Germination Testing at the Tasmanian Seed Conservation Centre

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The germination testing programme of the Tasmanian Seed Conservation Centre (TSCC) at the Royal Tasmanian Botanical Gardens (RTBG) began in August of 2006. At that time, we had two incubators and the programme was run solely by myself. Since 2006 the programme has grown to using 12 incubators and is almost totally performed by a team of eight volunteers, with myself orchestrating collections and conditions to be run. Tests are typically conducted on 9 cm petri dishes of 1% agar. These can either be plain water agar or agar with 0.01 M concentration of Potassium Nitrate or a 4% dilution of SmokeMaster Regen 2000. Those outside the discipline of seed science will be familiar with the role that smoke can have in promoting germination. Less well known is the potent effect that small amounts of nitrate can have as well. The levels used are typically available in bare soils but, need to be added to a test media to elicit germination. Our germination volunteer activity consists of setting up and scoring tests, as well as performing cut-tests at the end of trials. Determining testing regimes to be used on collections is overseen by myself, with the end point of achieving a greater than 75% germination result from the viable seed.

Figure 1. Germination test set-up equipment: petri dishes of agar, test sheets, sowing grid, seed boats and fine bamboo dibber. Photo: J. Wood

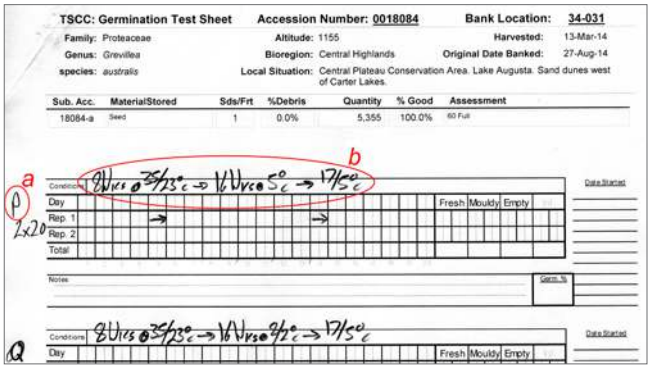

Figure 2. Typical TSCC test sheet: a) test plate code, b) test treatments.

Volunteer involvement in the programme began slowly. The nature of germination testing requires a very regular and consistent volunteer involvement. Scoring is typically carried out weekly and therefore is a major commitment in time, when the duration of testing is considered. Germination tests at the TSCC can take as little as five weeks but generally take about 20 weeks and in certain cases can take over two years. These time lengths are a consequence of dormancy types and level expressed by wild seed. Non-dormant, conditionally dormant and physically dormant seed can generally be germinated very quickly given appropriate conditions. Other dormancy types can impose a series of blocks that need to be overcome successively, leading to these longer durations. Partly due to this aspect, volunteer recruitment has been done slowly and applicants are specifically warned, prior to induction, about the level of commitment required. A fair degree of experience is required with germination testing to become fully competent. As such I sit in with new volunteers when they first start or get them to work alongside long serving volunteers for a few sessions and I then monitor them as they get started. Wild seed bank germination testing is particularly challenging as we typically test a vast range of plant families producing seeds with very different internal morphologies and successfully germinating in a variety of ways. As there is no single source for this information, volunteers are reliant on your expertise and resources to guide them through this work.

To aid with orientating around cut-testing we have a copy of A.C.Martin (1946). Although this article has little coverage of major Australian families, the article is still useful in getting a measure of internal morphology variation. It's highly unlikely that you will find volunteers with a wide knowledge of seed and seedling morphology. Therefore, I encourage my volunteers to come to me if they are not sure about anything, have questions about finishing/transfer of tests or notice anything odd on the plates. What this means in practice is that on volunteer days I can expect to be called on, between once an hour to every ten minutes on particularly intense days.

Figure 3. Sown and labelled 6 cm Petri dishes. Photo: J. Wood

It's not unfair to describe germination scoring as long periods of boredom punctuated with brief moments of excitement and this is explained in the process of volunteer introduction. It should also be appreciated that volunteer programs often have a group participation element that facilitates an important social engagement aspect to most activities. Our germination program doesn't really lend itself to that (doesn't necessarily exclude it) but it's important to explicitly talk about this aspect as well to properly inform expectations. Many of the failings in testing are due to momentary lapses in concentration (*e.g.,* miscounting of seedlings, placing plates in the wrong incubator, missing a transfer date) so it can be argued that a reduction in distractions is preferable. However, it's my observation that errors seem more likely to occur when volunteers have stressors outside of the volunteer activity. As such it's my recommendation that volunteers are supported to manage their workload to what they deem to be reasonable and not to take on too much. Additionally I try to separate our testing collections into two sets, one with collections that can be reasonably expected to germinate relatively quickly (<20 weeks) and the second with collections that will likely require lengthy, complex move-along trials (1-2+ years). I advise volunteers to take a mix of both, so they generally have some germination activity taking place and aren't spending months looking at petri dishes of seeds.

Table 1. Bicolour coding for incubator regimes at the TSCC.

Temperate regime	Colour code		Photo-peroid
00°C			10/14
08/02°C			10/14
05°C			10/14
12/00°C			10/14
10°C			10/14
17/05°C			10/14
15°C			10/14
22/10°C			10/14
20°C			10/14
27/15°C			10/14
25°C			10/14
32/20°C			10/14
30°C			10/14
35/23°C			14/10

Prior to 2019 test plates were labelled with accession number, date started, test code letter/s (Figure 1), replicate number (when applicable) and test conditions. Since early 2019 the TSCC has moved to a bicolour coding system for the incubator regimes (Figure 3 and Table 1), with colour coded printed labels (J8651) attached to plates, to replace writing the test temperature onto the plate. This system was adopted to help volunteers quickly spot plates going into the wrong incubator. Labels are attached so that part of the label runs out onto the side of the lid, so the bicolour code is visible for all plates in a stack. For move-along experiments the next regime label is placed on top of the previous label on the day of transfer. The system has been universally approved by the volunteers who like the extra safeguard in catching errors. So far, the system seems to be going well but we have had the occasional forgetting to update the label. Currently I'm attributing this to adjusting to a new testing practice (it's still too early to be sure) but ultimately no system can be foolproof.

Figure 4. Seedbank volunteers at the TSCC. Photo: J. Wood

I would gauge the TSCC volunteer germination group as a successful volunteer program. Although I have had about a fifth of applicants leave after 6 months the bulk of volunteers have been with the seedbank between 5–10 years. The TSCC volunteers are very self-motivated and find their work fascinating and rewarding. I don't shy away from sharing my frustration or excitement about test results and it's great to see that reflected by volunteers, particularly when they finally get some germination activity in a challenging collection. Although the primary goal of wild seedbanks is the *ex-situ* conservation of seed-bearing plants, the germination testing we conduct is equally as important. Testing is key to the functioning of a seedbank (why store seeds if you can't turn them back into plants?) however identifying techniques to germinate wild species has implications for the broader plant conservation community. As such the RTBG began sharing its germination data with the public since 2008 by placing it online. If you would like to see what we do you can find the TSCC Germination Database on the RTBG website (link below).

References

Martin, A. C. (1946). The comparative internal morphology of seeds. *The American Midland Naturalist*, 36(3), 513-660.

Resources

TSCC Germination Database
https://gardens.rtbg.tas.gov.au/conservation/tsccgerminationdatabase/

Seedbank Origami: envelopes, trays and boats
https://rtbg.tas.gov.au/wp-content/uploads/2020/07/RTBG_SeedBank_Origami.pdf

Ex situ management including seed orchard establishment for Native Guava (*Rhodomyrtus psidioides*) affected by Myrtle Rust

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In 2010, the plant pathogen *Austropuccinia psidii*, commonly referred to as Myrtle Rust, was detected in Australia for the first time on the NSW Central Coast. Over the subsequent decade Myrtle Rust's impact on many native Myrtaceae species has been significant, particularly for *Rhodomyrtus psidioides* (G.Don) Benth. or the Native Guava. Once common from Broken Bay on the NSW coast to south east Queensland and up to 120 km inland, in February 2019 the species was listed as Critically Endangered in NSW. *Rhodomyrtus psidioides* is severely threatened by Myrtle Rust over its entire range and characterised as 'extremely susceptible' to infection (Pegg *et al.* 2014; NSW Scientific Committee 2017). All plant parts have been documented as being affected including leaves, stems, flowers and fruits (Pegg *et al.* 2014; Carnegie *et al.* 2016; NSW Scientific Committee 2017). Damage to new foliage and subsequent failure to replace older leaves progressively weakens the plant, ultimately causing death. How long this process takes remains unclear. *Rhodomyrtus psidioides* readily suckers, however new growth is often rapidly overwhelmed by Myrtle Rust. Flowers and fruits are similarly affected and seldom manage to produce any viable seed, therefore *R. psidioides* struggles to reproduce either asexually or sexually in the wild and has suffered serious decline as a result.

Collecting seed or cuttings of *R. psidioides*, along with other Myrtle Rust susceptible species, was flagged as a high priority by Australian Plantbank collectors after the disease emerged in 2010. In the wild, plants were often covered in Myrtle Rust or had deteriorated to the point where taking cutting material was no longer feasible. Seed was often not viable, not filled or in such small numbers that it was impossible to determine seed storage behaviour. As a result, early seed collections were treated as orthodox and frozen at -18°C.

Conventional horticultural wisdom often prescribes ideals: plant material in good condition, pest and disease free, collected at a specific time of year, all supported by known data. The reality of working with many threatened species is that material may be limited and of poor quality, access to plants restricted due to rarity, location or other external factors, information on propagation and cultivation non-existent, and resources limited.