Germination Testing at the Tasmanian Seed Conservation Centre©

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ORIGINS OF THE TASMANIAN SEEDBANK

August 2005 saw the opening of the Tasmanian Seed Conservation Centre (TSCC) a seed banking facility located in a purpose-built laboratory at the Royal Tasmanian Botanical Gardens.

The seed bank is a product of the Joint Tasmanian-Millennium Seed Bank Project (MSBP), a project resulting from collaboration of the following:

- The Royal Tasmanian Botanical Gardens
- The Tasmanian Herbarium
- The Resource Management and Conservation Division of DPIW
- The Seed Conservation Department of the Royal Botanic Gardens, Kew (U.K.)

Kew's Seed Conservation Department in the U.K. is home to a global conservation program known as the Millennium Seed Bank Project (MSBP). This highly ambitious project aims to collect and bank seeds of around 24,200 species by the end of 2009. To meet this goal the MSBP jointly funds seed banking activities in around 36 different countries around the world, including Australia.

THE BASIC AIMS AND GOALS OF MILLENNIUM SEED BANK PROJECT

The Tasmanian members of the MSBP are collectively referred to as "SeedSafe." By the beginning of 2010 the SeedSafe group aims to:

- Collect and conserve 800 plant species native to Tasmania.
- Collect and conserve at least 60% of Tasmania's threatened flora.

Seeds and detailed field data are collected from naturally occurring, healthy populations in the wild. Back at the TSCC, seeds are carefully cleaned, dried, sealed in packages, and stored at -20 °C. In this way SeedSafe will ensure the long-term security and conservation of Tasmania's unique native plant species by providing:

- Ex situ support for plant conservation programs.
- Seed material to assist in the scientific study of our native plants.
- Long term preservation of plant biodiversity loss caused by environmental degradation.

Additionally, the SeedSafe program and TSCC will greatly enhance our knowledge of Tasmania's native flora, of which little is currently known. By collecting seeds in-situ, as well as field data and herbarium specimens, testing seed viability, uncovering germination requirements, and propagating plants, we expect to uncover a great deal about Tasmanian plant lifecycles, germination ecology, and environmental impacts.

PRINCIPLES OF SEED BANKING

Seed banking involves storing seeds for future use, therefore offering some insurance against the loss of plant species by extinction. Seed banking therefore is a form

of ex-situ conservation (off site) that complements better known in-situ conservation approaches. Of course banking or storing seeds of horticulturally significant species has been going on in some form since the advent of agriculture; at least 13,500 years ago. However, "conservation seed banking" of wild plant populations is a much more recent response to the global decline of plant biodiversity.

The process of seed banking basically involves:

- Collecting seeds from in situ wild plant populations.
- Drying the seeds down to approximately 15% relative humidity.
- Storing the seeds for future use at approximately -20 °C.

Seeds are nature's genetic storehouses; they occupy little space and require minimal attention over considerable periods of time. If banked correctly, most seeds can be grown into plants well into the future. Such an approach is recognised globally as a scientifically proven, highly efficient, and cost-effective method of conserving the variation within and between different plant species. Consequently in recent years modern, high-tech seed banks have been built in many countries around the world for the ex-situ conservation of wild plant species.

In the case of highly threatened species a seed collection can often represent a far greater number of potential genetic individuals (plants) than their habitat area can support in the wild. Within its short history the SeedSafe program has already put this potential to good use. A few examples of threatened species success stories include:

- **Hairy cliff-eyebright.** *Euphrasia phragmostoma* (Orobanchaceae) is a species endemic to a 1.6 km stretch of sea cliff on the Tasman Peninsula. Along this one stretch of cliff *E. phragmostoma* grows on narrow ledges and in cracks covering a total area of just over half a hectare. That's an in-situ population of around 700 individuals. By comparison, the seed collection for this species, safely stored in the ex-situ seed bank, is of over 27,000 viable seeds.
- **Davies' waxflower.** *Phebalium daviesii* (Rutaceae) is another endemic species which is currently known from a single 400-m stretch of river near St. Helens. This species was in fact believed to be extinct until it was rediscovered in 1990. Since discovery the population has declined from 50 plants to 20. The RTBG has maintained a collection of 32 individuals which are carefully propagated every year. In summer 2008–2009 the collection was harvested from and >34,500 were collected.

Once material has been successfully collected in the field it is transferred to the TSCC as quickly as possible and held within a drying room. The drying room is maintained at 15 °C and 15% RH, an internationally accepted regime for drying seed collections. In such conditions seeds dry down quickly and gently and do not over-dry. Once dried down the seed is cleaned as soon as possible. This is done with two main aims:

1) To reduce collection bulk as much as possible (e.g., surplus plant material and large bulky seed covering structures are removed).
2) To remove empty or infested seeds (this significantly improves the quality of the collection and also improves the longevity of the collection in storage).

Once the material is cleaned to the highest level practical the material is ready to be banked. This consists of ensuring seed are properly dried down, then sealing in air-tight foil laminate packets and placing in -20 °C storage. The collection can now be considered safe and secure. We can cut a sample of seed to make sure that the seed is full. But how can we be sure that the seed is alive?

GERMINATION TESTING FOR SEED BANKS

The germination of seeds is the ultimate measure of viability of any seed collection. It can also be argued that there is little point storing seeds for the future if we don't know how to germinate them. Therefore successful germination testing is critical to the function of any seed bank. For this reason the TSCC, like other seed bank programs, needs to determine and record the germination requirements for each seed collection it receives.

The majority of germination tests performed by the TSCC are carried out in 9-cm-diameter plastic Petri dishes containing 1% agar gel (effectively 99% water). Petri dishes are stacked inside plastic bags to avoid water evaporation, and these bags are then placed in germination incubators.

Currently the TSCC has five germination incubators. Three incubators run at 5, 15, and 20 °C constant temperatures and the remaining two run at alternating temperature regimes of 22/10 °C and 27/15 °C. Alternating temperature regimes simply attempt to mimic natural day/night temperature fluctuations. Light is provided by fluorescent tubes for 10 h each day, and in the case of alternating temperature regimes, light will coincide with the warmest temperature. The choice of a 10-h light, 14-h dark photoperiod was made to roughly approximate day length during the Tasmanian autumn and spring period; when we would expect the bulk of seed germination in Tasmania to take place.

The role of photoperiod in the initiation of flowering in plants is well known. Studies so far conducted would seem to indicate that seeds are largely insensitive to the day length, however only a tiny fraction of the world's flora has actually been subject to germination studies. The choice of photoperiod is therefore one that is deemed least likely to inhibit germination.

Once the seeds are sown the plates are inspected every 7 days and the number of germinated seeds is recorded. This is known as "scoring" and enables germination curves to be plotted at the end of the germination test. On average, a TSCC germination test will run for 56 days, but about 40% of tests run for up to 112 days, and the occasional test has been known to run for over 12 months.

At the end of a germination test, any nongerminated seeds are assessed using the cut test. Empty or infested seeds are excluded when calculating the overall percentage germination. In theory, germination percentages should not be significantly different to estimated viability percentages. Viable seeds that do not germinate may possess dormancy. Once the TSCC have uncovered germination requirements of a collection to achieve >75% germination, the germination test can be repeated approximately every 10 years to ensure that the seeds in the seed bank are maintaining their viability.

THE CHALLENGE OF GERMINATING WILD PLANTS

If a seed is viable, then it has the potential to become a plant. For this to happen

however, the seed must germinate, and for germination to occur, the seed requires (in almost all cases) the following:

1) Water (during absorption and subsequent stages of growth)
2) Oxygen (for respiration)
3) Temperatures adequate for metabolism and growth

Additionally some seeds also require light and therefore must be on the soil surface in order to germinate, and not buried beneath the soil surface.

Wild plant species show a huge variation in response to being given these basic requirements. Testing at the TSCC shows that about 45% collections pass in the first round of testing (Table 1). This first round generally consists of sowing at the five basic temperatures without any additional treatment. One notable exception to this are seeds from plant families known to have physical dormancy (hard seeded). With these families seeds are scarified (chipped with a scalpel) before placing under test conditions, unless there are strong indications that this is not necessary.

In the second round of testing simple stratification treatments are most regularly used and this gets a further 10% of collections to pass a test. With successive steps, testing becomes more complex with two or more stratification treatments and/or application of trigger regimes, like smoke, being used.

Collections passing and (more often) failing after the first round are predominately physiologically dormant and it is this form of dormancy that poses the largest barrier to successful germination of wild plants.

Table 1. Percentage of collections achieving greater than 75% germination: a sample of plant families held by the Tasmanian Seed Conservation Centre.

	Rounds of testing				Total	Collections
Families	1 (4)*	2 (3)	3 (3)	4 (2)	passes	(no.)
Myrtaceae	72%	11%	11%	0%	94%	18
Fabaceae	86%	0%	7%	0%	93%	14
Asteraceae	64%	15%	11%	2%	91%	66
Juncaceae	56%	25%	6%	0%	88%	16
Poaceae	61%	13%	3%	0%	76%	38
Cyperaceae	20%	16%	4%	4%	44%	25
Ericaceae (includes Epacrids)	13%	7%	7%	0%	27%	15

*Figures in brackets represent the typical number of tests run in each round.

Members of the Myrtaceae tend to be nondormant and germinate readily. Fabaceae typically have physically dormant seed which germinate rapidly if the seed coat is scarified. Asteraceae and Juncaceae show some physiological dormancy that is dealt with in the second and third rounds of tests. Presently our Poaceae collections pose a bigger challenge showing a higher number of physiological dormant seeds. However the grasses are far easier than the sedges (Cyperaceae) and the heaths (Ericaceae). These two families are classic in displaying deep and complex dormancy (data generated in April 2009).

But why do plants put up such barriers to germination? In the wild, at least, the process of germination represents a very risky step in the lifecycle of a plant. The majority of seed-bearing plants disperse seeds that are desiccation tolerant, which means they can withstand drying, and remain viable in the soil for months or even years. In contrast however, most seedlings are highly vulnerable. At the seedling stage most of the plant's features are poorly established; the roots are small, scant, and have limited access to water, the leaves are small limiting their ability to capture light, protective structures / compounds are often missing or minimal, and the process of seedling growth limits the plant's resources. Viewed in this light the possession of seed dormancy seems like a valuable trait. However to horticulturist, conservationist, and seed banks this trait frequently proves a frustration.

It is often assumed by some that those species possessing dormancy are mostly uncommon or rarer plant species and that common species germinate readily, however many notable weed species have seed dormancy. In fact it is often this trait that makes some weeds so hard to eradicate. By staggering germination over several seasons plant species develop semi-persistent, soil seed banks. Great for survival but means weeding practices have to be done persistently over long periods of time to ensure removal.

What is perhaps most remarkable about the dormancy behaviour exhibited by seed is that the trait can be very plastic. Depth of dormancy can vary across a species range, meaning different provenances will vary in response to a specific treatment. We also know that dormancy depth can vary year to year within the same population, in response to differing environmental conditions. For example plants from cool temperate regions may require longer periods of chilling if formed in cool, wet summer than in a long, hot summer. Such variability may in part explain why certain recommended techniques, may work poorly or even fail when others try them. This also means that just because I have successfully germinated a species once before, I can't assume that the same technique will achieve the same response in a second collection.

SHARING DATA

As the seed bank coordinator I'm tasked with trying to get the highest level of germination out of the collections I hold. But how should I approach this task?

As can be seen from the preceding paragraphs there are a huge number of variables in deciding what would be an appropriate approach to take. Physical dormancy is strongly linked to taxonomy and can be readily identified in laboratory tests. In contrast, dealing with physiological dormancy is a much bigger challenge. I look to the location, climate, the habitat, and the habit and life cycle of the plant to determine what factor may be significant. Of course what is hugely helpful is finding what techniques others have found successful. However for this information to be really useful it does need to include other data, particularly provenance and also storage conditions, harvest date, and sowing date. But accessing that kind of information is difficult.

With only 3 years under our belt the Tasmanian Seed Conservation Centre has run over 2000 germination tests on over 400 seed collections. All this germination data is carefully recorded onto the database system required to manage the

seed bank collections. In Summer 2007 the Royal Tasmanian Botanical Gardens (RTBG) began to look at the means to make this germination data publicly available and by Summer 2008 had made this a reality.

The TSCC Germination Database is available on the RTBG website at <www.rtbg.tas.gov.au/tasgerm>. In addition to making the database available we have also provided 18 pages of content explaining what is currently understood about seed germination and dormancy as well as how our laboratory techniques can be adapted to home use. This information is available at <www.rtbg.tas.gov.au/seedbio>.

Through the database and the support pages we aim to help those who are interested in germinating seeds. We would also hope to encourage others to share their knowledge. In a topic this big, a single individual's activity can achieve only a little. Working collectively we can perhaps crack the tough nut that is seed dormancy.