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Seed Banking (Conservation) in New Zealand: Supporting in Situ Conservation

Craig McGill[a1], Kate Nolan[2], Jessica Schnell[1], Marion MacKay[1], Cristina Winkworth[1], Georgina Aubia[1] and Vivienne McGlynn[1]
[1]School of Agriculture and Environment, College of Sciences, Massey University, Palmerston North, New Zealand. [2]Institute of Education, College of Humanities and Social Sciences, Massey University, Palmerston North, New Zealand.

C.R.McGill@massey.ac.nz

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ABSTRACT

The conservation status of New Zealand's indigenous flora is continuing to deteriorate with the number of species classified as Threatened or At Risk increasing. The in situ conservation efforts to preserve the flora need to be supported by ex situ conservation approaches. One such approach is seed banking where seed is collected and stored at low moisture, usually in equilibrium with 15-25% relative humidity and low temperature, usually -20°C as insurance against loss in situ. New Zealand has a long history of banking seed important to its primary industries, but, a much shorter history of banking seed of New Zealand's indigenous flora. A project to collect, study and conserve seed of New Zealand's flora began in 2013. Following specific protocols this project banked 327 seed accessions of 195 species from October 2013 to October 2017. The arrival of the disease Myrtle rust in New Zealand in 2017 has meant that since late-2017 the focus has been on banking seed of Myrtaceae collected by the Department of Conservation. To date 500 Myrtaceae collections have been collected and banked within the Myrtle rust response programme. The arrival of Myrtle rust in New Zealand and the potential threat it possesses to the New Zealand Myrtaceae has led to wider questions on what seed banking in New Zealand with the integration of Mātauranga Māori alongside seed bank activities should look like.

INTRODUCTION

New Zealand is listed as one of the world's biodiversity hot spots (Myer3 et al., 2000) with 80% of New Zealand's trees, ferns and flowering plants found nowhere else (https://www.doc.govt.nz/nature/native-plants/). However, New Zealand's unique plant biodiversity is under threat from diseases such as Kauri dieback (*Phytophthora agathidicida*) and Myrtle rust (*Austropuccinia psidii*) (de Lange et al., 2018) as well as climate change and habitat loss.

The status of New Zealand's vascular flora is continuing to decline. There are 403 species classified as Threatened (nationally critical, endangered or vulnerable). This is an increase from 289 in 2012. Similarly, the number of species classified as At Risk (Declining) has increased from 102 in 2012 to 158 in 2017. In contrast, the number At Risk (Recovering) has only increased from 7 to 8 (de Lange et al., 2013; de Lange et al., 2018). The worsening status of flora in situ means that conservation efforts in situ need to be supported by a range of other approaches. One such approach is seed banking where seed is stored at low temperature, usually around -20°C and at low moisture, usually in equilibrium with 15-25% relative humidity. Seed banks are a very efficient and relatively cost-effective way of holding a wide range of biodiversity within a relatively small area (Li and Pritchard, 2009). Seed banks provide an insurance policy against future changes that may threaten plant populations in the wild.

New Zealand has a long history of collecting and banking seed important to New Zealand's primary industries. One such seed bank is the Margot Forde Germplasm Centre, based at the AgResearch Grasslands Research Centre in Palmerston North. The Margot Forde Germplasm Centre was established in the 1930s to hold collections of the world's grassland genetic diversity.

These collections are a key contributor to the resilience of New Zealand's multi-billion dollar pastoral sector providing genetic diversity that can be used in breeding programmes to develop new cultivars in response to climate change or changing pest and disease pressures (Williams, 2010).

ESTABLISHMENT OF THE NEW ZEALAND INDIGENOUS FLORA SEED BANK

In contrast to the species utilized in primary production, banking of seed of New Zealand's indigenous flora is relatively recent. The New Zealand Indigenous Flora Seed Bank began in 2013 as a project to collect, study and conserve seed of New Zealand's flora. Prior to this banking of some seed of the New Zealand flora was achieved through funding from MWH New Zealand Limited. In 2013 funding from the Massey University Strategic Innovation Fund and the New Zealand Lottery Grants Board enabled the expansion of seed banking of New Zealand's indigenous flora through the creation of a partnership to bring together organizations with the expertise and / or facilities to achieve the collection, study and banking of seed of the New Zealand flora:

1. Massey University is coordinating the project, providing research expertise and resources for collecting and banking the seed collected as well as holding herbarium voucher specimens from the population from where seed is collected.

2. The Department of Conservation provides access to public conservation land, threatened species advice and connections for engagement with iwi / hapu. For the seed banking in response to Myrtle rust the Department of Conservation is undertaking the seed collection.

3. AgResearch provides drying facilities and storage for the banked seed, as well as seed collections' data at the Margot Forde Germplasm Centre (https://www.agresearch.co.nz/about/our-subsidiaries-and-joint-ventures/margot-forde-forage-germplasm-centre/).

4. Landcare Research provides taxonomic advice and duplicate storage for herbarium voucher specimens.

5. The New Zealand Plant Conservation Network gives access to its flora database and a network of members to contribute to seed collecting.

The New Zealand Indigenous Flora Seed Bank is part of the Millennium Seed Bank Partnership led from the Royal Botanic Gardens, Kew, UK. This is the largest ex situ conservation project in the world with partners in over 95 countries and with seed of 13% of the world's plant species banked (https://www.kew.org/science/collections/seed-collection/about-millennium-seed-bank). The Royal Botanic Gardens, Kew have provided scientific and technical advice for the New Zealand project.

NEW ZEALAND INDIGENOUS FLORA SEED BANK PROGRAMME

Target Species

At the establishment of the New Zealand Indigenous Flora Seed Bank four target species groups were identified. This was to give a collecting focus to the seed bank collection programme. Non-target species are still collected but the collection programme is built around the target species.

The four target groups were the Myrtaceae, the alpine flora, the Fabaceae and the Podocarps and other trees of the forest. The groups were targeted for a number of different reasons:

1. The Myrtaceae includes 28 species in New Zealand including pōhutakawa (*Metrosideros excelsa*), northern rātā (*Metrosideros robusta*), southern rātā (*Metrosideros umbellata*), mānuka (*Leptospermum scoparium*) and kānuka (*Kunzea* spp.). Myrtaceae are widespread throughout New Zealand and mānuka is a species of economic importance. At the time of the seed bank's establishment in 2013 the potential for the arrival of Myrtle rust in New Zealand made collection of the Myrtaceae a high collecting priority. As a result of the arrival of Myrtle rust on Raoul Island in April 2017 and on the New Zealand mainland in May 2017, all 28 of New Zealand's indigenous Myrtaceae are now classified as threatened (de Lange et al., 2018). One New Zealand Myrtaceae, swamp maire (*Syzygium maire*) is known to be desiccation-sensitive and therefore the seed cannot not be dried to the low moisture needed for banking under standard conditions. Alternative protocols will need to be developed for swamp maire.

2. The alpine flora, one-third of New Zealand's flora is found in the alpine zone. This includes around 500 species that are found exclusively in the alpine zone. Of the species in the alpine zone around 83% are endemic and about one-third are At Risk or Threatened. Those At Risk or Threatened include the alpine forget-me-nots (*Myosotis* spp.), alpine daphne (*Pimelea* spp.), hebe (*Veronica* spp.), the alpine daisies (*Celmisia* spp.), buttercups (*Ranunculus* spp.), speargrass (*Aciphylla* spp.) and gentians (*Gentianella* spp.).

Alpine flora has some protection in National Parks, but at the same time it is threatened by human pressure and browsing animals. The alpine flora is especially vulnerable to climate change, as the opportunity to migrate to new environments is limited. If lost, the alpine biodiversity can never be replaced, so there is some urgency to conserve these species. Seed banking of this group will safeguard the diversity of these species.

3. The Fabaceae which include some of the best-known flowering plants in the New Zealand flora, such as kowhai (*Sophora* spp.), the native brooms (*Carmichaelia* spp.) and two kākā beak species (*Clianthus puniceus* and *Clianthus maximus*). Of the 36 species in this group 26 are At Risk or Threatened (de Lange et al., 2018). There are eight species of kowhai and although kowhai is found in forest and scrub vegetation throughout New Zealand some species are limited in their distribution (Heenan et al. 2001).

The native brooms (*Carmichaelia* species), are predominantly South Island species, many with limited distribution and kākā beak (two *Clianthus* species) are restricted to a very few places in the North Island. Twenty species of *Carmichaelia* and both *Clianthus* are At Risk or Threatened, due to threats in their native habitats such as browsing pests and / or habitat loss. Four *Carmichaelia* (*C. corrugata*, *C. nana*, *C. petriei* and *C. torulosa*) have moved into a worse category since 2013 (de Lange et al., 2018). Seed banking of this group will safeguard these species until the threats in their habitats can be overcome.

4. The broadleaf and coniferous trees and shrubs are the backbone of the New Zealand's forests. Conifers from the family Podocarpaceae include rimu (*Dacrydium cupressinum*), tōtara (*Podocarpus totara* var. *totara*), miro (*Prumnopitys ferruginea*) and matai (*P. taxifolia*), while other indigenous conifers are kauri (*Agathis australis*), and the native cypresses kawaka (*Libocedrus plumosa*) and kaikawaka (*L. bidwillii*). Broadleaf trees include tawa (*Beilschmiedia tawa*), tītoki (*Alectryon excelsus* subsp. *excelsus*), kohekohe (*Dysoxylum spectabile*), and rewarewa (*Knightia excelsa*).

The shrub layer of the forest includes species like māhoe (*Melicytus* sp.), *Pittosporum* sp. and hebe. Nearly 300 tree and shrub species (on-shore and off-shore) are At Risk or Threatened, including kauri and the Myrtaceae which are threatened by disease. A number of species in this group are thought to be desiccation sensitive (for example, rimu, miro matati, tawa and kohekohe) and therefore cannot be banked under standard conditions. New protocols will need to be developed for these species.

The initial project duration was four years, from October 2013 through to October 2017. There were two key aspects to the initial project: 1. to develop a network of volunteer seed collectors to along with seed bank staff collect the seed and 2. to process and bank seeds.

Seed Collection

Prior to collecting, discussion is undertaken with relevant groups, including with iwi/hapu to ensure that appropriate permissions to collect are obtained. Once permission is obtained, strict protocols are followed when collecting seed to ensure that seed is collected with minimum disruption to the environment, including careful control of the numbers of seed collected so sufficient seed remains for natural recruitment, and to ensure that high quality collections that capture the maximum genetic diversity in the target population of the species or sub-species are collected. The higher the quality of the seed at collection the longer the potential storage life of the seed.

A network of volunteers has been trained through a series of regional work-shops. At the conclusion of the four-year pro-ject 162 volunteers covering most areas of New Zealand had been trained. Collectors have come from a range of backgrounds including Department of Conservation staff, regional and city council staff (including staff from Botanic Gardens), iwi / hapu groups, botanical / conservation groups, university / museum-based researchers and individuals with an interest in plant conservation.

Training includes a one-day practical collecting expedition and covers:

1. Assessing if a seed population was suitable for collection. This includes assessing both seed number and quality.

2. Sampling and collecting strategies. This is to ensure that the seed collected is representative of the population and captures as much of the genetic diversity in the population as possible.

3. Planning and risk assessment (including biosecurity).

4. Collecting of associated data and herbarium voucher specimens. This data is as critical as the seed itself. Without this data the value of the seed collected is greatly reduced. If the seed is used to re-establish a population this data is used to maximize the chances that the plants grown from the seed will re-establish in the wild.

5. Post-harvest handling of seed to ensure that the seed is received by the seed bank in as good condition as possible.

6. Obtaining permissions / permits to collect.

In addition to collecting by volunteers, the seed bank also undertook collecting expeditions. Over the four-year project time 30 collecting expeditions ranging from 1 to 14 days were undertaken. Most collecting expeditions were 1-3 days. The 14-day expedition was focused on collecting seed of the Alpine flora. In the four year of the project, 327 seed accessions of 195 species were banked.

Processing and Banking of Seed

Again, strict protocols are followed to ensure that during the processing and banking of the seed, loss of seed quality is minimized. The processing of the seed involves:

1. Drying the seed as quickly as possible to a moisture in equilibrium with 15% relative humidity. For the majority of seed there is a direct relationship between seed moisture and seed storage life with lower relative humidity / moisture meaning seed will remain viable (alive) in storage longer (Justice and Bass, 1978).

2. Removing the seeds from the fruits. This can be a dry fruit such as a pod or capsule, for example as in kowhai, or a fleshy fruit, for example as in *Coprosma* spp. As much of the fruit debris and any other vegetative material is then cleaned from seed. Extraction and cleaning is largely done by hand to minimize any damage to the seed; damage that can shorten the storage life of the seed. Once cleaned the number of seeds in the collection is determined by weight. Seed is then returned to 15% relative humidity to remove any moisture that may have re-entered the seed during extraction and cleaning.

3. Banking. Once re-dried the seed is split into two unequal size collections and sealed in water impermeable packaging for storage. The larger (main) collection remains in the seed bank and the other, smaller collection, is sent for safety duplicate storage. Safety duplicate storage will be at one or more of New Zealand's botanic gardens. This does not however exclude the possibility of safety duplicate storage at other locations within New Zealand. An accession number on the packages links the collections in the seed bank and safety duplicate storage to the data gathered during seed collection and processing / banking. This data is held in the seed bank database. After one month in storage a sub-sample of the seed from the main collection is assessed for quality and to confirm that the seed has survived the banking process and first month of storage.

4. Assessing the quality of the seed. Seed, if large enough, is x-rayed to determine if the seed is full i.e., contains an embryo and food reserves and / or whether the seed has been predated by insects. Without an embryo and food reserves the seed will not develop into a plant. Insects can also deposit eggs in the developing seed, these hatch into larvae that feed on the seed. Larvae or the damage caused by them can be seen on the x-ray. The ultimate aim is to determine the percentage of healthy (full) seed in the seed lot that can be converted into a plant.

5. To determine the amount of full seed that is viable (alive not dead) a sub-sample of seed is germinated. Seed of many wild species, including species in the New Zealand flora have complex dormancy mechanisms within them. In the natural environment these are designed to disperse the seed in time and space. Nonetheless in the seed bank seed dormancy is problematic. To determine the full normal germination potential of the seed the dormancy in the seed needs to be alleviated. Assessing normal germination will indicate if the seed is viable and whether the resultant seedling has all the structures needed to develop into a healthy plant. For many species in the New Zealand flora the dormancy-breaking techniques are not known. As a result, at the end of the germination test any seed that has not germinated is stained with Triphenyl tetrazolium chloride. This chemical will stain viable tissue red. The test, the "Tz test", will indicate which seed is viable but not necessarily whether the viable seed will produce a healthy seedling.

6. From the seed weight, x-ray, germination and Tz test results the number of viable seed available for restoration if needed can be determined.

7. The viability of collections are monitored over time to determine how quickly the seed is losing viability and whether the population needs to be recollected. Most collections in the New Zealand Indigenous Flora Seed Bank were collected in 2014 or later so no re-checking of the collections has occurred as yet.

Missing Knowledge

Relatively little is known about the seed biology and reproductive phenology of many of New Zealand's indigenous flowering species. There is a need to understand New Zealand's indigenous plant ecologies in specific locations and regions across New Zealand to enable effective seed collecting. Data is missing on:

1. Sensitivity to drying. A number of species in the New Zealand flora are known to produce seed that will not tolerate drying. Species producing seed known to be desiccation sensitive (or recalcitrant) include nīkau (*Rhopalostylis sapida*), swamp maire (*Syzygium maire*) and kohekohe (*Dysoxylum spectabile*). For others such as miro (*Prumnopitys ferruginea*) and mātai (*P. taxifolia*) the storage behaviour of is uncertain (http://data.kew.org/sid/). The extent of desiccation sensitivity in the New Zealand flora is not known. Of the approximately 3000 named and unnamed taxa in the New Zealand flora less than 200 have had their storage behaviour reported (http://data.kew.org/sid/). For those sensitive to drying different storage approaches will be needed. Two research projects are underway at Massey University to develop storage protocols using cryopreservation (freezing the seed or the seed embryo in liquid nitrogen (-196°C)) for swamp maire (PhD research) and kohekohe (Masters research).

2. Optimum time for seed collection. For maximum storage life there is an optimum time for seed collection. The phenological information needed to enable the optimum time for collection to be determined is not

available for most species. The optimum date for seed collection will vary across regions and between seasons. Collecting of the phenological data provides an opportunity for citizen science projects locally, regionally and nationally with the potential to grow scientific capability to ensure that we know at what time to collect indigenous seed for maximum storage potential.

3. Seed storage life. The time for which seed of a given species will retain viability in storage will vary between and within families. For example, seed of Orchidaceae are short-lived in storage (Hay et al., 2010). In contrast most species within the Malvaceae are long-lived in storage whereas within the Fabaceae longevity can be variable across species (Long *et al*., 2015). The length of time most species can be stored before viability declines is not known (collections are monitored to determine when viability begins to decline) nor why seed of some species retains viability in storage longer than other species. A research project (PhD) is underway to determine why orchid seed is short-lived in storage.

4. Dormancy and germination. To assess the quality of the seed coming into the seed bank and to be able to convert the seed into plants the ability to germinate the seed is essential. Seed of many species in the New Zealand flora have complex dormancy mechanisms, which need to be alleviated to allow germination to begin. For many species seed dormancy-breaking protocols still need to be developed. Equally for other species protocols may have already been developed within the wider plant conservation community. A key role for the New Zealand Indigenous Flora Seed Bank may be as a data warehouse where knowledge around germination and dormancy-breaking requirements is held not only for use in banking but also in local and national restoration projects where plant supply will be greatly enhanced if every seed can be converted into a plant.

Impact of Myrtle Rust on the New Zealand Indigenous Flora Seed Bank

The arrival of Myrtle rust in 2017 considerably changed the operational activities of the New Zealand Indigenous Flora Seed Bank. The New Zealand Indigenous Flora Seed Bank is supporting the Myrtle rust response being led by the Ministry of Primary Industries and the Department of Conservation. The seed bank is processing and banking seed of New Zealand's indigenous Myrtaceae being collected by the Department of Conservation. This programme is the largest targeted seed collection programme ever undertaken in New Zealand (https://www.doc.govt.nz/nature/pests-and-threats/diseases/myrtle-rust/). As part of this programme a further 50 collectors, predominantly DOC staff have been trained in sustainable seed collecting in 2018. To date 500 Myrtaceae collections, representing 14-and-a-half million full seeds, have been collected and banked. This adds to the 24 Myrtaceae collections made prior to the arrival of Myrtle rust.

Although collections of seed of non-Myrtaceae are still being sent in by trained collectors for banking, processing and banking Myrtaceae seed is now the primary focus of the seed bank.

SEED BANKING IN NEW ZEALAND: THE FUTURE

The New Zealand Indigenous Flora Seed Bank began as a four-year project to collect, study and conserve seed of New Zealand's flora. The arrival of Myrtle rust in New Zealand and the potential threat it possesses to the New Zealand Myrtaceae, including iconic species such as the two New Zealand

species of pōhutukawa (*Metrosideros excelsa* and *M. kermadecensis*), has raised the profile seed banking in New Zealand and the role it can play within New Zealand's internal biosecurity.

This has led to wider questions on what seed banking in New Zealand with the integration of Mātauranga Māori alongside seed bank activities should look like. Should New Zealand follow the traditional centralized seed banking model, or should the New Zealand model be built around a decentralized seed bank. Whichever model is used, at the centre of any seed bank programme would be coordinated research activity, seed storage, information / data sharing and project management. This hub will serve a range of connected groups (scientists, educators, iwi / hapu, community leaders, conservationists) to support and resource local and regional seed conservation activities. A hub and spoke model will facilitate community engagement in the project through education outreach, possibly with the New Zealand botanic gardens who already undertake considerable education outreach, and citizen science.

The long-term vision should be to facilitate the development of networked collaborations that result in sustainable conservation and preservation activities for New Zealand, locally, regionally and nationally. The aim of the seed bank is to partner with other ex situ and in situ conservation activities at the individual, iwi / hapu, community, regional, national and international levels to help ensure the continued existence of indigenous plant life and the biodiversity it contains.

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