characters can be distinguished qualitatively (color, shape, and texture) and quantitatively (length, width, thickness and weight). Both are used to support identification and classification processes for taxonomy purposes. So far, qualitative characters are more often used in seed identification than quantitative characters. Quantitative characters or numerical characters, namely seed morphometry, are rarely used in seed identification. The purpose of this study was to determine the most influential seed morphometric characteristics in the identification process. The seeds used were associated species of *Musa acuminata* Colla from Bromo Tengger Semeru National Park (BTSNP). The methods used were measurement of length, width, thickness, and weight, determination of Eccentricity Index (EI) and Flatness Index (FI) by observing color, texture and shape of the seeds of 12 species. The color, texture and surface characters of the seeds were converted into numerical characters through scoring method. Data were analyzed by PCA and cluster analysis using PAST ver.3.0. The results showed that based on the influence of morphometric characters, there were three seed groupings of associated species of *M. acuminata* from BTSNP, i.e. (I) group of species influenced by the color, weight and texture characters; (II) group of species influenced by length, width, thickness and FI characters; and (III) group of species not influenced by all characters. The seeds of *Pinanga coronata*, *Ricinus communis* and *Solanum betaceum* had the most specific morphometric characters making them easy to distinguish from others. Seed groupings of *Amaracarpus* sp., *Cayaponia laciniata*, *Eumachia montana* and *Zapoteca tetragona* differed from other species due to significant their length, width, thickness and FI values. Thus, numerical characters can be used as one of the supporting characters in the process of grouping species.

**Key words:** Bromo Tengger Semeru National Park, identification, morphometry, seed, supporting characters

## INTRODUCTION

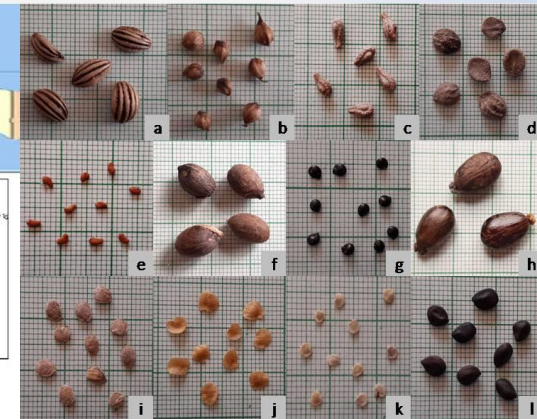
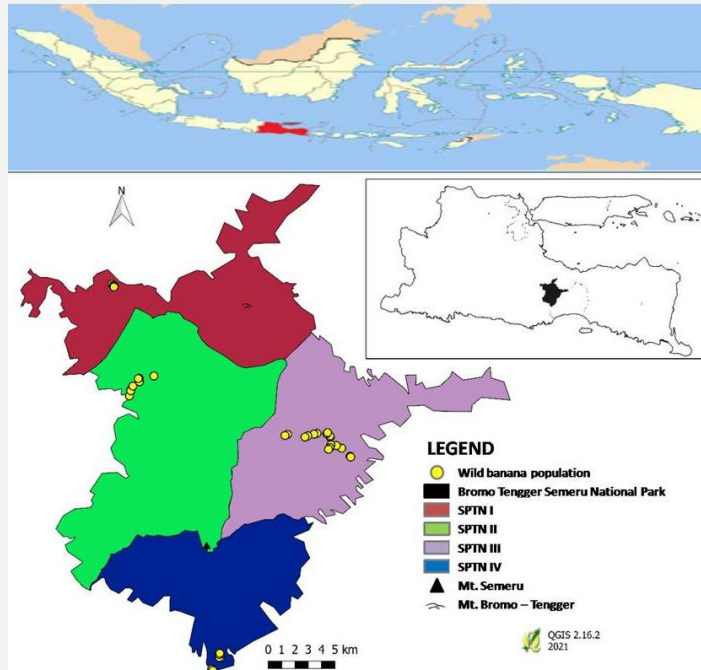
Morphological characters of seeds are very important in seed conservation efforts, especially in ecology and seed biology science. The variation of seeds morphological characters is very high, which is shown through qualitative and quantitative characters. The numerical morphological characters can be analyzed through morphometry. Morphometric characters are able to contribute in supporting genera, species and intraspecies level of identification.

Bromo Tengger Semeru National Park (BTSNP) is one of the national parks in East Java. Based on the wild banana seed exploration results, there are found associated plants were fruit. Associated plant species in a plant community are related to the interaction between one species and another.

The purpose of this study was to determine the most influential seed morphometric characteristics in the identification process.

## MATERIAL & METHOD

This study was conducted on November 2020 – April 2021 at In-vitro and Seed Bank Laboratory of Purwodadi Botanic Garden. The seed materials of associated plants with *M. acuminata* were obtained from BTSNP. The morphometric characters of 12 seed species were determined by measuring the length, width, thickness, and weight, Eccentricity Index (EI) and Flatness Index (FI). Data were analyzed by PCA and cluster analysis using PAST ver.3.0.



12 seed species of associated plants with *Musa acuminata* from BTSNP; a. *Amaracarpus* sp., b. *Acronychia trifoliolata*, c. *Cayaponia laciniata*, d. *Eumachia montana*, e. *Fragaria blumei*, f. *Pinanga coronata*, g. *Phytolacca icosandra*, h. *Ricinus communis*, i. *Solanum betaceum*, j. *S. capsicoides*, k. *S.pseudocapsicum*, dan l. *Zapoteca tetragona*. Scale 1 square = 1 mm.



# Seed Morphometry of Associated Species of *Musa acuminata* Colla from Bromo Tengger Semeru National Park, East Java, Indonesia

Dewi Ayu Lestari<sup>1\*</sup>, Elga Renjana<sup>1</sup>, Linda Wige Ningrum<sup>1</sup>, Apriyono Rahadiantoro<sup>1</sup>, Elok Rifqi Firdiana<sup>1</sup>, Trimanto<sup>1</sup>, Shofiyatul Mas'udah<sup>1</sup>, Lia Hapsari<sup>1</sup>, Dian Latifah<sup>2</sup>

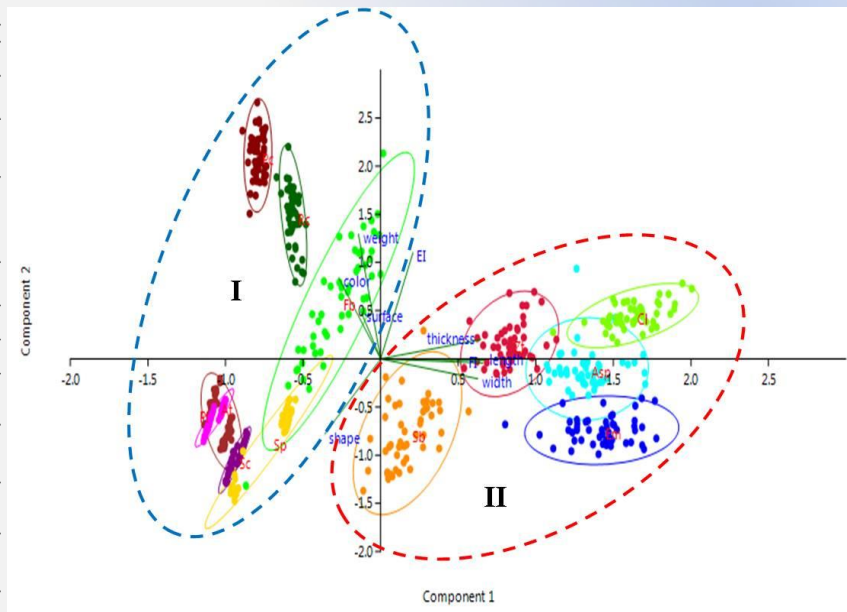
<sup>1</sup>Purwodadi Botanic Garden – Research Center for Plant Conservation and Botanic Garden, Indonesian Institute of Sciences (LIPI)

<sup>2</sup>Cibodas Botanic Garden – Research Center for Plant Conservation and Botanic Garden, Indonesian Institute of Sciences (LIPI)

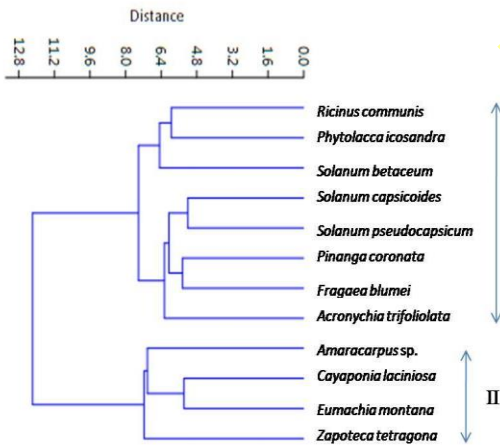
Email: [chunyang.dee@gmail.com](mailto:chunyang.dee@gmail.com)\*



Species Name	Family	Quantitative character				Qualitative character		
		Length (mm)	Width (mm)	Thickness (mm)	Weight (g)	Color	Texture	Shape
<i>Acronychia trifoliolata</i> Zoll. & Moritz	Rutaceae	0.39 ± 0.04	0.33 ± 0.03	0.33 ± 0.03	0.01 ± 0.005	Pale brown to dark brown	to Rough stringy	and Spheroid
<i>Amaracarpus</i> sp.	Rubiaceae	6.98 ± 0.41	5.58 ± 0.35	2.52 ± 0.35	0.04 ± 0.04	Pale brown to dark brown	with Rough, notched and semi-circle	Broad elliptic
<i>Cayaponia laciniosa</i> (L.) C. Jeffrey	Cucurbitaceae	6.47 ± 0.33	3.39 ± 0.14	3.41 ± 0.35	0.03 ± 0.003	Cream	Rough, have cuneiform pattern at the middle of seed shape	Cuneiform
<i>Eumachia montana</i> (Blume) I.M. Turner	Rubiaceae	6.93 ± 0.49	5.51 ± 0.39	2.43 ± 0.33	0.03 ± 0.006	Grey	Rough semi-circle	and Ovoid to broad elliptic
<i>Fragaea blumei</i> G.Don	Gentianaceae	2.43 ± 0.44	1.46 ± 0.20	0.82 ± 0.16	0.002 ± 0.0006	Brown	Rough (like cracks)	Fusiform to cuneiform
<i>Phytolacca icosandra</i> L.	Phytolaccaceae	0.29 ± 0.01	0.25 ± 0.01	0.16 ± 0.01	0.006 ± 0.0004	Black	Shiny smooth	and Semi-deltoid to spheroid
<i>Pinanga coronata</i> (Blume ex Mart.) Blume	Arecaceae	1.04 ± 0.06	0.69 ± 0.05	0.69 ± 0.04	0.33 ± 0.05	Black	Ribbed stringy	and Broad ovoid to elliptic
<i>Ricinus communis</i> L.	Euphorbiaceae	1.38 ± 0.09	0.84 ± 0.06	0.58 ± 0.03	0.29 ± 0.07	Dark brown with cream to pale brown pattern	Smooth shiny	and Broad elliptic with blunt base
<i>Solanum betaceum</i> Cav.	Solanaceae	4.01 ± 0.56	3.77 ± 0.57	0.67 ± 0.14	0.003 ± 0.0003	Pale brown	Cream colour hairs	Thin (plateriform) and ovoid
<i>S. capsicoides</i> All.	Solanaceae	0.48 ± 0.03	0.41 ± 0.03	0.06 ± 0.01	0.003 ± 0.0005	Golden yellow	Rough with texture pattern	Tiny, spheroid to narrow square
<i>S.pseudocapsicum</i> L.	Solanaceae	0.26 ± 0.01	0.25 ± 0.01	0.04 ± 0.01	0.002 ± 0.0005	Cream yellowish white	to Rough	Thin (plateriform) and broad kidney
<i>Zapoteca tetragona</i> (Willd.) H.M.Hern.	Leguminosae	5.55 ± 0.33	3.85 ± 0.35	2.59 ± 0.31	0.04 ± 0.006	Black	Smooth shiny	Oblong fusiform to



PCA results from 12 seed species morphometric of associated plant with *Musa acuminata* in BTSNP; At=*Acronychia trifoliolata*, Asp=*Amaracarpus* sp., Cl=*Cayaponia laciniosa*, Em=*Eumachia montana*, Fb=*Fragaea blumei*, Pc=*Pinanga coronata*, Rc=*Ricinus communis*, Sb=*Solanum betaceum*, Sc=*S. capsicoides*, Sp=*S. pseudocapsicum*, Pi=*Phytolacca icosandra*, dan Zt=*Zapoteca tetragona* (red code is code for seed species; blue code is code for morphological character; Cluster I in blue ellips and cluster II in red ellips)



Cluster analysis result from 12 seed species morphometric of associated plants with *Musa acuminata* in BTSNP

### CONCLUSION

Quantitative morphological characters such as length, width, thickness and FI values became the most influential morphometric characters in identifying seed plants found to be associated with *M. acuminata* in BTSNP. Qualitative morphological characters such as seed shape and color have no effect because the variations are too diverse, thus affecting subjectivity.

### ACKNOWLEDGEMENT

We would like to thank the Millennium Seed Bank Partnership, Royal Botanic Gardens, Kew and the Research Center for Plant Conservation and Botanic Garden – LIPI for funding the exploration of wild banana seeds and associated species in BTSNP. Mr. Sumaji, Mr. Sampun, Mr. Didik Purnomo and the team from BTSNP for assisting in the implementation of the exploration. Ms. Lutvinda Ismanani and Mr. Hari Harmawan for those who help in the technical processing of the seeds.



# Desiccation tolerance of seeds and germination characteristics of tree species growing in the Ryukyu Islands, Japan

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Forest Tree Breeding Center, Forestry and Forest Products Research Institute (FTBC, FFPRI)

## Introduction

The Ryukyu Islands are one of the most biologically diverse areas in Japan, with a high percentage of endangered and endemic species as well as a large number of species belonging to various taxonomic groups. For these reasons, this area is expected to be registered as a World Natural Heritage site by International Union for Conservation of Nature (IUCN) in 2021. Ex-situ conservation is important for preserving current biodiversity and diverse genetic resources. In particular, seed banks are one of the most effective ways to conserve the forest's genetic resources in a limited space. However, there is still limited information on the desiccation tolerance of seeds, which is a key factor in the feasibility of seed bank conservation. In addition, details regarding the germination conditions of many tree species, especially those in the tropics, are also limited.



## Study aims

In this study, we investigated the desiccation tolerance and germination characteristics of seeds of tree species growing in Iriomote Island, one of the Ryukyu Islands.

# Study species

					SID by RBG Kew		
	Family	Species	Distribution	Rad list	Species	Genus	Germination condition
1	Hernandiaceae	<i>Hernandia nymphaeifolia</i>		-	ND	ND	ND
2	Lauraceae	<i>Machilus thunbergii</i>		-	Recalcitrant	-	ND
3	Pandanaceae	<i>Pandanus odoratissimus</i>		-	ND	ND	ND
4	Hamamelidaceae	<i>Distylium racemosum</i>		-	ND	ND	ND
5	Cannabaceae	<i>Trema orientalis</i>		-	Uncertain	Orthodox?	ND
6	Fagaceae	<i>Quercus miyagii</i>	Endemic	-	ND	Recalcitrant	ND
7	Euphorbiaceae	<i>Mallotus japonicus</i>		-	Orthodox	-	ND
8		<i>Melanolepis multiglandulosa</i>		-	ND	ND	ND
9	Phyllanthaceae	<i>Antidesma pentandrum</i>		-	ND	Orthodox	ND
10		<i>Flueggea trigonoclada</i>		-	ND	Orthodox	ND
11	Clusiaceae	<i>Calophyllum inophyllum</i>		-	Recalcitrant?	-	ND
12	Salicaceae	<i>Idesia polycarpa</i>		-	ND	ND	ND
13	Rutaceae	<i>Melicope triphylla</i>		-	ND	Orthodox	ND
14		<i>Zanthoxylum ailanthoides</i>		-	ND	Orthodox	ND
15	Cornaceae	<i>Alangium premnifolium</i>	Endemic	-	ND	ND	ND
16	Primulaceae	<i>Ardisia sieboldii</i>		-	ND	Recalcitrant	ND
17	Symplocaceae	<i>Symplocos cochinchinensis</i>		-	ND	ND	ND
18	Icacinaceae	<i>Nothapodytes nimmonianus</i>		EN	ND	ND	ND
19	Rubiaceae	<i>Psychotria asiatica</i>		-	Orthodox	-	ND
20	Boraginaceae	<i>Ehretia dicksonii</i>		-	ND	Orthodox	ND
21	Oleaceae	<i>Fraxinus griffithii</i>		-	ND	Orthodox	ND
22		<i>Osmanthus marginatus</i>		-	ND	Orthodox ? /Recalcitrant	ND
23	Lamiaceae	<i>Callicarpa japonica var. luxurians</i>		-	ND	Orthodox	ND
24		<i>Clerodendrum trichotomum</i>		-	ND	Orthodox	ND
25		<i>Premna serratifolia</i>		-	ND	ND	ND
26		<i>Vitex quinata</i>		CR	ND	Orthodox ? /Recalcitrant	ND
27	Aquifoliaceae	<i>Ilex rotunda</i>		-	ND	Orthodox	ND
28	Goodeniaceae	<i>Scaevola taccada</i>		-	Orthodox	-	light 8/16; 25°
29	Staphyleaceae	<i>Turpinia ternata</i>		-	ND	ND	ND
30	Annonaceae	<i>Monoon liukuense</i>		CR	ND	ND	ND

- 30 species belonging to 23 families and 30 genera of woody species growing in Iriomote Island, Okinawa Prefecture.

- For 11 species, no information on other species belonging to the same genus was found in the SID.

- Includes rare and/or endemic species.

# Materials and methods

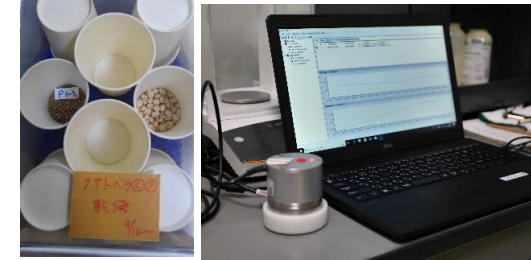
## Desiccation treatment

- The following three desiccation treatments were performed on the cleaned seeds.

Control: No treatment.

Drying treatment: seeds dried at 15% humidity and 20° C.

Wet treatment: Seeds stored at more than 80% humidity at 20° C.



## Viability test

- Seeds soaked in water overnight were cut and stained with tetrazolium (TTC) solution.
- Seeds with stained embryos were considered viable.



## Germination test

- After overnight water absorption, sow on 1% agar medium.
- 10-60 seeds x 1-2 replicates, 20°C, 16h dark, 30°C, 8h light.
- Germination was checked twice a week, and seeds were considered to have germinated when they showed roots of about 2 mm.
- Seeds that did not germinate for 6 weeks after sowing or 4 weeks after the last germination were dissected to check their condition.





# Results: Desiccation tolerance

	Species	Desiccation tolerance	Seed dormancy
1	<i>Hernandia nymphaeifolia</i>	Orthodox	No
2	<i>Machilus thunbergii</i>	Recalcitrant	No
3	<i>Pandanus odoratissimus</i>	Orthodox	No
4	<i>Distylium racemosum</i>	Orthodox	No
5	<i>Trema orientalis</i>	Orthodox	No
6	<i>Quercus miyagii</i>	Recalcitrant	No
7	<i>Mallotus japonicus</i>	Orthodox	No
8	<i>Melanolepis multiglandulosa</i>	Orthodox	No
9	<i>Antidesma pentandrum</i>	Orthodox	No
10	<i>Flueggea trigonoclada</i>	Orthodox	No
11	<i>Calophyllum inophyllum</i>	Orthodox	Yes?
12	<i>Idesia polycarpa</i>	Orthodox	No
13	<i>Melicope triphylla</i>	Orthodox	No
14	<i>Zanthoxylum ailanthoides</i>	Orthodox	-
15	<i>Alangium premnifolium</i>	Orthodox	No
16	<i>Ardisia sieboldii</i>	Orthodox	-
17	<i>Symplocos cochinchinensis</i>	Orthodox	Yes
18	<i>Nothapodytes nimmonianus</i>	Orthodox	No
19	<i>Psychotria asiatica</i>	Orthodox	No
20	<i>Ehretia dicksonii</i>	Orthodox	Yes
21	<i>Fraxinus griffithii</i>	Orthodox	No
22	<i>Osmanthus marginatus</i>	Recalcitrant	-
23	<i>Callicarpa japonica var. luxurians</i>	Orthodox	No
24	<i>Clerodendrum trichotomum</i>	Orthodox	-
25	<i>Premna serratifolia</i>	Orthodox	-
26	<i>Vitex quinata</i>	Orthodox	No
27	<i>Ilex rotunda</i>	Orthodox	Yes
28	<i>Scaevola taccada</i>	Orthodox	No
29	<i>Turpinia ternata</i>	Orthodox	No
30	<i>Monoon liukuense</i>	Recalcitrant?	No

- The range of viability rate  
Control: 50-100%.  
Drying treatment: 65-100%  
Wet treatment: 0-100%
- For the three species of *Machilus thunbergii*, *Quercus miyagii* and *Osmanthus marginatus*, the drying condition viability rates were 27%, 0%, and 23%, respectively, more than 70 percentage points lower than the control and wet condition. These were considered to be recalcitrant seeds with no desiccation tolerance.
- For the remaining 25 species, no decrease in viability rate was observed upon drying (= orthodox seed).

# Results: Germination characteristics

- The germination experiments were conducted on 23 tree species from which sufficient seeds could be collected.
- The germination was observed in 19 species. The speed of germination was different among the species, and the final germination rate also varied.
- The remaining four species did not germinate even after 6 weeks. Many of these seeds were healthy when cut, and were therefore considered dormant. Some treatment may be necessary for the germination of these species.

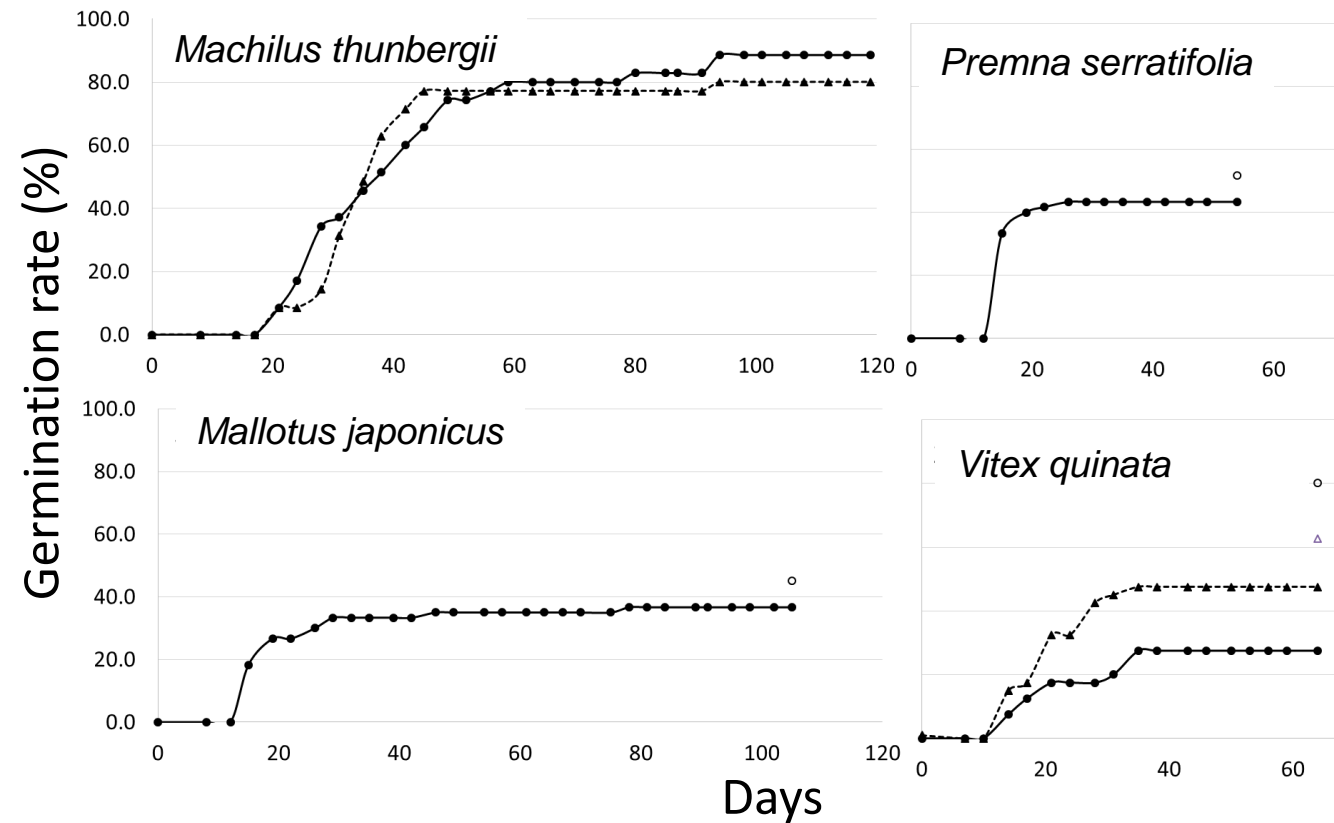


Fig. 1 Variation in germination speed in control seed.

This study revealed desiccation tolerance of seed and germination characteristics of 30 species including rare and endemic trees. It is necessary to investigate appropriate storage and germination conditions for these species in the future.

# Does fire promote germination and seedling emergence in Australian alpine species?

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- In fire prone ecosystems, some species have adapted to increase their germination in response to fire, taking advantage of the post-fire environment.
- Fire stimulated germination is a well-studied strategy in Australia, yet it has never been investigated in Australia's alpine flora.
- Studies of Australian alpine seed and fire are interesting because, unlike other alpine communities across the globe, the Australian Alps experience widespread fires every 50 - 150 years.
- This evolutionary timescale of fire suggests that alpine seed may respond to fire cues.

This study aims to provide some of the first insights into the effect of fire on:

1. Alpine seed germination
2. Seedling emergence from the alpine soil seed bank



The Australian Alps are a highly diverse bioregion with many endemic alpine specialists.

# Hypotheses and questions

## How does fire in the Australian Alps affect...

### Seedling emergence?

**Questions:**

*Do burnt soil seed banks produce a greater*

- a) abundance*
- b) diversity of seedlings?*

We predict that burnt soil seed banks will produce a greater abundance and diversity of seedlings.

Soil seed bank experiment



### Seed germination?

**Questions:**

*Does heat and smoke increase the*

- a) final % germination?*
- b) germination speed?*

*Does smoke\*heat have a greater effect compared to these cues acting alone?*

We predict that some alpine species will increase their germination in response to fire cues, particularly with the combination of heat and smoke.

Lab germination experiment



# Burnt soil seedbanks produced a lower diversity of seedlings

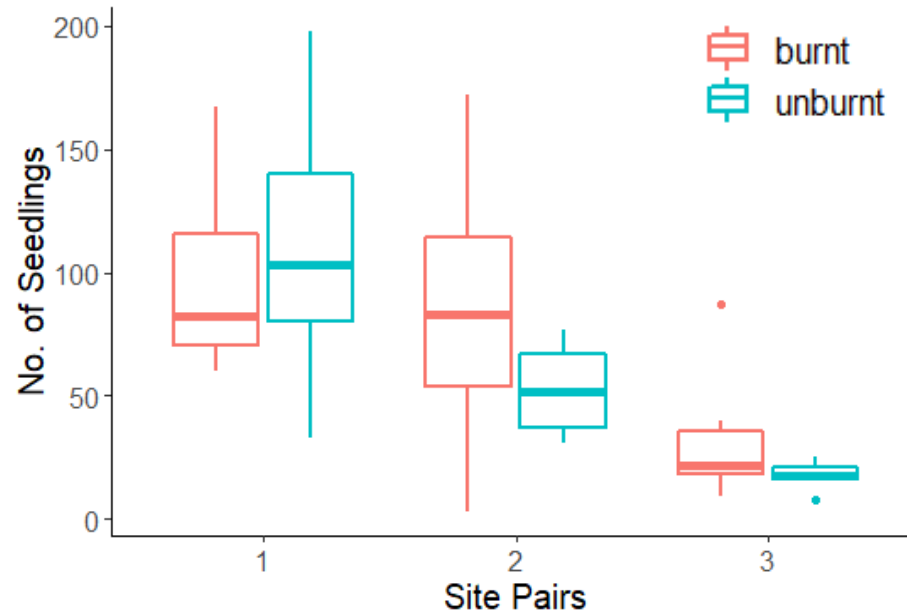
## Methods:

- Soil samples were collected one year post-fire from six burnt and unburnt sites in subalpine elevations. Sites were paired based on elevation.
- Soil samples were spread onto trays and placed in the glasshouse.
- Emerging seedlings were marked and identified.
- Seedlings of each morphotype were potted for identification

## Results:

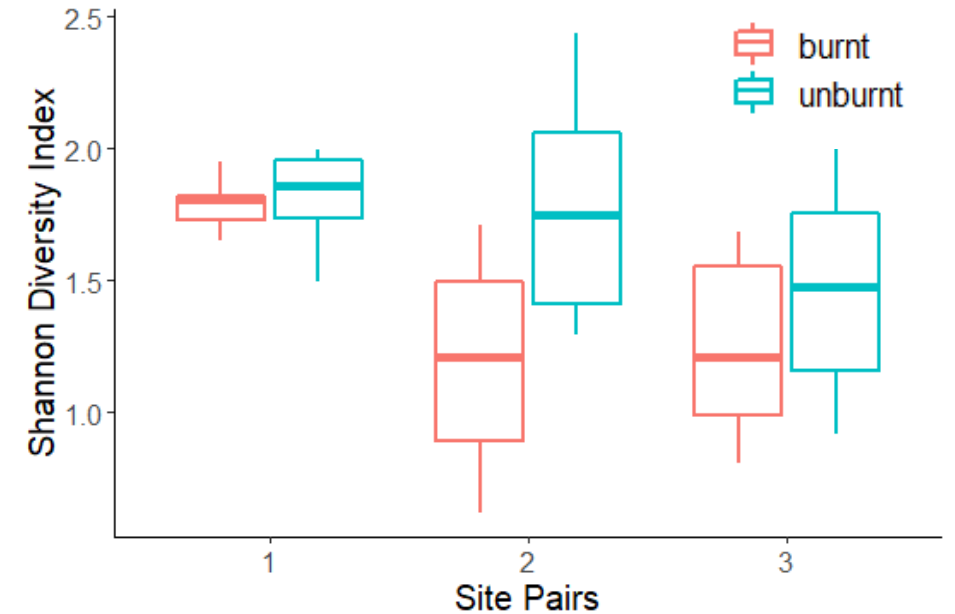
Do burnt soil seedbanks produce a greater...

a) Abundance of seedlings?



No, burnt soil seedbanks did not significantly differ in the number of emergent seedlings compared to unburnt seedbanks.

b) Diversity of seedlings?



No, burnt soil seedbanks produced a lower diversity of seedlings compared to unburnt seedbanks.

Sampling more sites at a larger range of elevations would be needed to confirm these trends.

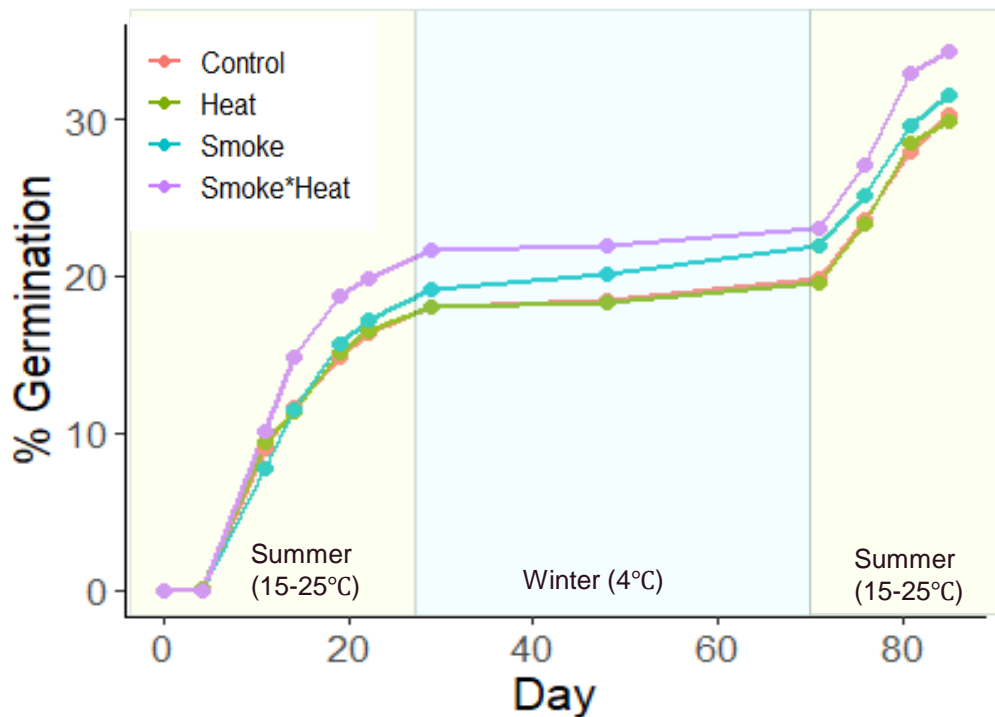
# Heat and smoke do not largely stimulate the germination of alpine seed

## Methods:

- 25 alpine species were selected to represent a variety of families, lifeforms, regeneration and germination strategies. Seed was obtained from the National Seed Bank, ACT.
- Seeds were subjected to heat and smoke factorial treatments.
- All seeds were subjected to temperatures mimicking an alpine year for 14 weeks to account for those species that require cold stratification.

## Results:

Preliminary data suggests that for the majority of alpine species, heat and smoke treatments have little effect on germination. The combination of heat and smoke seems to have a larger effect compared to these cues acting alone.



Average germination of all 25 species with factorial heat\*smoke treatments. Differences in treatments are likely caused by outlier species e.g. *Epacris paludosa*.

## A notable exception...

*Epacris paludosa*, a shrub found in boggy areas, responds particularly well to smoke. This is common in the *Epacris* genus and may represent the retention of an evolutionary trait.



## Why study alpine seed and fire?

- Studies are fundamentally interesting considering the unusual relationship between fire and an alpine environment.
- The germination requirements of many Australian alpine species remain unknown.
- Fire in the Australian Alps is becoming more frequent, it is important that we understand how the flora will respond.



## Future directions

- Further investigation is required to confirm the trends presented in this study.
- Future studies should incorporate a broader sampling of soil seed banks particularly at higher elevations.
- Fire appears to increase germination in some alpine species. Germination testing with a wider range of species may reveal more effective germination techniques.

### Acknowledgments

Staff and volunteers at the National Seed Bank. Australian Mountain Research Facility staff, volunteers and summer students. Plant Services – ANU. Nicotra Lab – ANU. For help with seedling identification: Brendan Lepschi, Neville Walsh, Keith McDougall. Photos © ANBG

# Urgently seed banking the tropics: *ex situ* conservation of Australian tropical mountain flora

\*Gemma Hoyle<sup>1</sup>, Amelia Stevens<sup>1</sup>, Lydia Guja<sup>1</sup>, Karen Sommerville<sup>2</sup>, Ganesha Liyanage<sup>2</sup>, Stuart Worboys<sup>3</sup> and Darren Crayn<sup>3</sup>

1. National Seed Bank, Australian National Botanic Gardens, ACT 2. The Australian PlantBank, The Royal Botanic Gardens and Domain Trust, NSW 3. Australian Tropical Herbarium, James Cook University, QLD



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Australian tropical montane flora  
requires *innovative* conservation  
action NOW!<sup>1</sup>

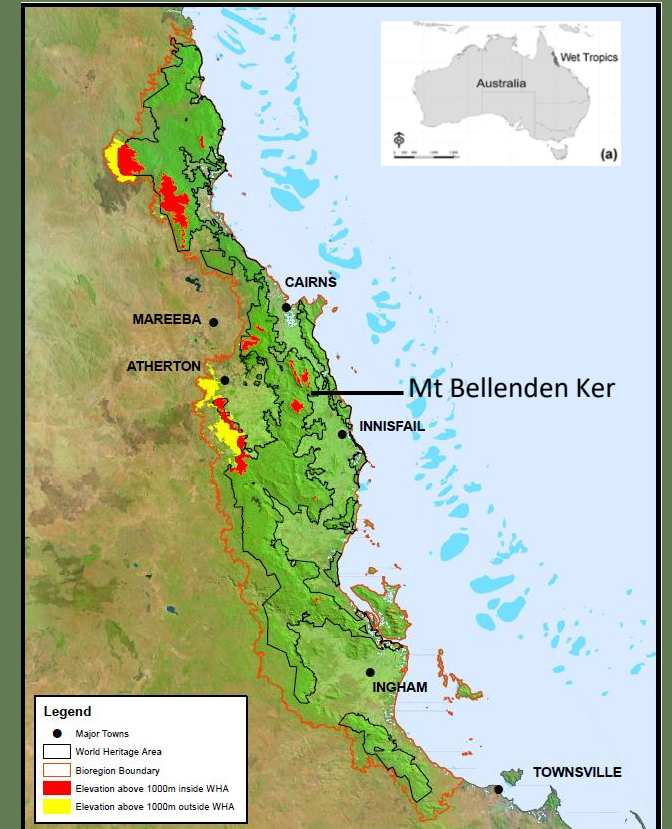
Tropical mountain cloud forests (TMCF) are defined by high altitude rainforest and frequent and/or persistent ground-level cloud. In Australia, TMCF is restricted to the Wet Tropics World Heritage Area of northeast Queensland (Fig. 1).

Montane tropics are highly threatened by climate change due to:

- Steep environmental gradients
- Reportedly narrow thermal tolerances of tropical species
- Anticipated reduction in cloud cover leading to decreased water input and changes in light quality/quantity<sup>2</sup>

Australia's TMCF are home to >70 endemic plant species. Modelling studies predict suitable habitat will decline by at least 60 % for 37 of these species and will be eliminated altogether for seven by 2085<sup>3</sup>.

With this literature review and preliminary investigations we explored the **application of *ex situ* seed banking** to mitigate species extinction in Australia's TMCF. **Search terms** included: TMCF, TM top, TM forest/rainforest, lower/upper montane cloud/rainforest.



**Figure 1.** Map of the Wet Tropics bioregion. Mt Bellenden Ker (1,593 m) is the wettest place in Australia. Mean annual rainfall measures 8,100 mm. Most of the rain falls during the 'wet' season, however, vegetation strips the clouds of moisture during the 'dry' season which evens out seasonality. Air temperatures range from 4 – 30 (mean 14 ± 3) °C.



# Seed collecting



Conservation action in Australia's TMCF presents significant challenges:

- Populations of target species are often only accessible by foot.
- Fruiting doesn't follow seasonal predictability as in temperate climates.
- Obtaining permission and permits to search for seeds can be time-consuming.
- Add to this flooding, mud, leeches and the fact that individuals of target plants are often present at very low densities in diverse forests.

Despite the challenges, mature seed has been sourced and **> 90 collections** have been made to date, mostly during dry season expeditions (Fig. 2).

With viable, genetically diverse seed collections, seed banking becomes a possible *ex situ* conservation tool for safeguarding TMCF plant germplasm into the future.

However, very little is known about the suitability of seed banking for TMCF species, or the germination techniques required to regenerate stored seed.

Often pole pruners are the only practical way of obtaining seed from canopy trees. With a reach of up to 16m, they are awkward to carry, difficult to set up and potentially dangerous to use.

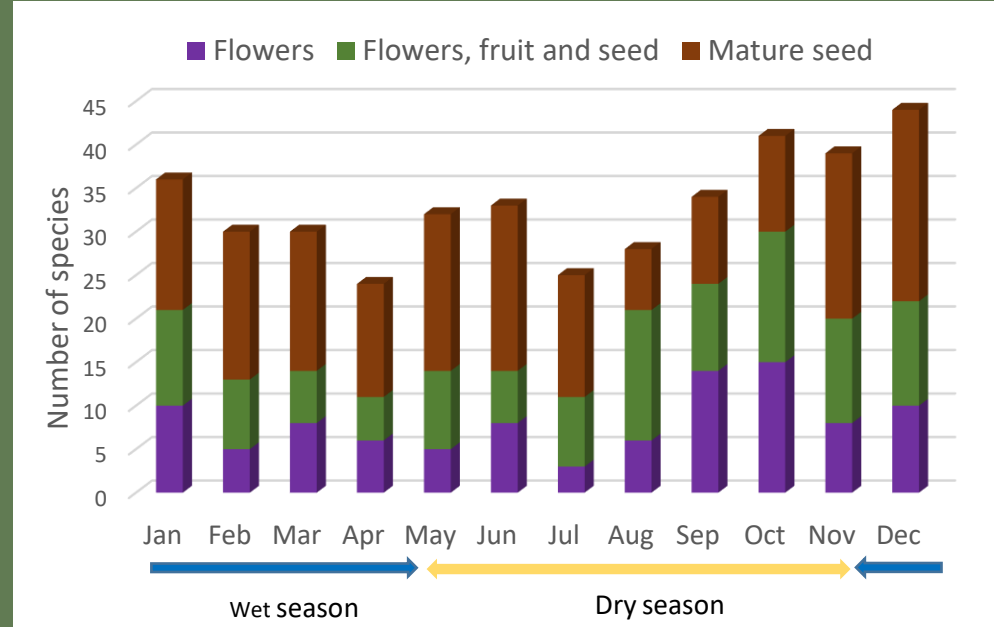


Figure 2. Number of Australian TMCF target\* species bearing flowers and/or mature seed each month. Seed has successfully been collected during the dry season. Data is derived from herbarium specimens held by the Australian National Herbarium (CANB) and the Australian Tropical Herbarium (CNS). \*We have counted 73 target plant species endemic to the Wet Tropics bioregion.



*Rhodamnia longisepala*  
Myrtaceae



*Melastroma malabathricum*  
Melastomataceae



*Lenbrassia australiana*  
Gesneriaceae



*Pittosporum rubiginosum*  
Pittosporaceae



*Aceratium doggrellii*  
(in a cassowary poo)  
Elaeocarpaceae



*Lomatia fraxinifolia*  
Proteaceae



*Mischocarpus macrocarpus*  
Sapindaceae



*Myrsine subsessilis*  
Primulaceae



*Mischocarpus Lachnocarpus*  
Sapindaceae

# Tropical seed storage

Successful seed banking requires seeds to tolerate drying (3 - 7 % moisture content) and freezing (-20 °C).

Up to 81.5 % of seed-bearing plants in tropical / subtropical rainforests are predicted to be suitable for seed banking<sup>4</sup>. However, the majority of species have not yet been tested for storage behaviour<sup>5</sup>.

The known behaviour of other species in a genus can be a useful predictor of seed storage behaviour in untested species<sup>6</sup>. Of the 57 Australian target TMCF genera;

- Species in up to 21 genera are potentially suitable for seed banking (Table 1).
- Species in nine genera are likely to be too sensitive to drying.
- Data is currently lacking for 27 genera<sup>7,8</sup>.

Physical characteristics such as seed coat permeability, fresh seed moisture content, seed dry weight and seed coat ratio can be used to predict the likely response to drying<sup>8</sup>.



*Pittosporum* sp.



*Lenbrassia Australiana*



*Aglaia sapindina*



*Denhamia fasciculiflora*



*Laccospadix* sp.

To determine the storage behaviour of previously unstudied species, fruit is collected at maturity and seeds are extracted as soon as possible. Seed coat permeability, fresh seed moisture content, seed dry weight and seed coat ratio are calculated. If these data do not provide a clear indication of storage behaviour, seeds are germinated after drying to ~5 % moisture content, and after drying and storage at -20 °C. The latter tests depend entirely on our ability to germinate the seeds (see following slide).

Family	Genus	Spp. tested	% O	% I	% R
<b>Apocynaceae</b>	<i>Parsonsia</i>	4	100	0	0
<b>Araliaceae</b>	<i>Hydrocotyle</i>	3	100	0	0
	<i>Polyscias</i>	2	100	0	0
	<i>Trachymene</i>	4	100	0	0
<b>Araucariaceae</b>	<i>Agathis</i>	4	50	25	25
<b>Clusiaceae</b>	<i>Garcinia</i>	11	18	0	82
<b>Cunoniaceae</b>	<i>Caldcluvia</i>	1*	100	0	0
	<i>Ceratopetalum</i>	1	100	0	0
	<i>Eucryphia</i>	1	100	0	0
<b>Ebenaceae</b>	<i>Diospyros</i>	32	66	6	28
<b>Elaeocarpaceae</b>	<i>Elaeocarpus</i>	3	0	0	100
<b>Ericaceae</b>	<i>Dracophyllum</i>	1	100	0	0
	<i>Leucopogon</i>	3	100	0	0
	<i>Rhododendron</i>	37	100	0	0
<b>Lamiaceae</b>	<i>Prostanthera</i>	3	100	0	0
<b>Lauraceae</b>	<i>Cinnamomum</i>	9	11	11	78
	<i>Cryptocarya</i>	2	0	0	100
	<i>Endiandra</i>	1	0	0	100
	<i>Litsea</i>	4	0	0	100
<b>Myrtaceae</b>	<i>Leptospermum</i>	31	100	0	0
	<i>Micromyrtus</i>	1	100	0	0
	<i>Ptilidostigma</i>	1*	0	0	100
	<i>Rhodamnia</i>	2*	100	0	0
	<i>Syzygium</i>	11	0	0	100
<b>Orchidaceae</b>	<i>Dendrobium</i>	5	100	0	0
<b>Piperaceae</b>	<i>Peperomia</i>	7	100	0	0
<b>Rubiaceae</b>	<i>Psychotria</i>	1	100	0	0
<b>Rutaceae</b>	<i>Flindersia</i>	2	100	0	0
<b>Sapotaceae</b>	<i>Planchonella</i>	1*	0	0	100
<b>Solanaceae</b>	<i>Solanum</i>	154	100	0	0

**Table 1:** Genera containing target TMCF species for which storage behaviour of one or more related species is known.

Storage behaviour is categorised as:

- Orthodox, tolerant of drying and freezing (O)
- Intermediate, partially tolerant of drying or freezing (I)
- Recalcitrant, sensitive to drying (R)

## *Ex situ* germination

Seed banking relies on reliable techniques to germinate stored seed.

Studies on tropical montane flora overseas have reported 75 % of species exhibited dormancy while approx. 11 % were non-dormant<sup>9</sup>.

Preliminary studies suggest that we can germinate Australian TMCF seed *ex situ*:



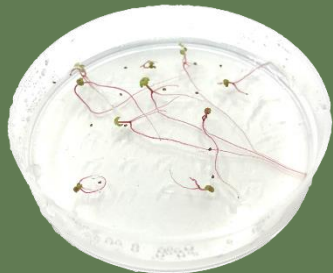
*Lenbrassia australiana* (Gesneriaceae)

- Non-dormant seed
- Light requirement for germination



*Gahnia sieberiana* (Cyperaceae)

- Possible dormancy, germination promoted by scarification and GA<sub>3</sub>
- 27/18°C light/dark 12/12 hr, germination commenced within 3 weeks and reached +80 %
- No light requirement



*Melastoma malabathricum* (Melastomataceae)

- 25°C, light/dark 12/12 h, germination commenced within 2 weeks and reached +80 %
- Non-dormant seed
- Light requirement for germination

## *In situ* seed ecophysiology

We know very little about what regulates regeneration from seed and species distribution of Australian TMCF *in situ*.

For example, light quality and quantity varies enormously within a TMCF, depending on canopy density and cloud immersion.



Studies overseas have reported:

- Seeds beneath a canopy gap germinated more than those beneath the forest canopy<sup>10</sup>.
- After a gap had been colonized, shading by vegetation prevented seeds of some species from germinating<sup>11</sup>.
- The ratio of red to far-red light (R:FR) influenced germination of tropical seeds: the bigger the seeds, the greater the R:FR needed for germination<sup>12</sup>.
- Seeds buried for months required longer exposure to light than freshly matured seed<sup>13</sup>.



# The Tropical Mountain Plant Science Project

The literature, together with our preliminary findings, supports *ex situ* seed banking as an applicable conservation tool for this unique and at-risk flora.

Our multi-organisational project, funded by the Ian Potter foundation, aims to:

‘...secure the future of Australia’s climate-threatened tropical mountaintop plants... by building a multi-strategy, *ex situ* conservation reserve to ‘backup’ at-risk wild populations, and support research, display and education.’

Australian TMCF seed and plant collections made to date provide significant research opportunities. Our project is committed to:

- Classifying seed storage behavior
- Diagnosing and classifying dormancy types
- Defining germination temperature thresholds
- Investigating light requirements for germination
- Investigating plant physiological temperature thresholds
- Investigating population genetics

Future work will continue to synthesise this cumulative knowledge for holistic conservation and management of Australia’s unique and urgently threatened TMCF plant species.



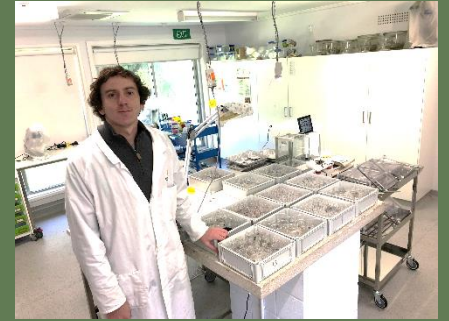
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# Temperate grasslands seed longevity: essential for effective *ex situ* seed banking

\*Nicholas Lloyd<sup>1</sup>, Jodi Price<sup>1</sup>, Gemma Hoyle<sup>2</sup> and Lydia Guja<sup>2,3</sup>

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3. Centre for Australian National Biodiversity Research, (a joint venture between Parks Australia and CSIRO), ACT, Australia



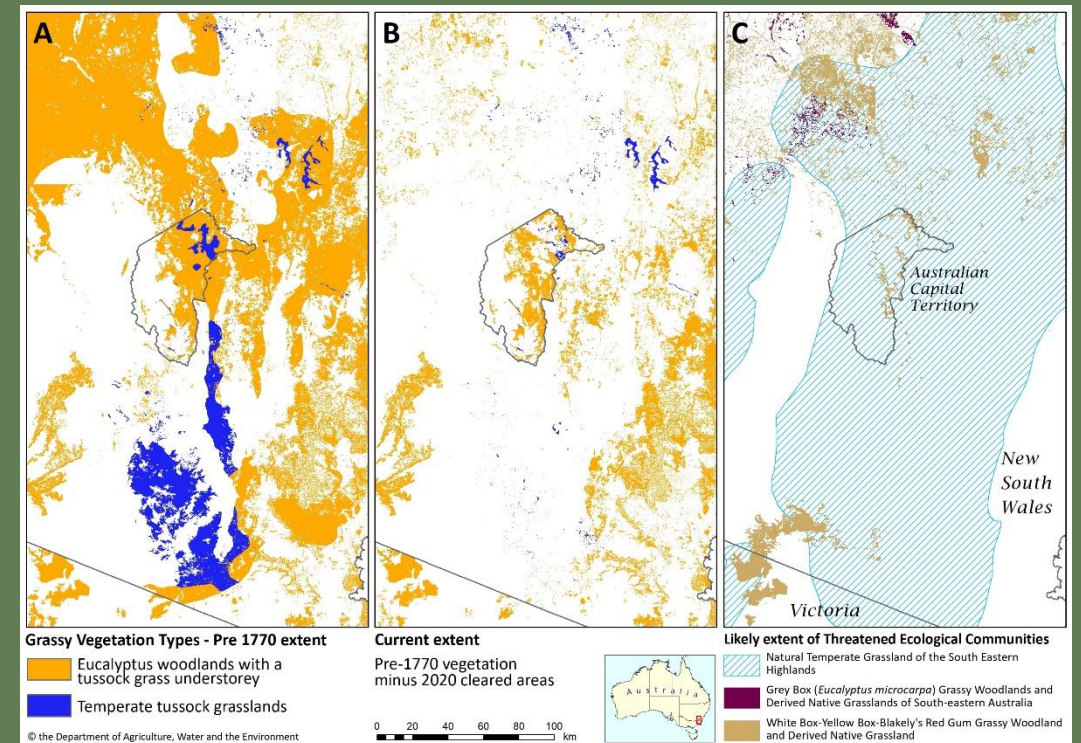
\*Email: nicklloyd777@gmail.com



Since European colonisation, Australian temperate grassy ecosystems have been subjected to severe ecological alteration and degradation.

Less than 10 % of the *Natural Temperate Grasslands of the South-Eastern Highlands* remain today and these plant communities are currently listed as endangered or critically endangered<sup>1</sup>.

Less than 5 % of the *Yellow Box, White Box and Blakely's Red Gum Woodland and Derived Native Grasslands* remain in good condition<sup>2</sup>.



Map shows the distribution of south-eastern Australian grasslands prior to 1770 (A) and today (B). Map C depicts where associated threatened ecological communities are likely to exist today<sup>3</sup>.

# *Ex situ* seed conservation for endangered ecological communities

*Ex situ* seed banking is an effective method to safeguard threatened plant communities against extinction and is utilised to secure grassy ecosystem species.

Seed longevity is defined as the life span of a seed and is important in *ex situ* seed banking to inform seed storage conditions and seed collection management.

Previous studies have explored seed longevity of Australian species:

- Life form and various other traits were associated with seed longevity in a study of 172 species<sup>4</sup>.
- Seed longevity was related to elevation and seed mass in alpine seeds<sup>5</sup>.

However, seed longevity of most grassy ecosystem species is unknown.



A collection of *Brachyscome scapigera* (Asteraceae) seeds from the National Seed Bank, Canberra. *B. scapigera* is a perennial herb common in temperate grassy ecosystems and was included in this study.

This study aimed to calculate the comparative seed longevity of south-eastern Australian grassy ecosystem species.

It was hypothesised that seeds would be relatively short-lived and that longevity would be related to various seed characteristics including life form.

This work will inform the *ex situ* conservation of threatened grassland communities.

# Methods

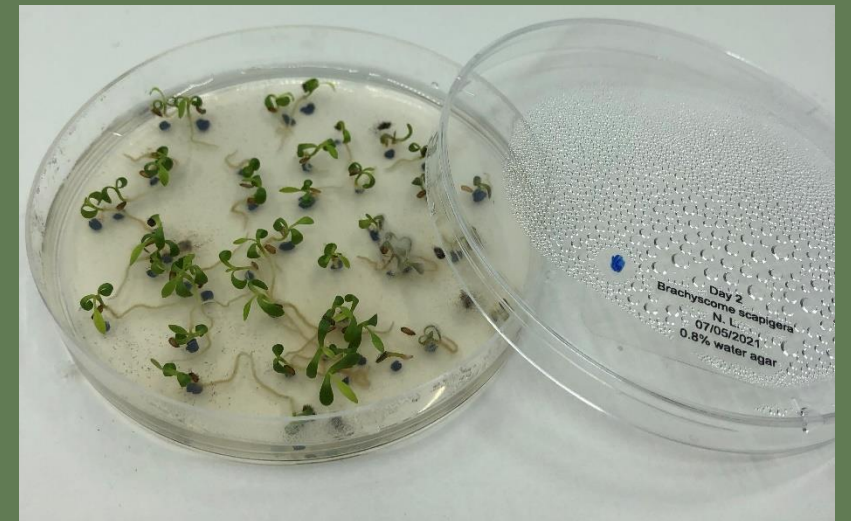
- Seeds of 28 species were subjected to an artificial ageing test<sup>6</sup>, reducing longevity to an observable time-span!
- To rapidly age the seeds, they were subjected to a regulated climate of 45°C and 60 % relative humidity for up to 250 days.
- At various intervals, seeds were taken out of ageing conditions and tested for viability in germination tests.



*Acacia dealbata* (Fabaceae) seeds.

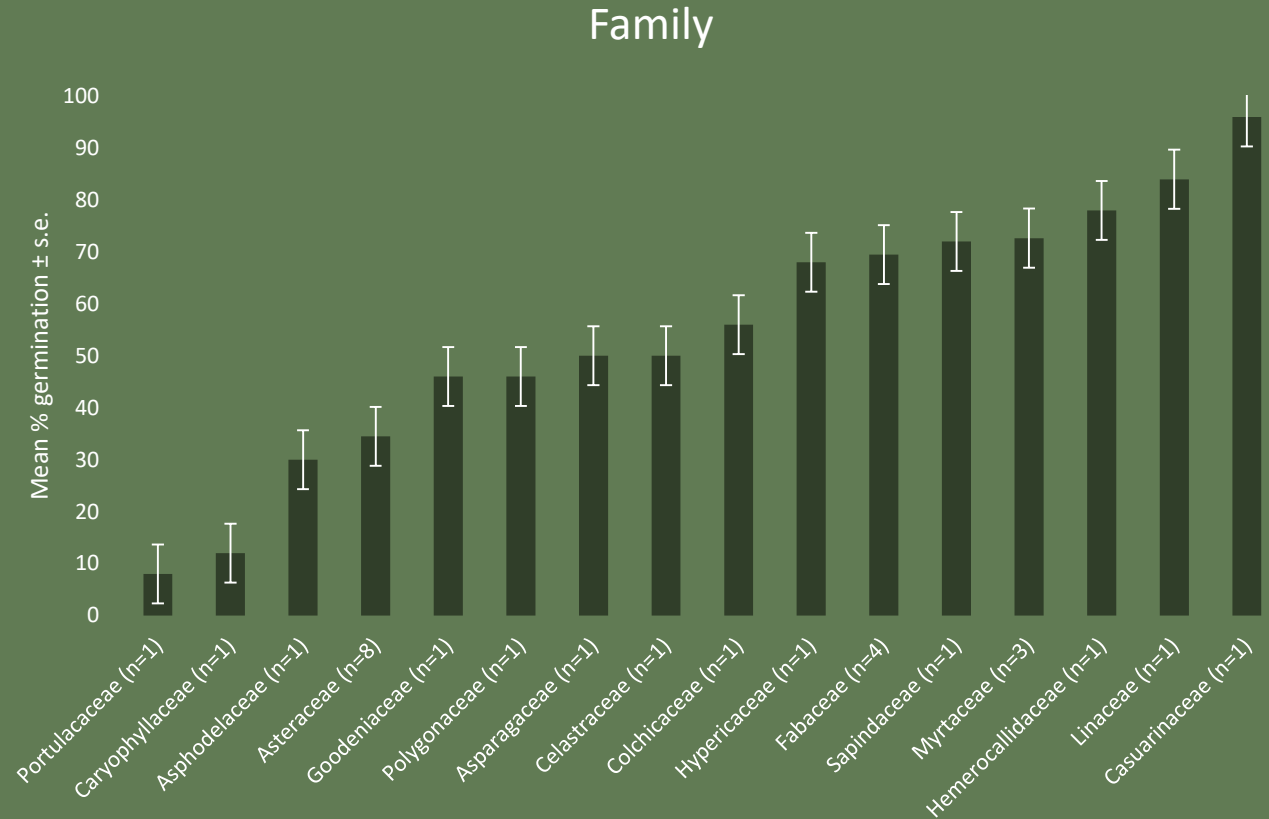
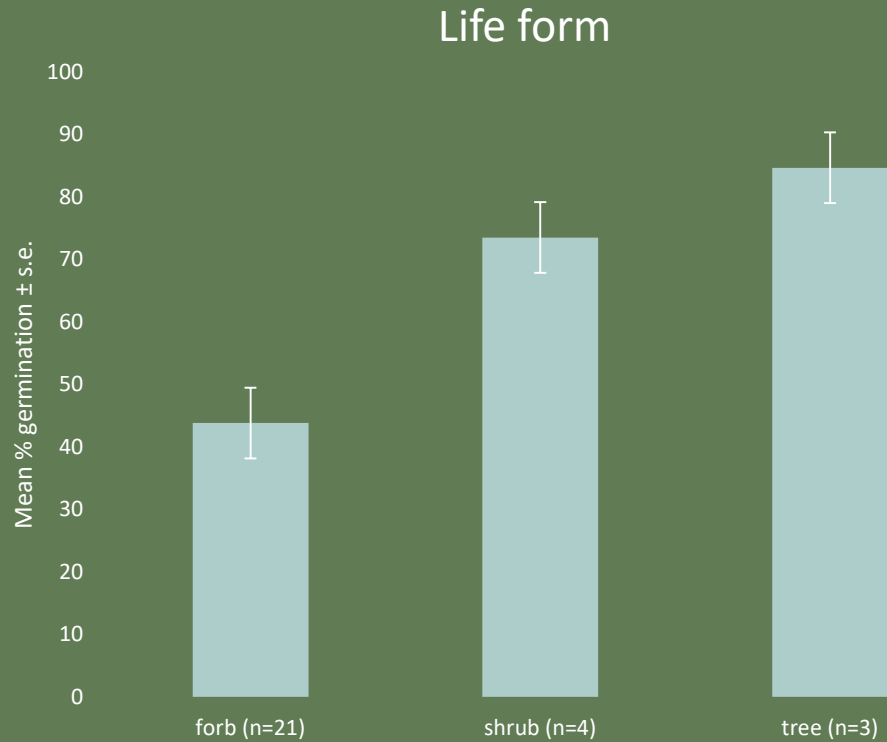


Seeds enclosed in a temperature- and humidity-controlled electrical box.



*Brachyscome scapigera* (Asteraceae) seeds germinating after two days of ageing.

# Seed germination after 15 days of ageing



Results so far suggest that typically larger life forms such as trees and shrubs are longer-lived than smaller, herbaceous life forms.



## Next steps:

- We are currently preparing to subject 30+ more grassy ecosystem species to artificial ageing including grasses, forbs, shrubs and trees.
- $p_{50}$  is defined as the time (in days) taken for viability of a seed collection to decrease by 50 %.
- We will use  $p_{50}$  values to compare longevity with that of seeds from other plant communities in Australia and overseas.
- Seed and plant traits will be analysed for correlation with longevity.

## Significance:

Ex situ conservation: Results will inform seed collection management, e.g. shorter-lived seeds will be prioritised for collecting and more regularly re-tested for viability and germinability.

Further study: Findings will inform the study of seed longevity *in situ*, i.e. persistence in the soil seed bank.



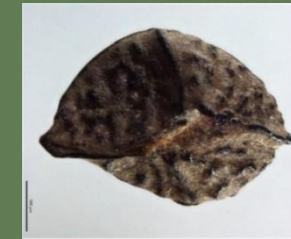
*Daucus glochidiatus* (Apiaceae)



*Mirbelia oxylobioides* (Fabaceae)



*Linum marginale* (Linaceae)



*Bulbine bulbosa* (Asphodelaceae)

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# Tracing the Origin of Native Seed/SEARCH Across Political, Social, Organisational, and Cultural Geographies

Christopher Peter Sauer

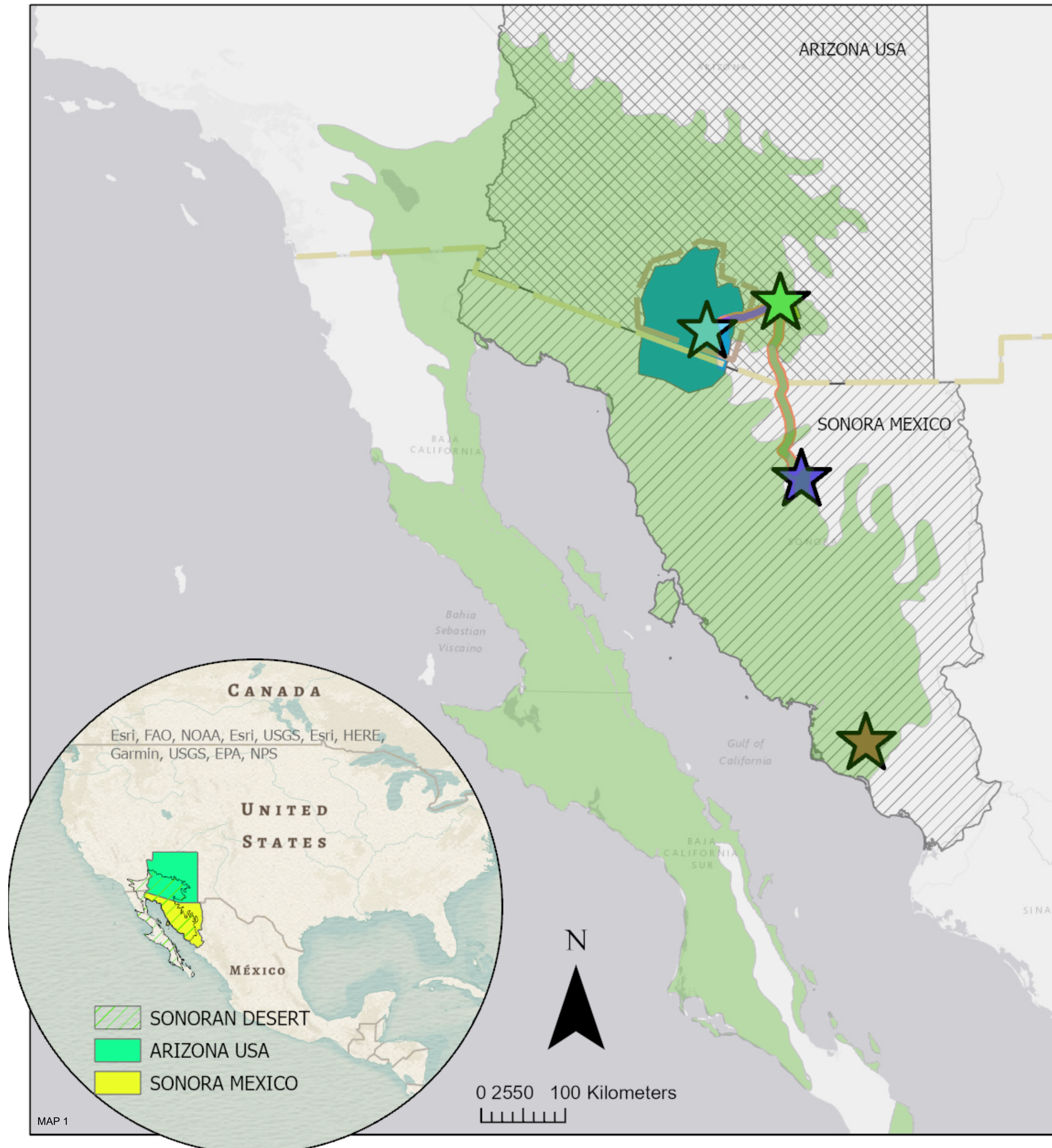
University of Queensland, Brisbane QLD Australia

## ABSTRACT

Native Seed/SEARCH (NS/S) is a seed bank and non-profit organization located in Tucson, Arizona. Their mission is to conserve and promote the arid-adapted crop diversity of the Southwest in support of sustainable farming and food security. Founded in 1983, NS/S is the brainchild of a group of ethnobotanists, archaeologists, and anthropologists associated with the University of Arizona's Office of Arid Lands Studies program.

In the early 1980s, the founders of NS/S were employed by Meals for Millions, a local NGO distributing seeds supplied by a US Department of Agriculture food security program. These seeds included modern hybrid broccoli and brussels sprouts which were not well-adapted to the ecological conditions of the Sonoran Desert. Participating Southwestern farmers, including on the Tohono O'odham American Indigenous Nation, began to request more suitable arid-adapted seeds. However, most of the known suitable varieties could only be sourced from rural communities in Sonora, Mexico. This posed a problem because MFM only allowed distribution of seeds produced within the United States. Along with their publicly stated mission of protecting biodiversity, NS/S was created to resolve this boundary of seed acquisition and distribution.

This poster presents a visual and textual representation of NS/S origins to analyse how the mismatch of political, social, cultural, and environmental geographies give rise to new organizational forms and seed preservation practices. In doing so, the poster reveals how, on the one hand, political boundaries restrict the circulation and distribution of native seeds, while on the other hand, social and cultural networks along with new organizations can be deployed to match the practicalities of local ecologies. The result is NS/S - an innovative community seed bank that has become an integral player in the long-term development of southern Arizona's vibrant native foods culture.



## TRACING THE ORIGIN OF NATIVE SEED/SEARCH ACROSS POLITICAL, SOCIAL, ORGANIZATIONAL, AND CULTURAL GEOGRAPHIES

- SONORAN DESERT
  - ARIZONA USA
  - SONORA MEXICO
- LOCATION**
- CIMMYT, Obregon Sonora
  - Native Seed/S Office, Tucson Arizona
  - Seed Collection, Cucurpe Sonora
  - Tohono O'odham Nation
- ROUTE**
- Cucurpe-Tucson
  - Tucson-Tohono O'odham
- NATIONAL BOUNDARIES**
- Tohono O'odham Nation
  - US/Mexico Border
- TERRITORY**
- Cucurpe, Sonora, Mexico
  - Tohono O'odham 2005 Bi-National
  - Tucson, AZ, USA

# Tracing the Origin of Native Seed/SEARCH Across Political, Social, Organizational, and Cultural Geographies

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## INTRODUCTION

### SONORAN DESERT:

The desert is a unique ecological zone that unifies a set of cultural geographies and social networks that are, at the same time, divided by political and organizational boundaries.

#### Ecological features of the Sonoran Desert

- 310,362 sq km of high green deserts located along the Sea of Cortez.
- Two rainy seasons within ~ 11 in/25 cm.
- Home to endemic plant (e.g., the iconic saguaro cactus) and animal life species (e.g. desert bighorn sheep) that evolved during the last post-glacial period.

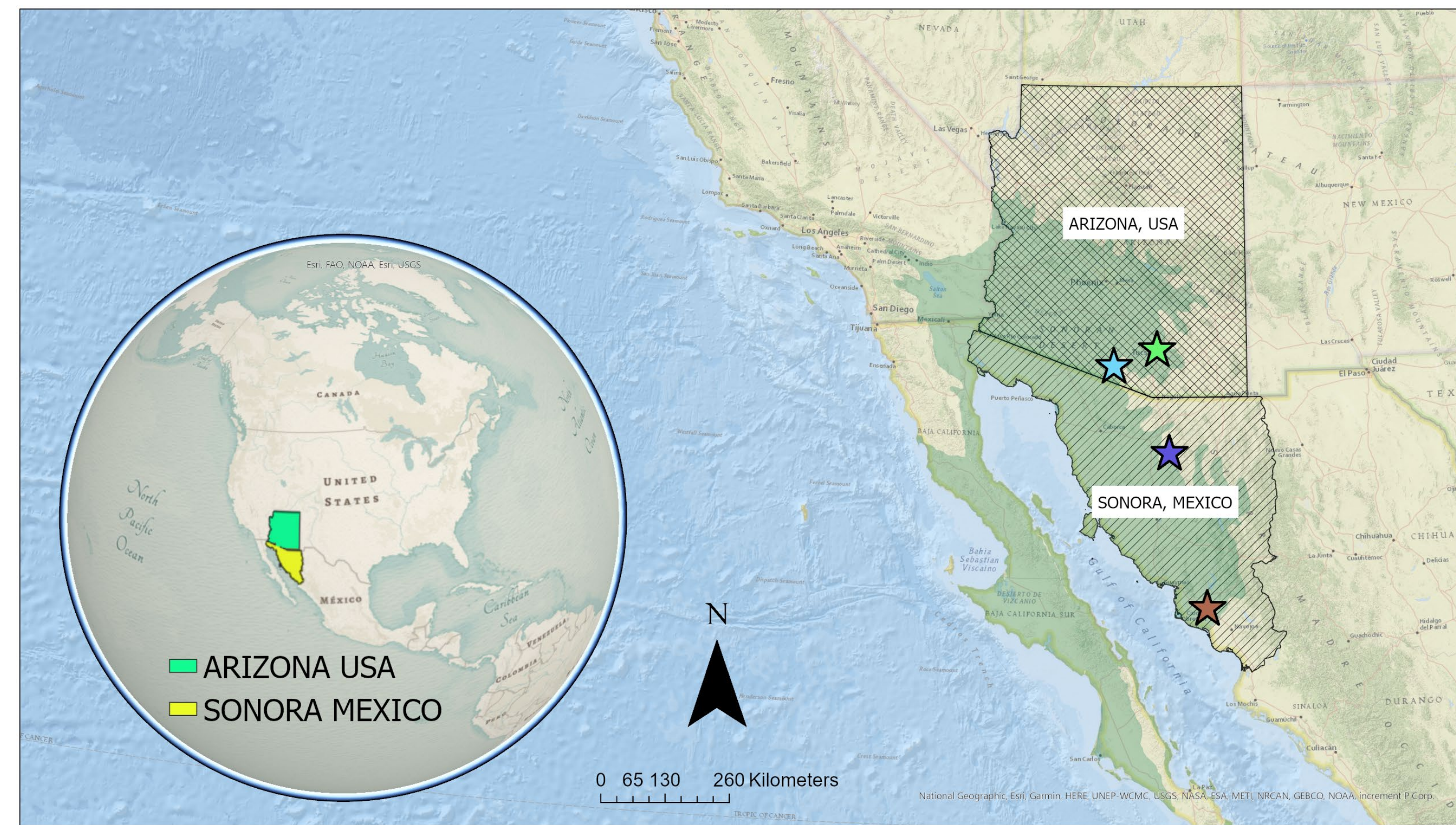
#### Political features of the Sonoran Desert

- Spans the US-Mexico border, including portions of Arizona (USA), Sonora (Mexico), and the Tohono O'odham Nation.
- The TO nation land is held in trust by US gov't, but portions of the tribe are also in Mexico.
- Historically part of Spanish colonial empire in the 1600s and portions were eventually ceded by Mexico to the USA under the Gadsden Purchase in 1854.
- Cultural networks are diverse and transcend both sides of the border with Mexican *mestizo* (mixed-race), Indigenous, and Anglo-Saxon communities.

**Cultural features:** Archaeological evidence indicates that Indigenous American groups have actively cultivated local plants in the Sonoran Desert since at least 3000 BC. Currently, desert farming and agriculture is common on both sides of border, while recent border "securitization" has disrupted historic cross-border family connections.

#### Social & Institutional Networks

- Concerns regarding the impact upon biodiversity from the Green Revolution gave rise to a number of institutions committed to addressing this issue in both the US and Mexico since the 1970s.
- This was not an abstract concern within the Sonoran Desert. The International Maize and Wheat Improvement Center (CIMMYT) research center in Obregon Sonora was one of the first important sites of Green Revolution research.
- In Tucson AZ the interdisciplinary Office of Arid Land Studies was established at the University of Arizona (UA) in 1974. In the 1970s UA was a hub of geoscientists, ecologists, archaeologists and anthropologists during a time of growth in population and establishment of new federal National Parks.
- Within Tucson and the Southwest the founders worked across a variety of academic, government and community employment categories while pursuing higher degree research.



MAP 2 INTRODUCTION

## INTRODUCTION

### LOCATION

- ★ CIMMYT, Obregon Sonora
- ★ Native Seed/S Office, Tucson Arizona
- ★ Seed Collection, Cucurpe Sonora
- ★ Tohono O'odham Nation
- ▨ SONORA MEXICO
- ▨ ARIZONA USA
- ▨ SONORAN DESERT

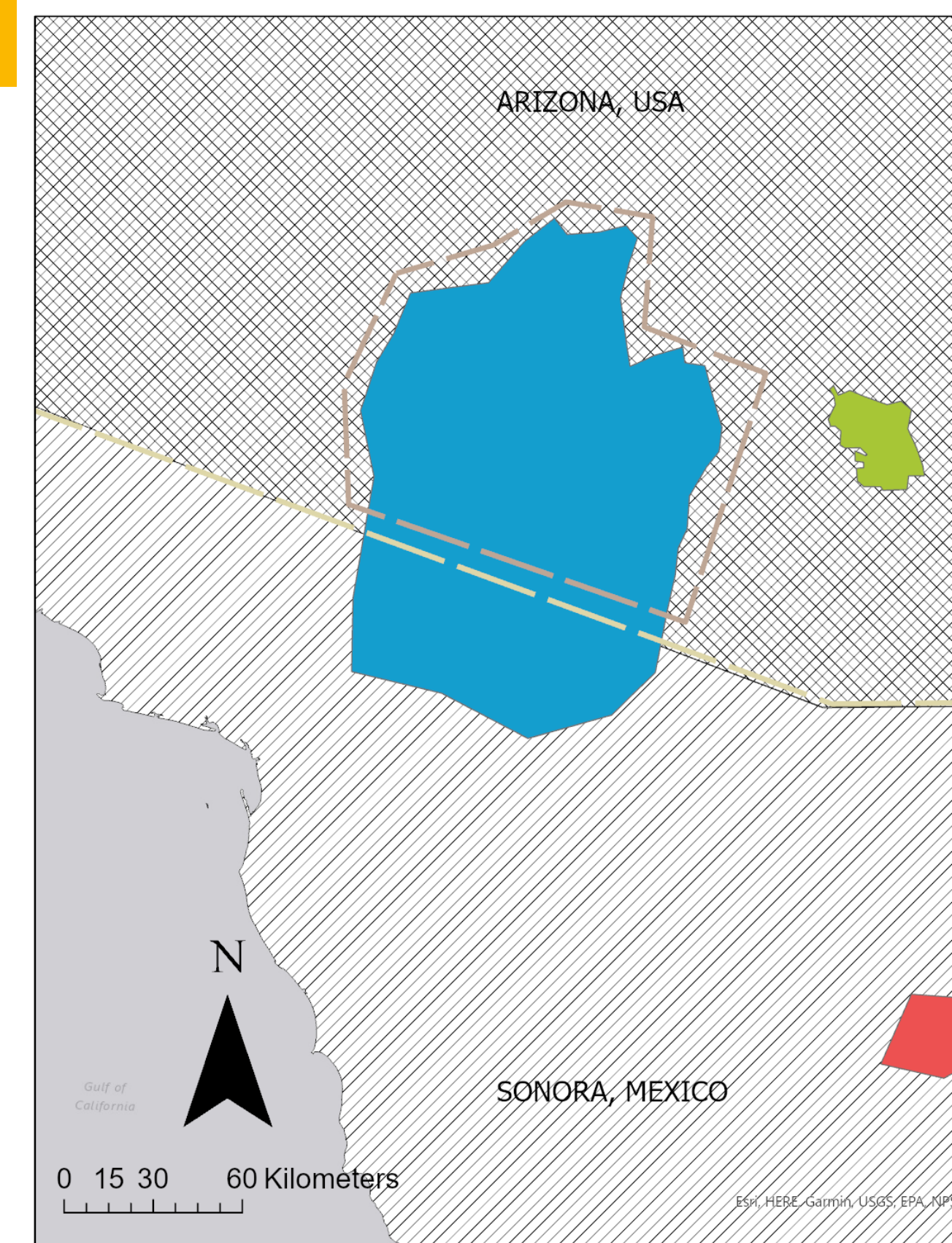
## BOUNDARIES AND GEOGRAPHIES

### Organizations: Political restrictions of funders lead to new organizational configurations

- NS/S founders were employed by Meals for Millions (MFM), a community development organization distributing seeds as part of a US Department of Agriculture food security program, including modern hybrid seeds ill-suited for the desert.
- Participating Tohono O'odham farmers began to request seeds, including a small tepary bean *Phaseolus Acutifolius* Variety Latifolius, more suitable for the Sonoran Desert no longer available in Arizona but still found in Mexico.
- MFM policies only allowed the distribution of seeds from the USA.

### Cultural Geographies

- The culture and cuisine of Sonoran resonated with this cohort because it related to multi-cultural life within Tucson, one of the oldest cities in the USA founded by Spain in 1775. The academic cohort of the UA and the NS/S founders embraced a Tucson history that features a mix of Indigenous/Spanish/Mexican/USA cultures with different languages including English, Spanish, Tohono O'odham.
- Their academic life as researchers mixed with a Sonoran Desert farming culture including plant varieties and meal recipes.



MAP 3 MULTIPLE BOUNDARIES

## MULTIPLE BOUNDARIES

- NATIONAL BOUNDARIES
- - - Tohono O'odham Nation
- - - US/Mexico Border
- TERRITORY
- Cucurpe, Sonora, Mexico
- Tohono O'odham 2005 Bi-National
- Tucson, AZ, USA
- ▨ SONORA MEXICO
- ▨ ARIZONA USA

# Tracing the Origin of Native Seed/SEARCH Across Political, Social, Organizational, and Cultural Geographies

Christopher Peter Sauer

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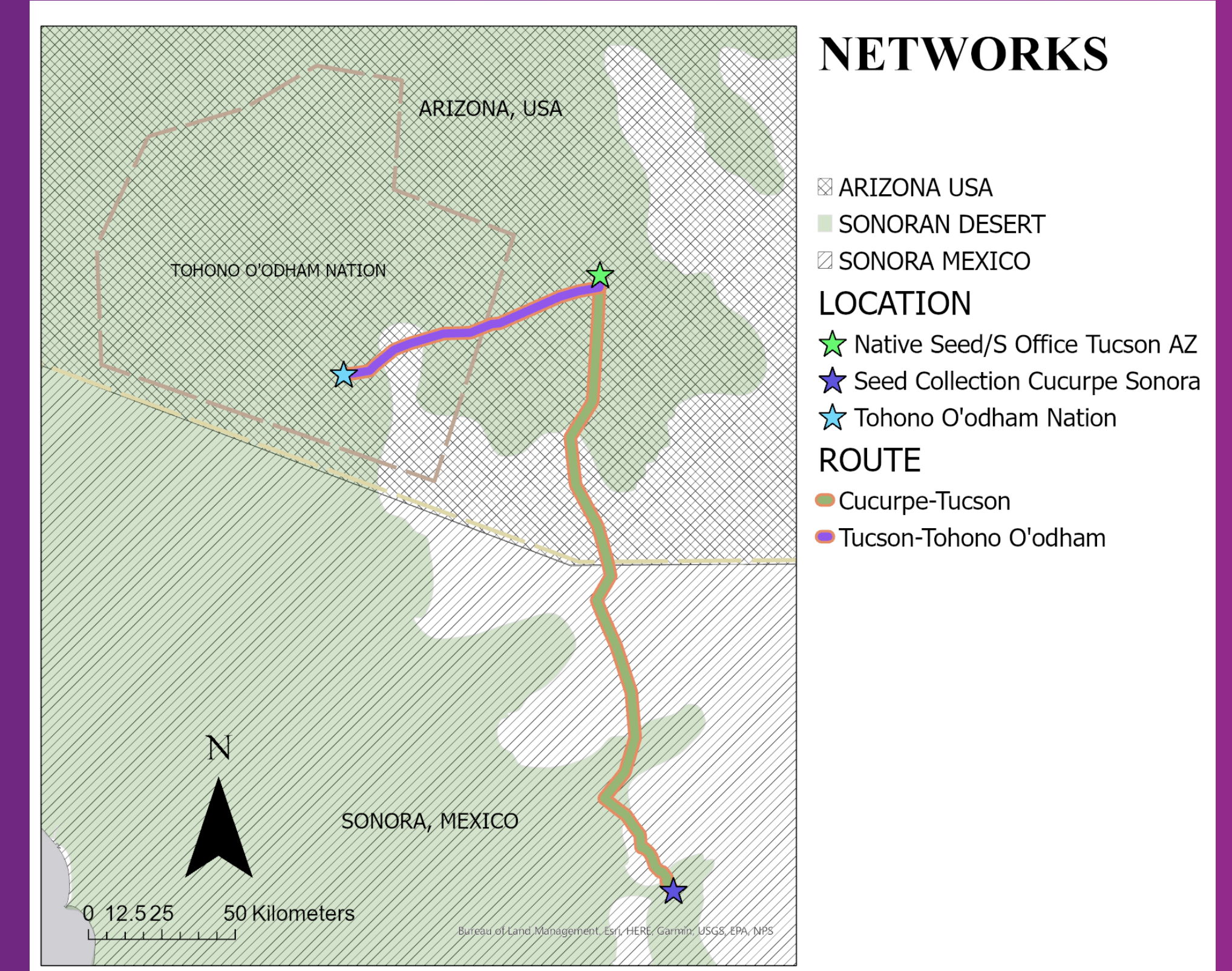
## NATIVE SEED/SEARCH

An organization capable of transcending political, social, organizational and cultural divides while restoring biological diversity.

- Established in 1983 by Karen Reichardt, Gary Nabhan, Barney Burns and Mahina Drees, it is a seed bank that aims to preserve the rich prehistoric and historic biodiversity of the Sonoran Desert.
- The four founders were inspired to work outside of various limitations to their activities by founding their own organization.
- Seed collection included benefit sharing such as seed repatriation to Indigenous people, including distribution programs for Native American farmers and internships.
- The founders were researching the cross-border ecological region of the Sonoran Desert. Articles and books include topics of ethnobotany, archaeology, anthropology, and they have had long careers.
- As researchers and graduate students they also worked in regional community organizations.

## NETWORKS – POLITICAL, SOCIAL, ORGANIZATIONAL, CULTURAL

- Native Seed/SEARCH utilized an extensive set of connections with influential people and organizations to develop grant writing capabilities for their fundraising and development. NS/S was able to activate these networks to gather resources such as sharing space with local zoos, botanical gardens, and help with purchasing buildings and grow-out farms.
- Social networks were grounded in their academic community which spanned a number of research institutions and included informal social gatherings within Tucson.
- The University of Arizona cohort met for food-centric social gatherings which emphasized local and culturally meaningful foods. These were dubbed the “Menudo (Mexican stew) Society” and “Tepary Bean Burrito” and fostered a sense of community and connection among this academic cohort in the mid-1970s.
- The potlucks involved a merging of Mexican, Indigenous and US cultural cuisine and activity.
- The culture, cuisine and plants of the Sonoran Desert resonated with this cohort because it related to the long standing multicultural life within Tucson.



MAP 4 NETWORKS

## CONCLUSIONS

These boundaries, geographies and networks provide insight into the many layers of Native Seed/SEARCH as a seed bank organization. The founders drew upon their networks to work across various forms of political, social, organizational and cultural geographies.

The restriction by MFM to using seeds sourced within the USA, prohibiting the distribution of varieties then only available from isolated communities like Cucurpe Mexico, provided an incentive to start a seed preservation non-profit organization that could work across limits.

There was support and interest from the community at the beginning of NS/S's activities, including coverage in the press, which helped encourage the founders to begin their organization on a shoestring budget of \$390.

The barriers created by the multiple overlapping borders and national boundaries, instead of limiting activity, helped foster a sense of mission and purpose that was part of the inspiration of NS/S.

## FURTHER RESEARCH

What are the intellectual property provisions, if any, of the NS/S collection practices among Indigenous people in the past and present? Are there access and benefit sharing agreements?

Create a complete timeline of seed collections dates and locations including the collection of the small tepary bean *Phaseolus Acutifolius* Variety Latifolius from Cucurpe.

How did research activities at UA support Tucson community food justice organizations and activities? How did these organizations shape and influence research activity?

In what way did NS/S food related events and fundraisers, such as Fiesta de los Chiles and Agave Fest, help create the vibrant food culture the led to Tucson's 2015 designation as a UNESCO City of Gastronomy?

Can lessons and examples from Tucson be used to promote Australian local and Indigenous food?

## ACKNOWLEDGMENTS

This research was conducted by the ARC Industrial Transformation Training Centre for Uniquely Australian Foods (IC180100045) and funded by the Australian Government.

Thank you to Tom Sheridan, Gary Nabhan and Allison Fish. Maps and photos by CP Sauer.



PICTURE 1 SONORA DESERT

# Tracing the Origin of Native Seed/SEARCH Across Political, Social, Organizational, and Cultural Geographies

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## APPENDIX: Timeline.

20000 ka to 1800 ka									
20,000 ka	14,500 ka	10,000 ka	5,000 ka	3,000 ka	1,250 to 1,500 ka	1530s AD	1600 to 1800s AD	1750s AD	
Last glacial maximum	Pre-Clovis migrations to Americas	Mexican monsoon climate developed	Early Southwest Indigenous crops	Tucson Indigenous agriculture	Large scale Hohokam irrigation agriculture	Spanish explorers in Sonoran Desert	Jesuit Mission agriculture introduced European plants, large Indigenous population decline	Tohono O'odham rebellions	
1800 CY to 1970 CY									
1854		1874 - 1916		1937		1944		1966	
USA acquires territory from Mexico including Arizona		Tohono O'odham reservations created		Tohono O'odham Nation adopt a constitution		Meals For Millions created a soybean based Multi-Purpose Food to end world hunger.		Nobel prize winning Green Revolution scientist Borlaug comes to research center in Obregon	
1970 to 2000 CY									
1974	1975	1978	1983	1986	1990	1990s	1994	1995	1998
Founding of UA Arid Land Studies	Harlan article "Our Vanishing Genetic Resources"	Nabhan & Sheridan article about Cucurpe, "Living with a River: Traditional Farmers of the Rio San Miguel"	Founding of Native Seed/SEARCH	Inaugural NS/S "Fiesta de los Chiles"	Nabhan receives MacArthur Foundation fellowship	Research programs in the Mexican states of Sonoran and Chihuahua	Intellectual Property Rights conference UA Indian Studies Program and AZ State Museum	Purchase of NS/S office	NS/S Purchases 60 acre grow-out farm

# Seed-based conservation of Norfolk Island's rare and endemic plants

\*Leah Dann<sup>1</sup>, Salit Kark<sup>1</sup>, Mark Scott<sup>2</sup>, Nigel Greenup<sup>2</sup>, Melinda Wilson<sup>2</sup>, Lydia Guja<sup>3</sup>

1. The University of Queensland, QLD 2. Parks Australia, Norfolk Island National Park 3. National Seed Bank, Australian National Botanic Gardens, ACT

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Australian Government  
Director of National Parks



Australian National  
Botanic Gardens



Friends of the  
Australian National Botanic Gardens



National Environmental Science Programme



Abstract

Introduction

Methods

Results

Discussion



Basemap from ESRI National Geographic, created in QGIS

## Norfolk Island

Diverse plant communities threatened by deforestation, biological invasions, & small endemic population sizes

Seed banking & restoration activities are key to conservation success

Requires seed biology & propagation knowledge

We created a handbook of seed collection, processing, & propagation information for 19 native plant species



*Myoporum obscurum*; PC Mark Scott



PC Emily Dann



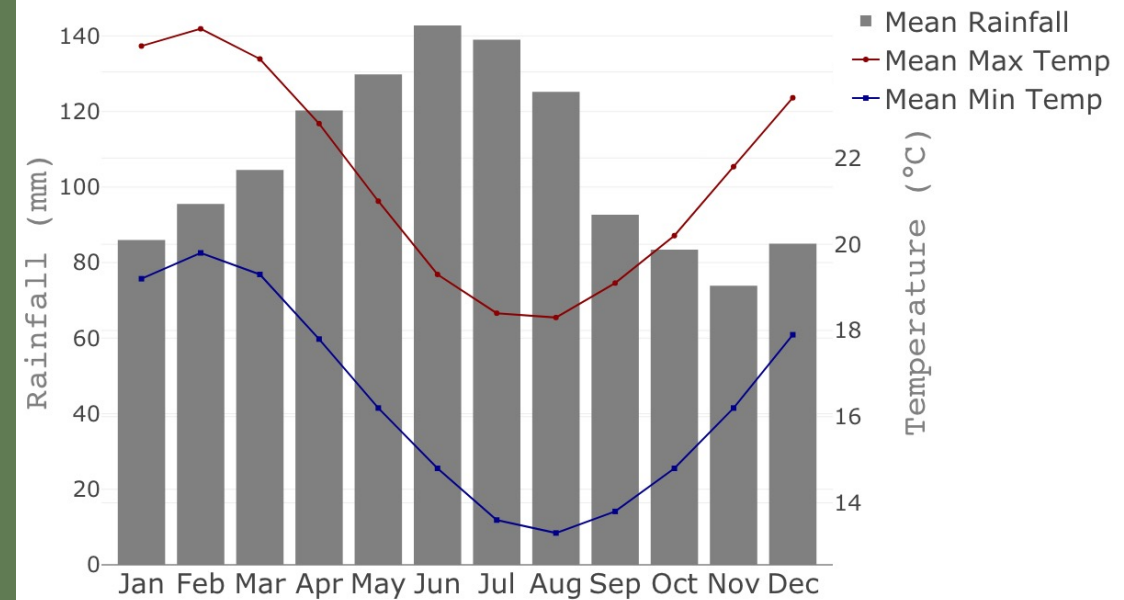
*Myoporum obscurum*; PC Mark Scott

## Background

- Globally, hundreds of plant species have gone extinct since the 1600s
- Most recent plant extinctions have occurred on islands
- Islands have a high proportion of the world's endemic and threatened species

## Norfolk Island

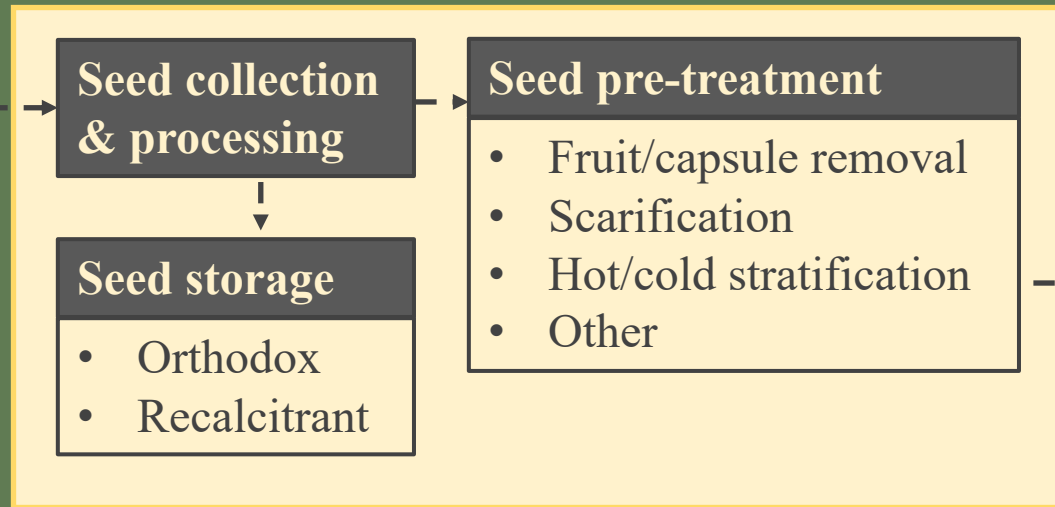
- Subtropical volcanic island in the South Pacific
- Has 33 Environment Protection and Biodiversity Conservation Act (EPBC) listed endemic plant species
- Native vegetation is threatened by habitat loss, invasive plant and animal species, and lost mutualisms



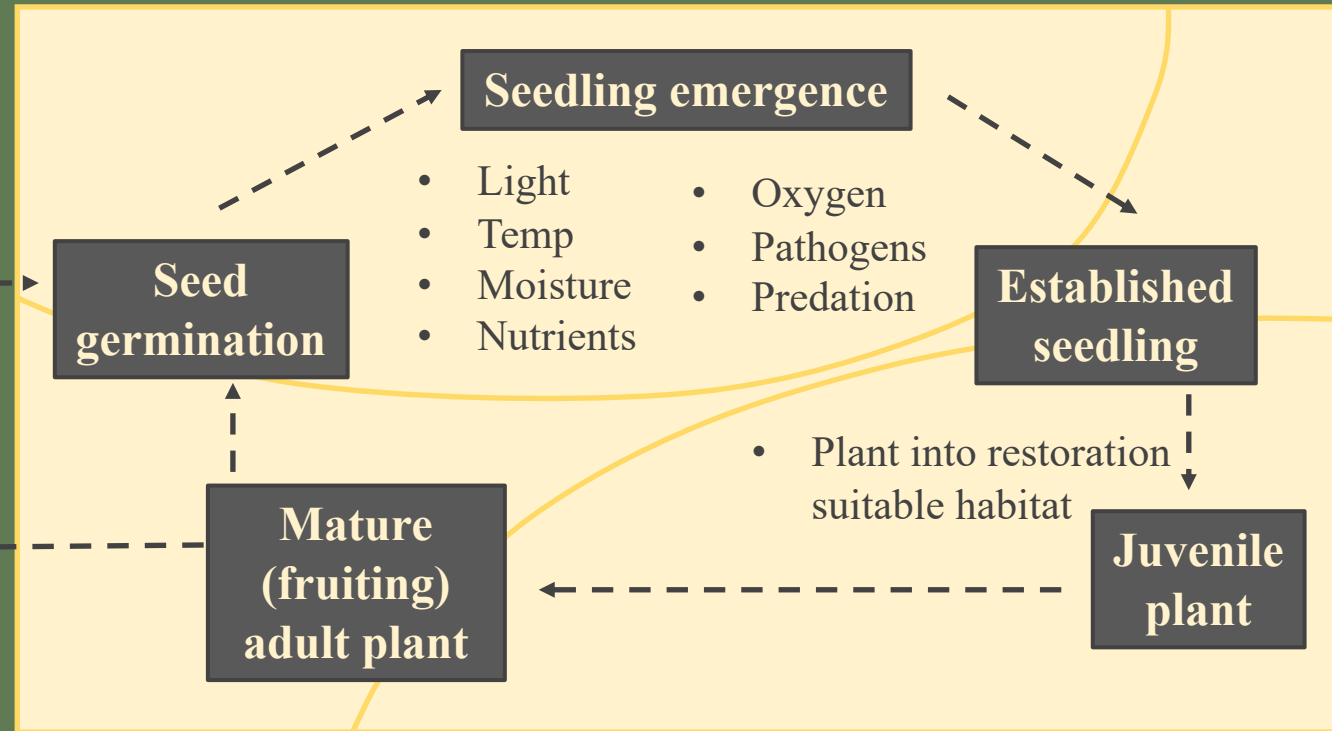
Top: Norfolk Island climate data, averaged from 1939-2021, collected from the Australian Government Bureau of Meteorology (accessed Aug 2021)

Bottom: Various plant communities and seed species on Norfolk Island (PC Dann unless otherwise stated)

## Seed Use & Storage Considerations



## Planting & Growth Considerations



The handbook was created using information gathered through field and nursery experiments, local and practitioner knowledge, and literature and database searches.

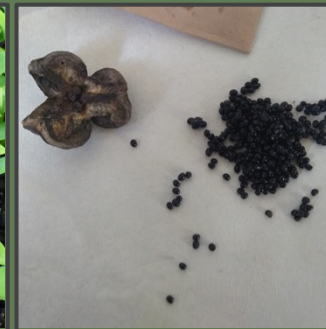
The figure above outlines some considerations for seed-based propagation, which we discuss in the handbook for each species.



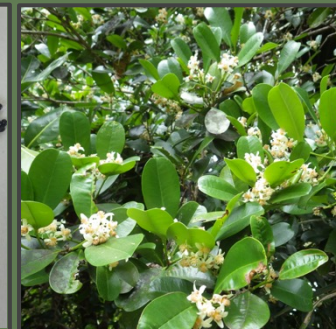
*Ungeria floribunda* emerging seedling; PC Mark Scott



*Euphorbia norfolkiana* seedlings; PC Mark Scott



*Pittosporum bracteolatum* capsule/seeds; PC Leah Dann



*Baloghia inophylla* mature flowering plant; PC Mark Scott



## *Wikstroemia australis*

**Common Name:** Kurrajong

**Family:** Thymelaeaceae

**Status:** Critically Endangered<sup>1</sup>

**Range:** Endemic to the Norfolk Island Group<sup>1,2</sup>

**Growth Form:** Small tree<sup>2,3</sup>

**Plant Description:** This small, fast-growing tree can grow up to 8m tall but is typically shorter. The leaves are smooth, opposite, and elliptical to lanceolate with entire margins (approximately 3-7 cm long, 2-3 cm wide). The tree will often have both yellow and green leaves. It is semi-deciduous, and at times will drop all of its leaves before replacing them, possibly associated with dry conditions. The flowers are long (~4 mm), thin and a yellowish-green colour<sup>2,3</sup>

**Fruit/Seed Description:** The fruit is ovoid to egg shaped, about 4mm long, and turns brick red when ripe<sup>2</sup>

**Habitat:** Adaptable and hardy. Found in drier areas of the rainforest, open areas, slopes, and dry ridges. Tends to grow well in disturbed areas<sup>2,3</sup>

**Light (for plant growth):** Moderate to high light levels. Prefers a light gap over complete shade

**Moisture:** Tolerant of both dry and moist environments

**Seed Collection:** Bag seeds before they ripen to protect from rat and insect predation. Collect seeds when fruit is red in colour. Collection times vary, but typically ripe seeds can be found anywhere from winter to summer (May to November, January)

**Seed Storage:** Orthodox (inferred)<sup>4,5</sup>

**Seed Dormancy:** Not dormant (inferred)<sup>5</sup>

**Seed Propagation:** Remove fruit from seeds before planting. Sow at medium density and cover with a few millimetres of seed raising mix

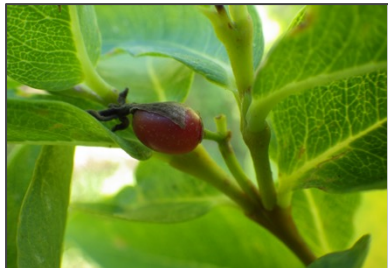
**Time to Emergence:** Approximately 25-55 days

**Time to Maturity:** Approximately 1 year

**Other Information:** The bark of this tree was often used to make rope and twine and for tying bags<sup>3,6</sup>

### Literature Cited

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3. Mills, K. (2010b). The Flora of Norfolk Island, 11: Field survey and assessment of the critically endangered endemic plant *Wikstroemia australis* (Kurrajong).
4. Royal Botanic Gardens Kew. (2021) Seed Information Database (SID). Version 7.1. From: <http://data.kew.org/sid/> (August 2021)
5. Iitiaty, Y., Brescia, F., Bordez, L., Gensous, S., McCoy, S., & Fogliani, B. (2020). Implications of seed traits for restoration of ultramafic plant taxa from the Goro plateau in the subtropical hotspot of New Caledonia. Restoration Ecology, 28(6), 1505-1516. Supplementary material. <https://doi.org/10.1111/rec.13248>
6. Coyne, P. 2011. Norfolk Island's Fascinating Flora. Petaurus Press, ACT.



Photos top to bottom (PC Mark Scott): *Wikstroemia australis* fruit/seed (top left), seedling (top right), flowers, juvenile plant, mature plant.

## A sample species page from the handbook

## Seed Handbook Information

- Norfolk Island and its vegetation
- Seed collection, storage/dormancy, processing, & propagation
- Individual pages with info about each species

Table showing species names, status, fruit colour, and typical fruiting months

Scientific Name	Common Name	Status	Fruit/capsule colour when ripe	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
<i>Abutilon julianae</i>	Norfolk/Phillip Island Abutilon	Critically Endangered*	Dark brown capsule												
<i>Araucaria heterophylla</i>	Norfolk Island Pine	Unlisted*	Brown scales												
<i>Baloghia inophylla</i>	Bloodwood	Unlisted	Dark brown												
<i>Boehmeria australis subsp. australis</i>	Nettle Tree, Tree Nettle	Critically Endangered*	Brown, green, or cream filaments												
<i>Celtis paniculata</i>	Whitewood	Unlisted	Blueish-black												
<i>Coprosma baueri</i>	Coastal Coprosma	Endangered*	Orange												
<i>Coprosma pilosa</i>	Mountain Coprosma	Endangered*	Purple												
<i>Cordylone obtecta</i>	Ti, Rauti, cabbage tree	Vulnerable	White or blueish-purple												
<i>Elaeodendron curtispiculum</i>	Maple	Unlisted	Blueish-black to dark green												
<i>Euphorbia norfolkiana</i>	Norfolk Island Euphorbia	Critically Endangered*	Brown												
<i>Hibiscus insularis</i>	Phillip Island Hibiscus	Critically Endangered*	Brown capsule												
<i>Meryta angustifolia</i>	Narrow-leaved Meryta	Vulnerable*	Dark greenish-purple												
<i>Meryta latifolia</i>	Broad-leaved Meryta	Critically Endangered*	Dark greenish-purple												
<i>Myoporum obscurum</i>	Popwood, Sandalwood	Critically Endangered*	Pinkish-purple												
<i>Nestegis apetala</i>	Ironwood	Unlisted	Yellow, pink, red, or purple												
<i>Pittosporum bracteolatum</i>	Oleander	Vulnerable*	Yellow to brown capsule												
<i>Rhopalostylis baueri</i>	Norfolk Island Palm	Unlisted	Red												
<i>Ungeria floribunda</i>	Bastard Oak	Vulnerable*	Brownish-yellow capsule												
<i>Wikstroemia australis</i>	Kurrajong	Critically Endangered*	Brick red												

\* = Endemic to the Norfolk Island Group

# Outcomes

The Norfolk Island seed handbook:

- Provides information about seed collection, processing & propagation of some of Norfolk Islands vital plant species
- Consolidates insights developed by researchers, practitioners, and local gardeners over many years
- Helps set the direction for further research into the seed ecology of Norfolk Island's endemic species
- Aims to be a tool that will optimize germination success, improve seedling establishment, and expand seed-based restoration efforts



*Ataucaria heterophylla*; PC Leah Dann



*Baloghia inophylla* capsule/seed  
PC Mark Scott



*Celtis paniculata* seedling  
PC Mark Scott



*Ungeria floribunda* capsule  
PC Mark Scott



*Abutilon julianae* seedling  
PC Mark Scott



*Hibiscus insularis* flower  
PC Mark Scott