Florabank Guidelines

Module 11

Seed Germination and Dormancy

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Key points

Understanding the requirements for seed germination, and the germination potential of the seed lot, can improve the success of seed-based propagation or restoration.

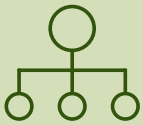
Germination testing determines the germination potential of a seed lot.

Seeds require appropriate moisture, temperature, oxygen and light conditions for germination.

Test germination of fresh seeds prior to storage to provide baseline information on germination potential and viability, and determine if dormancy is present.

Reasons why seeds do not germinate: the seeds are dead, not filled, dormant, or the test conditions provided were not appropriate.

A dormant seed does not have the capacity to germinate in a specified period of time under physical environmental factors that otherwise are favourable for its germination.



Identifying the class of dormancy provides direction to best select an approach to alleviate that class of dormancy.

Clues for conditions that overcome dormancy can be sourced from the seed's natural environment.

Ex situ treatments to stimulate germination and alleviate dormancy include smoke, scarification, stratification, after-ripening and chemicals such as Gibberellic Acid.

Introduction

The initial success of ecological restoration projects utilising seeds hinges on the ability of seeds to germinate and emerge – whether they are directly sown into a restoration site (and germinate immediately or form a soil seedbank), or used to propagate seedlings that are later planted out as tubestock. Knowledge of the conditions required for seed germination can also help understand the dynamics of natural soil seedbanks, which can inform conservation management decisions and natural regeneration practices.

Filled seeds may not germinate, or have a low germination percentage, for one of three reasons: they are dead (non-viable; also includes empty and seeds eaten by predators) (see Module 10 – Seed Quality Testing); the conditions provided were not appropriate for germination; or they are dormant. Understanding the conditions that alleviate dormancy and the requirements needed for seeds to germinate are thus essential and can be tested by applying experimental treatments during seed germination tests. Considering the temperature and light conditions that the seeds experience in their natural environment following dispersal, and/or when seedling emergence occurs in situ, can provide vital clues for germination requirements and/or dormancy alleviating treatments. This approach can not only improve the success of experimental treatments, but also lead to a greater understanding of a species' ecology (i.e. germination conditions that favour emergence and successful establishment in situ).

This module outlines methods to identify optimal conditions for seed germination, and, if seeds don't germinate because they are dormant, how to classify dormancy and select appropriate treatments to alleviate dormancy. First, we step through a general protocol for undertaking seed germination testing - from sampling and design to implementation, monitoring and data analysis. We then provide a brief overview of the different types of seed dormancy mechanisms. Lastly, we provide guidance on selecting treatments to overcome dormancy under ex situ conditions.

Seed germination testing

Germination testing determines the germination potential of a seed lot (ISTA 2020). Seeds require appropriate moisture, temperature, oxygen and light conditions for germination. Conditions are often, but not always, species-specific and variation can even exist among populations within a species (Beardsell and Mullett 1984; Close and Wilson 2002) due to genetic or paternal/maternal environment differences (Hoyle et al. 2008a; Rix et al. 2012; Satyanti et al. 2019).

Germination testing may be undertaken by the organisation to whom the seeds belong (e.g. seed collector, seed store, native nursery, restoration practitioner), or outsourced to an external organisation. Even if seed germination testing is outsourced to an external laboratory or consultant, the person requesting the tests will still need to understand which tests to request, and how to interpret the results. It is useful to know what level and rate of germination may occur under nursery or field conditions, especially if seeds are to be used for propagation of seedlings,

or in direct seeding. In these cases, germination testing should ideally be done by mimicking natural conditions (e.g. using fluctuating diurnal temperatures similar to the germinating season) or choosing the appropriate season for germination under natural conditions, if temperature controlled facilities are not available.

Some practical guidance on germinating particular species can be sourced as follows:

- Scientific journals and papers published online, such as within:
  - Australian Journal of Botany
  - Australasian Plant Conservation
  - Seed Science Research
  - Seed Science and Technology
- Review papers, such as
  - The process of germination in Australian species (Bell 1999)
  - Seed dormancy and germination stimulation syndromes for Australian temperate species (Merritt et al. 2007)
  - The ecology, dormancy and germination of Australian native seeds (Commander et al. in prep.)
- Books, such as
  - Growing Australian Native Plants from Seed (Ralph 2003)
  - Seed Collection of Australian Native Plants (Ralph 1993)
  - Australian Seeds (Sweedman & Merritt 2006)
  - Plant Germplasm Conservation in Australia (Offord & Meagher 2009, Martyn Yenson et al. 2021)
  - Pilbara Seed Atlas and Field Guide (Erickson et al. 2016)
  - Banksia Woodlands: A restoration guide for the Swan Coastal Plain (Stevens et al. 2016)
  - Australian Rainforest Seeds: A Guide to Collecting, Processing and Propagation (Chapman et al. 2020)
- Online databases, such as
  - Royal Botanic Gardens, Kew – Seed Information Database (SID)
  - The Australian Seed Bank
  - Seeds of South Australia
  - Royal Tasmanian Botanic Gardens, germination database
- Botanic Gardens (through Botanic Gardens Australia and New Zealand), Research Institutions, The Australian Seed Bank Partnership, and 'Friends of' groups (e.g. Friends of Kings Park)
- Native seed suppliers and native plant nurseries (e.g. see RIAWA, ANPSA)
- Native Plant Societies
- And see also publications in the references and further reading section

Information to assist with determining suitable conditions for germination can also be sourced from climate information, for instance using data from the Bureau of Meteorology, Worldclim, or installing a weather station or data loggers in the species' habitat.

When to test germination

Germination tests should initially be undertaken on fresh seeds prior to storage to provide baseline information on germination potential and viability. Germination testing of fresh seeds can help determine whether or not dormancy is present.

Seed age and storage conditions influence germination, which means that over time, the germination percentage can change. Germination may decrease due to viability loss, or increase due to dormancy loss. Viability loss during storage depends on the species and dormancy type, storage conditions and seed longevity (see also Module 10 – Seed Quality Testing). If storage conditions are sub-optimal, germination may decline rapidly during storage due to viability loss. Under some storage conditions (generally warm and dry), seed germination of some species may initially increase over a short period of time (weeks or months) as dormancy is lost (see after-ripening in the section below on physiological dormancy).

Given the changes in germination that may happen during storage, seeds should be tested for germination prior to storage, and again when they are taken out of storage, prior to sale or use in propagation or restoration.

For seeds in storage, the frequency of re-testing during storage depends on the expected longevity of the collection, and the end-use of the collections. In general, seeds in long-term storage should be tested every 10 years (Davies et al. 2015). Australian species have very diverse longevity in storage. Australian alpine and temperate seeds from cool, wet environments are shorter-lived than their relatives from other habitats (Merritt et al. 2014, Satyanti et al. 2018), so they need to have shorter testing intervals. For further discussion of seed longevity and re-testing intervals, see Merritt et al. (2021). For seeds in conditions suitable for short (<5 years) and medium (5-10 years) term storage, we recommend that viability, and possibly also germination, is tested at the end of the recommended storage period and/or prior to sale (see Modules 9 – Seed Drying and Storage and 10 – Seed Quality Testing). More frequent testing during short and medium-term storage, and analysis of viability changes over time, may be useful to refine re-test intervals and better understand the lifespan key restoration species.

Potential changes to germination following storage means it is essential that the seed lot is properly labelled and records about the storage history and germination test dates are kept. Germination can then be tracked alongside storage history and be compared to previous test results. Potential issues in seed quality or storage conditions can be flagged if multiple tests are carried out over time.

If optimum conditions for germination or dormancy alleviating treatments are unknown, test as soon as possible, because (i) it is important to test fresh seed, and (ii) suitable conditions and treatments can take considerable time to identify. If seeds are to be propagated in a nursery to produce seedlings, it may be prudent to determine the optimal conditions for germination through germination testing under controlled conditions prior to sowing the seeds in the nursery.

What to test

In general, the intact **diaspore** (or dispersal unit) should be used in germination testing, and throughout this module we have used the word seed to mean diaspore. In some species, such as those of *Acacia* and *Eucalyptus*, this will be the seed, as these genera mostly have dehiscent fruits (i.e. fruits that open to release the seeds). In other cases, such as for species of *Eremophila* and Poaceae (grasses) the diaspore is a fruit. If seeds within indehiscent fruits do not germinate because they are dormant, then extracting seeds from fruits may increase germination. However, keep in mind that extracting seeds from indehiscent fruits may not be a practical pre-treatment on a large scale, so understanding how to germinate the diaspore is useful. Seeds may be extracted from fleshy fruits, especially if fruits contain multiple seeds (e.g. *Solanum*). (See also Module 8 – Seed Processing). Seed fill for some species is often low (e.g. Rutaceae (Martyn et al. 2009)), hence, low germination should be expected, and seed fill should be examined (see also Module 10 – Seed Quality Testing). Seed appendages (such as arils) should not need to be removed.

Representative sampling

Taking a representative sample is the first step in any seed test and a vital one for ensuring that the test results are not biased. Any test must be performed on a sample that is large enough to meet testing requirements (see section 'Sample size and replication') and that is also representative of the whole seed lot.

To take a representative sample, first uniformly mix the seed lot. This can be done by:

- shaking up the seed lot in its container
- emptying the seed lot from one container to another
- mixing by hand
- mixing by mechanical means

Large quantities of seeds may have to be mixed in batches.

The next step is to take a primary sample of seeds from the seed lot, then randomly extract seeds for the test. See also Module 10 – Seed Quality Testing, for detailed instructions of sampling techniques, as well as ISTA (2020) and ISTA (International Seed Testing Association) videos on hand halving and the spoon method. The amount of seeds required is determined by the number of tests to be conducted and the number of seeds per replicate for each test (see section 'Sample size and replication').

Ensure that the objective of the germination test is clear before mixing any seed lots – for example, if seed lots are separated into different populations, keep these seed lots separated if you wish to determine inter-population differences.

Although **dormancy class** (see section 'Seed dormancy classification and alleviation') is likely to be constant within a species, other germination traits may not be. For instance, the degree of dormancy, germination percentage, optimal germination temperature, and **germination rate** (how fast seeds germinate), may differ between populations and collection years, due to maternal environment and/or genetic differences (Rix et al. 2012; Rix et al. 2015) as well as storage environment and duration.

Designing germination tests

Ex situ germination test conditions

To provide adequate moisture, seeds may be incubated on moistened paper (such as filter paper, glass fibre filter paper or germination test paper), irrigated growing media (e.g. seed raising mix) or sterilised sand, or solidified **water-agar** (generally between 0.7% – 1% w/v is used) (Figure 1). Seeds of most species should not be submerged in water, as they will not receive sufficient oxygen. Various containers can be used, but the Petri dish (either plastic or glass) is one of the most useful containers for small numbers of seeds. Glass Petri dishes may be sterilised (for instance under high temperature and pressure in an autoclave) before use to help prevent the spread of contamination, if it occurs, and can be re-used. Parafilm or clingwrap wrapped tightly around the circumference of the container may help reduce the rate of moisture change and loss in the germination environment. Alternatively, Petri dishes can be carefully placed into snap-lock plastic bags. Other, low-cost containers include plastic food containers, pots or punnets (Figure 2).

Remember to label the containers with the species name, accession number or seed lot number, test start date, replicate number, pre-treatments prior to incubation, and incubation conditions or treatments used during incubation (e.g. temperature, light). Petri dishes and plastic containers can be labelled with permanent marker, whilst pots and punnets can be labelled with tags or nursery markers. Record all of the information separately too, such as on a record sheet or in a spreadsheet.

Conditions required for germination vary considerably across genera and species, and practitioners should research appropriate literature and consult experts to determine the best conditions for particular taxa. In practical situations, there are many variables which will influence germination, some of which are temperature, light, smoke and the age of seeds. Clues for conditions required for germination can be sourced from the seed's natural environment. For instance, check which season seeds naturally germinate and what the soil temperatures are during that season. If the season of germination is not known, then the season(s) with reliable rainfall may indicate when seeds germinate. Other clues to investigate include understanding where seeds germinate in the soil profile, or whether they germinate immediately after fire.

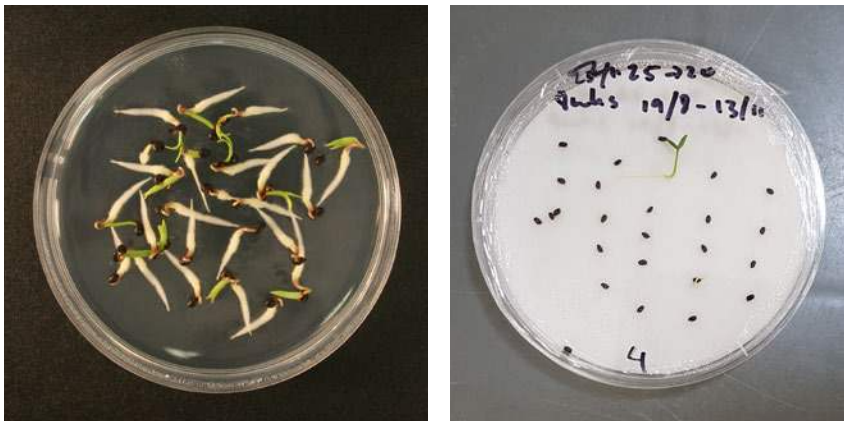


Figure 1. Testing seed germination on water agar (left) and on filter paper (right) in Petri dishes. (Photos: L. Commander)

Figure 2. Testing seed germination in punnets of soil / sand in a glasshouse. (Photo: L. Commander)

Temperature

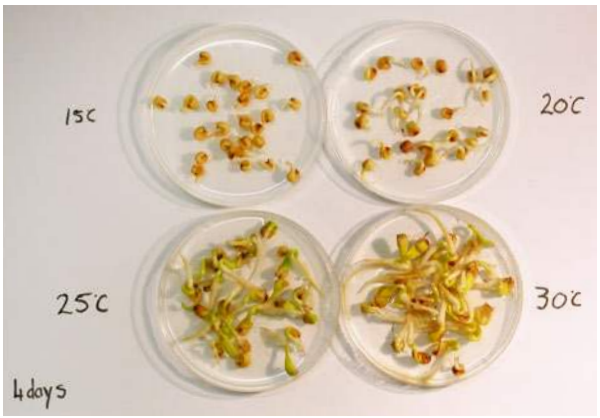


Figure 3. Temperature affects germination rate and final germination percentage. (Photo: L. Commander)

Seeds germinate over a range of temperatures. While this range may be wide or narrow, the appropriate temperature conditions depend on the species and the climate from which the seeds were sourced. The optimum temperature is the temperature at which there is the highest proportion of germinated seeds, and the germination is the fastest (Figure 3). Optimal germination temperature usually corresponds with the temperature during the season that seeds usually germinate in their natural habitat. If seeds are incubated above or below their optimum temperature, then the maximum germination potential may be underestimated. As a general guide, seeds are able to germinate to some degree from 10°C to 35°C, with examples of optimal temperatures of tropical and temperate species ranging from around 25 to 35°C and 10 to 25°C respectively. Seed testing can be undertaken at constant temperature, or at alternating temperatures to mimic natural diurnal temperature ranges. Alternating temperatures use a higher day temperature and lower night temperature. Alternating and constant temperatures may result in different amounts of germination. Some species may have greater total germination at an alternating temperature regime compared with a constant temperature regime, whereas others have higher germination at constant temperatures compared with alternating temperatures. These differences may be species-specific and depend on factors including seed size, growth form and altitude (Liu et al. 2013).

Seed germination testing can be done in incubators, which, like refrigerators, can be set to specific temperatures, and can also be set to alternating temperatures (Figure 4). However, if incubators are not available, germination testing can be done at room temperature, if room temperature is fairly constant (i.e. there is temperature control), and the temperature within the range that is optimal for seed germination (e.g. room temperature of 25°C may be too warm for optimal germination of some species). Record the temperature of the room (during the day and at night) and report it with the results (Figure 5).

Germination testing can also be done in a nursery, such as a cool room, shadehouse or glasshouse – again, if the temperature is suitable, or if the facility is in the same climatic zone as the seed source and seeds are sown during the season in which they naturally germinate. Ensure that the seeds are kept moist and that there are no seed predators. Germination testing in nursery facilities without temperature control may be restricted to certain times of the year.

Using dataloggers to record temperatures during testing can be useful both to keep track of day and night temperatures if seeds are not tested under controlled temperatures, but also to keep track of the temperature within facilities, in case of equipment or power failures (Figure 5). Recording the temperatures during testing can ensure that the test is repeatable, or help explain any unexpected germination failures.

Figure 4. Various incubators used to control temperature for germination testing. (Photo: L. Commander)

Light

Seeds of some species require light in order to germinate, while others require darkness, and some are not sensitive to light conditions (Baskin and Baskin 2014). If the light requirement of a species is unknown, then a sample can be incubated in darkness and another in light. Seeds in the Asteraceae commonly require light for germination (Merritt et al. 2006), and *Asterolasia* (Rutaceae) has recently been identified to require light (Collette & Ooi, 2017).

To keep seeds in the dark, Petri dishes can be wrapped in aluminium foil, or placed in lined boxes. If the sensitivity of a species to light is unknown, seeds tested in the dark should not be exposed to any light until the end of the testing period (Luna et al. 2004).

For further information on how to check germination while seeds are incubated in the dark, and which wavelengths of light typically promote germination, see Martyn Yenson et al. (2021), Luna et al. (2004) and Baskin and Baskin (2014).

Figure 5. A small datalogger can be used to record temperatures during incubation of germination tests, either within a cabinet or within a room. (Photo: L. Commander)

Smoke

Smoke from fires stimulates seed germination of many Australian species. A number of compounds within smoke have been found to be responsible for this effect (Flematti et al. 2010). To increase germination of smoke-responsive species, seeds can be treated with aerosol smoke, imbibed for a short period of time (e.g. 24 h) in an aqueous smoke solution (smoke water), or treated with a compound isolated from smoke, such as karrikinolide or glyceronitrile. Smoke water (Figure 6) contains toxic compounds, so it is not generally recommended as an additive to Petri dishes – instead seeds can be imbibed in smoke water then transferred to Petri dishes. Undiluted smoke water can also have an adverse effect, so ensure

Figure 6. Smoke water. (Photo: N. Emery)

that the dilution (~1 to 10% v/v) is correct. Different techniques of making smoke water may produce different concentrations, so it may be prudent to test each batch with a range of dilutions on a species with a known smoke response (e.g. see Tieu 1999). Smoke water is also available for purchase, in which case it is wise to test each batch you receive using seed of known smoke responsive species. Other smoke products include smoked vermiculite and smoke primers. When testing seeds of a species for the first time, or testing a new product or treatment, always test a control batch of untreated seeds so that the treatment can be assessed.

Instructions for treating seeds with aerosol smoke, or making smoke water can be found online. To expose seeds to aerosol smoke, the following equipment is required: a non-flammable receptacle for the burning plant material (e.g. a metal bin), an air inlet fan to allow air into the receptacle, and a cooling pipe to carry smoke from the receptacle to a tent or small shed which contains the seeds (Figure 7). To make smoke water the set-up is similar, except the cooling pipe leads to a receptacle of water, through which the smoke is bubbled, with a vacuum to assist in drawing the smoke through. Any plant material can be used to create smoke, not just native plants, for instance, hay can be used. Ensure that permissions are sought, a risk assessment is undertaken, fires are only lit when and where they are legally allowed, and authorities are informed in case members of the public call the fire service if they notice smoke. Wear appropriate protective equipment to offer protection from heat and smoke.

Many plant families have seeds that respond to smoke or smoke compounds. Families and key genera include:

- Apiaceae e.g. *Actinotus* (Baker et al. 2005).
- Asteraceae e.g. *Schoenia* (Plummer et al. 2001).
- Dilleniaceae e.g. *Hibbertia* (Hidayati et al. 2012).
- Goodeniaceae e.g. *Goodenia* (Commander et al. 2017), *Scaevola* (Guja et al. 2010).
- Gyrostemonaceae e.g. *Codonocarpus*, *Gyrostemon, Tersonia* (Baker et al. 2005).
- Haemodoraceae e.g. *Anigozanthos* (Downs et al. 2014), *Conostylis* (Turner et al. 2009).
- Myrtaceae e.g. *Baeckea* (Thomas et al. 2003).
- Poaceae e.g. *Austrostipa, Eragrostis, Panicum* (Clarke et al. 2005), *Triodia* (Commander et al. 2017).
- Proteaceae e.g. *Grevillea* (Commander et al. 2017).
- Rutaceae e.g. *Asterolasia* (Collette and Ooi 2017), *Boronia* (Mackenzie et al. 2016), *Diplolaena* (Commander et al. 2009), *Philotheca* (Collette and Ooi 2020).
- Solanaceae e.g. *Anthocercis* (Commander et al. 2009), *Solanum* (Commander et al. 2008).
- Stylidiaceae e.g. *Stylidium* (Norman et al. 2006).

A longer list of additional genera can be found online.

Figure 7. To create smoke, hay can be placed in a metal bin and ignited, and the smoke then fed through a pipe either into an enclosed space to treat seeds with aerosol smoke, or bubble through water to create smoke water. (Photos: L. Commander)

Seeds of some species are not initially smoke responsive following dispersal, but may first require exposure to other environmental conditions (Merritt et al. 2007). For instance, some species exhibit an interaction between heat and smoke, or soil burial and smoke.

If a germination test is performed after seeds have been treated with smoke or smoke products, ensure that these treatments are stated alongside the results of the germination test.

In situ (field) test conditions

Testing germination responses in situ could be sufficient if you are certain the seeds have the potential to germinate. However, keep in mind that results achieved in situ may vary from those under ex situ conditions. Ex situ laboratory or nursery test conditions are designed to optimise germination to see the maximum potential germination, and that is likely to be higher than in situ germination and emergence. Under in situ conditions, temperature may fluctuate widely, soil may dry out rapidly, and seeds may be either surface sown or buried, meaning they will be exposed to different levels of light. In spite of the challenges, in situ germination and/or emergence testing can be useful, particularly for informing seeding rates for direct seeding.

It is important to note that if seeds are sown, it is seedling emergence that is assessed rather than germination potential itself (Figure 8). It may also be useful to use the information from both approaches. For example, results from ex situ germination testing, especially when based on temperature conditions that occur in the field, can inform germination potential, and be followed by an in situ seedling emergence trial, the results of which may inform seeding rates for restoration.

Figure 8. Measuring seedling emergence of sown seed in situ. (Photo: L. Commander)

Sample size and replication

When testing germination, it is necessary to decide on the number of seeds in the test (sample size). These seed lots may be split into replicates with equal numbers of seeds. Replication allows the estimation of variability of results, and means that if something happens to one replicate (e.g. contamination), then results can still be obtained from the other replicates. The replicates then can be randomly allocated to treatments (e.g. light or dark). If seeds are subjected to a treatment (e.g. smoke), there should also be a control (e.g. no smoke) which is left untreated.

There is no standard sample size for germination testing of wild seed. For many seed lots of internationally traded agricultural, forestry and horticultural species, a 400 seed test is a statutory country requirement or mandated by the seed sales contracts (see ISTA 2020). However, even for ISTA, when testing expensive seeds or small seed lots, fewer than 400 seeds can be used. Assuming a high level of seed fill, we recommend that at least four replicates of 25 to 50 seeds be used where possible (see Davis et al. 2015), ensuring no more than 10% of the collection is used overall. Also ensure that seeds are taken from a random sample (see section Representative

Sampling). This number of seeds per replicate is important to ensure that the results can be clearly interpreted statistically. If the collection is smaller than 1000 seeds, use fewer replicates but where possible do not use fewer than 10 seeds per replicate. Table 1 provides a guideline for sample size. Replicates may be assigned to individual containers (Petri dishes, punnets, pots, trays) (Figure 9). Note that some seeds are so large that only a few fit into a container, meaning higher numbers of replicates across multiple containers may be necessary.

If seed fill is very low, then remove empty seeds prior to the test if it is possible to do so. For instance, using x-ray analysis, it is often possible to select only filled seeds. Likewise, a vacuum separator could be used to separate heavy (filled) from light (**unfilled**) seeds (see other methods in Module 8 – Seed Processing). If removing empty seeds is not possible, then increase the number of seeds per replicate. To adjust sample size to account for seed fill, see Davies et al. (2015).

Figure 9. Replicates can be assigned to petri dishes. (Photo: L. Commander)

Table 1. Selecting the number of seeds per replicate and the number of replicates for each treatment or germination test, based on the collection size (from Davies et al. 2015).

Collection size	Seeds per replicate	Number of replicates	Total number of seeds per sample
≥ 1000	25 – 50	4 – 10	100 – 500
≥ 500	25	2 – 4	50 – 100
≥ 250	10	2 – 4	20 – 40
< 250	No test	0	0

Contamination of germination tests

Sometimes, germination tests can become contaminated with bacteria or fungi (Figure 10). Fungal contamination sometimes indicates that a seed may be non-viable, and this may be more frequent when the germination unit is a fruit, or if the species is collected from a warm, wet environment such as a rainforest. Changing the germination medium can sometimes reduce contamination issues, for instance by switching from agar or filter paper to sand. Sterilising the germination medium may help – soil or potting mix can be steam sterilised or sterilised by heating in an oven, while sand, filter paper and agar can be autoclaved. Also, try increasing the distance between the seeds on the medium. That way, if one seed becomes contaminated, the contamination is less likely to spread to the others. When using Petri dishes, placing the seeds on the dishes within a **laminar flow cabinet** and/or not opening the lids of the dishes as frequently when checking germination may be beneficial for minimising contamination by microorganisms (Figure 11).

If there is contamination of seeds in the germination test, then consider using a fungicide, or surface sterilising the seeds prior to the next test to limit contaminants. A common method of surface sterilisation is to place seeds in a 0.5% solution of bleach (sodium hypochlorite; NaOCl) for 10 minutes and then rinse with running water for 1 minute (Davies et al. 2015). Household bleach can be used, but take care to ensure it is sufficiently diluted and be aware that in solution, sodium hypochlorite easily decomposes, so its strength decreases with age. However, this method of sterilisation may positively or negatively impact the germination, so it may be wise to investigate any effects using a control (untreated seeds). Also, ensure that seeds are not too dry (i.e. 5% moisture content) when soaked in bleach, otherwise they may be subject to imbibition shock, i.e. it is preferable equilibrate seeds at ambient relative humidity prior to soaking, if they have been dried in a drying room at 15% RH. Surface sterilisation may be less effective for seeds with hairy seed/fruit coats, in which case a couple of drops of detergent or 1% Tween® (a surfactant) may increase effectiveness. Previcur® may be used as a fungicide. When using bleach or fungicide, ensure that the directions on the label are followed, a material safety data sheet is obtained, a risk assessment is performed, appropriate personal protective equipment is worn, and precautions are made in case of an incident (e.g. eyewash station nearby).

Figure 10. Petri dishes can become contaminated with bacteria and/or fungi. (Photo: L. Commander)

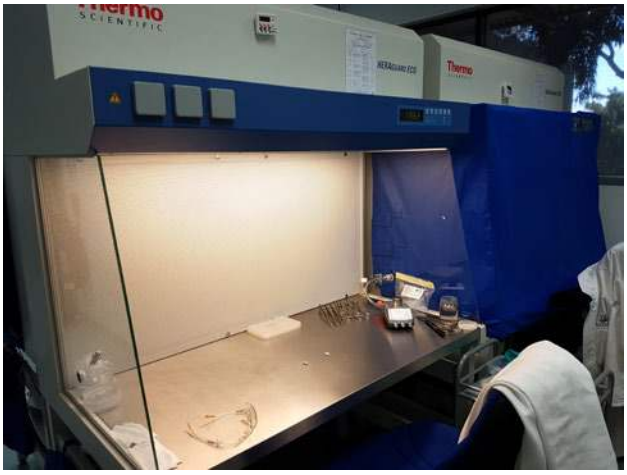


Figure 11. A laminar flow cabinet for sterile work. (Photo: L. Commander)

Recording germination and test duration

Germination is the process that starts with water uptake and finishes with the elongation of the embryonic axis (Bewley & Black 1994). Once germination is complete, the radicle (root) will have penetrated its covering structures (e.g. seed coat). When testing germination, record the number of germinated seeds per replicate at regular intervals and at the conclusion of the test.

Seeds may be fast or slow to germinate, depending on intrinsic factors, test conditions and presence of dormancy. Some species may germinate within a week, others may take one month, some more. Since the length of the incubation period depends on the germination rate (speed) of the seeds, and can be between one and four weeks (Baskin and Baskin 2014; ISTA 2020), plan to allow at least four weeks for germination testing, longer if expected for the test species or if germination time is unknown. It may be useful to incubate the seeds until no further germination

has been observed for two weeks, to see how many seeds germinate in total, and how long this takes. Note that for seeds firstly exposed to a treatment, the incubation period is additional to the treatment time.

If nursery facilities are available, the germinants from the test can be grown on in pots. Observations of seedling development can determine if a germinant develops into a normal seedling. Observing the growth and morphology of the seedlings can assist in field identification at a later date, or to confirm the identity of the species. Seedlings can be harvested and pressed or photographed at regular intervals to compile a seedling identification guide. Plants can be grown on to contribute to a living collection or seed production area, or be planted out in restored areas.

Reporting and interpreting results

The germination percentage can be calculated using the formula below (Equation 1). The percentage can be averaged across the replicates.

$$Germination\ (\%) = \frac{number\ of\ germinated\ seeds}{total\ number\ of\ seeds} \times 100$$

Equation 1. Germination percentage.

When the germination percentage is reported, it is also useful to report the total number of seeds tested (e.g. 42 out of 50 seeds germinated), the number of replicates used for each test condition, incubation conditions, including the temperature and light conditions, and also the duration of the germination test.

Another response variable is the time to x% germination, e.g. time to 50% of maximum/final germination (t_{50}) (McNair et al. 2012), which describes the germination rate.

Data can be presented using column or bar charts of mean and standard error of final germination %; line or scatter plots showing cumulative germination; or box and whisker plots.

To statistically analyse seed germination tests, a two-sample binomial test or a chi-squared test (χ^2) can compare the total number of germinated seeds between two samples against the expected, as long as the total number of incubated seeds is known. If comparing multiple treatments (i.e. a factorial experiment), use a generalised linear model (GLM) or a generalised linear mixed model (GLMM) with a logit link function (for binomial data). Survival analysis can characterise the pace and distribution (shape) of germination. If germination is assessed repeatedly, a time-to-event model fitting could be used to analyse the germination curve. Probit analysis (GLM or GLMM with a probit link function) can be performed if independent samples are used, i.e. scored once only.

For further information on data analysis, summary and presentation, see Chapter 7 in Plant Germplasm Conservation in Australia (Martyn Yenson et al. 2021), McNair et al. (2012), Hay et al. (2014), Carvalho et al. (2018), and Gianinetti (2020).

Why didn't the seeds germinate?

In some cases, seed lots do not germinate, or have a low germination percentage. The possible reasons why seeds do not germinate are:

- the seeds are dead (non-viable);
- the seeds were not filled because they were empty, immature, or eaten by insects;
- the test conditions provided were not appropriate;
- the seeds are dormant; or
- the seeds were removed or eaten by fauna (in nursery trials).

If seeds have been incubated in Petri dishes or similar, and not in soil, then seeds can be removed from the test conditions to examine which of these reasons may be responsible for the lack of germination.

Firstly, perform a cut test or tetrazolium test on ungerminated seeds at the conclusion of the germination test (see Module 10 – Seed Quality Testing). The cut/tetrazolium test will reveal if any of the ungerminated seeds are alive (with the potential to germinate), or if any are dead, empty, under-developed or predated (which cannot germinate). A limitation of this method is that cut tests only give an indication of viability. Also, seeds may lose viability during incubation. Following a cut/tetrazolium test, the "Germination % of Viable seeds" or "Viability adjusted Germination (VaG)" can be calculated using Equation 2.

$$Viability\ adjusted\ germination\ (\%) = \frac{number\ of\ germinated\ seeds}{total\ number\ of viable\ seeds} \times 100$$

Equation 2. Viability adjusted germination percentage

Performing a cut test prior to a germination test may quickly assess seed fill without needing to wait for the results of a germination test, and also prevent wasted resources if seed lots with low seed quality are used for germination testing. See also Module 10 – Seed Quality Testing, for advantages and disadvantages of these two tests.

If any of the ungerminated seeds are **viable**, try setting up a new germination test and incubate a new sample of seeds under different conditions, such as a higher or lower temperature, or in light, unless seeds have already been incubated under conditions previously determined to be suitable.

Viable seeds that haven't germinated under environmental conditions usually suitable for germination, within a specified time period (about four weeks, although up to 6 weeks may be more appropriate in some instances), are considered dormant (Baskin and Baskin 2004; Baskin and Baskin 2014).

Seed dormancy classification and alleviation

What is seed dormancy?

Seed dormancy is 'a seed characteristic, the degree of which defines what conditions should be met to make the seed germinate' (Vleeshouwers, 1995). A dormant seed therefore, is one that does not have the capacity to germinate in a specified period of time under any combination of normal physical environmental factors (temperature, light/dark, etc.) that otherwise are favourable for its germination, i.e. after a seed becomes non-dormant (Basin and Baskin 2004). The function of seed dormancy is to prevent germination when conditions might be conducive for germination but are unfavourable for seedling establishment. For instance, seeds of some species require a period of warm dry conditions to break dormancy, an advantageous mechanism in environments where rainfall is rare in summer but reliable in winter. This would prevent germination in response to summer storms, after which seedlings would die due to lack of ongoing soil moisture, for example, and ensure emergence occurs with consistent rainfall in the winter.

Why do we need to classify dormancy?

Identifying the class of dormancy is helpful, as it provides direction to best select an approach to alleviate that class of dormancy. Dormancy class is generally consistent within a species, however the degree of dormancy may differ between seed lots.

To classify dormancy, the following information must be known: whether or not the seeds are permeable to water, whether or not the seeds germinate within about 4 weeks, and whether the seeds have developed or under-developed **embryos** (Baskin and Baskin 2014). Then, a dichotomous key can be used to determine the class of dormancy (Figure 12). Under this approach, dormancy is classified into five classes: physical, physiological, morphological, combinational (physical + physiological), and morphophysiological (physiological + morphological).

For classifying dormancy, it is best to use fresh seeds. Generally, if seeds are dormant, firstly determine whether or not the seeds imbibe water. Seeds that do not imbibe may exhibit physical or combinational dormancy (although it should be noted that combinational dormancy is extremely rare in Australian native species). If the seeds can imbibe water (i.e. are permeable), the next step is to examine the embryo. If the seed contains a fully developed embryo, then the seed may possess physiological dormancy. If the seed contains an underdeveloped embryo, then the seed may possess morphological or morphophysiological dormancy (Figure 13).

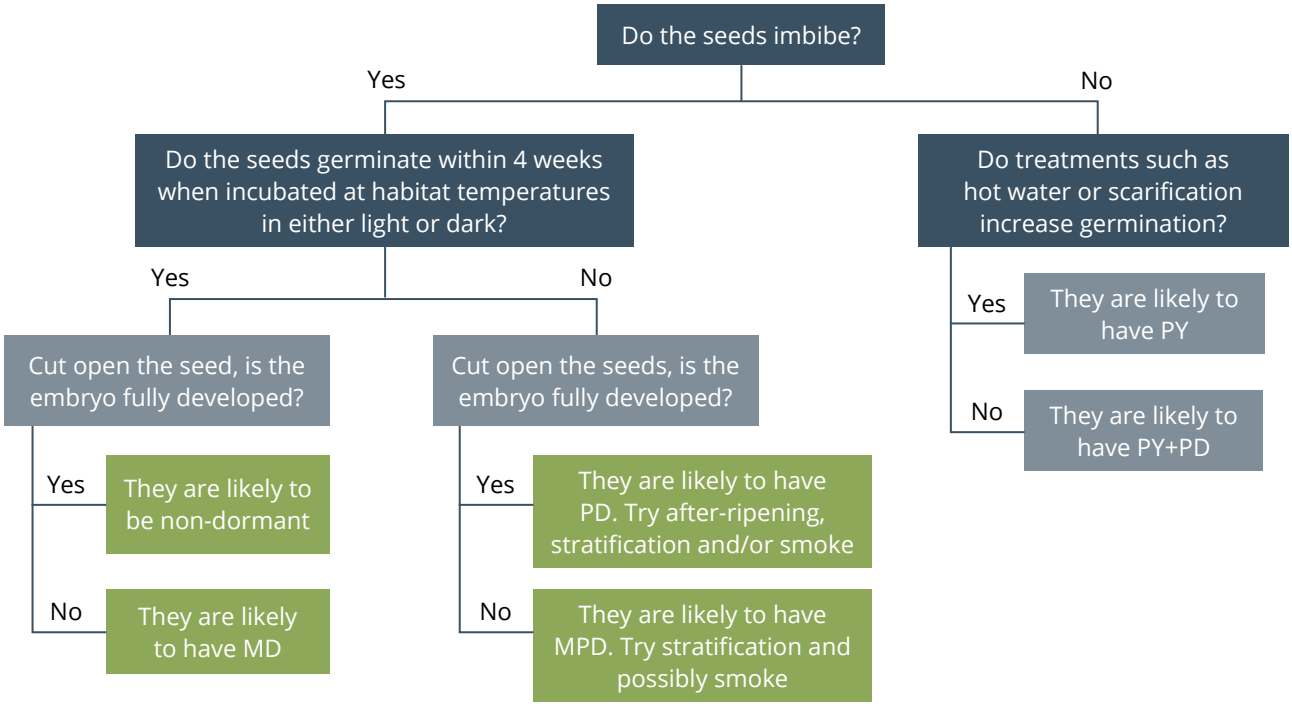


Figure 12. Process and decision tree for determining the type of dormancy present in viable seeds. To assess whether or not the embryo is fully developed, seeds should be cut open prior to incubation. To assess whether or not the embryo grows within the seed prior to germination, seeds can be dissected periodically during incubation, and the embryo measured. PD=Physiological dormancy; PY=Physical dormancy; MD=Morphological dormancy; PY+PD=Combinational dormancy; MPD=Morphophysiological dormancy.

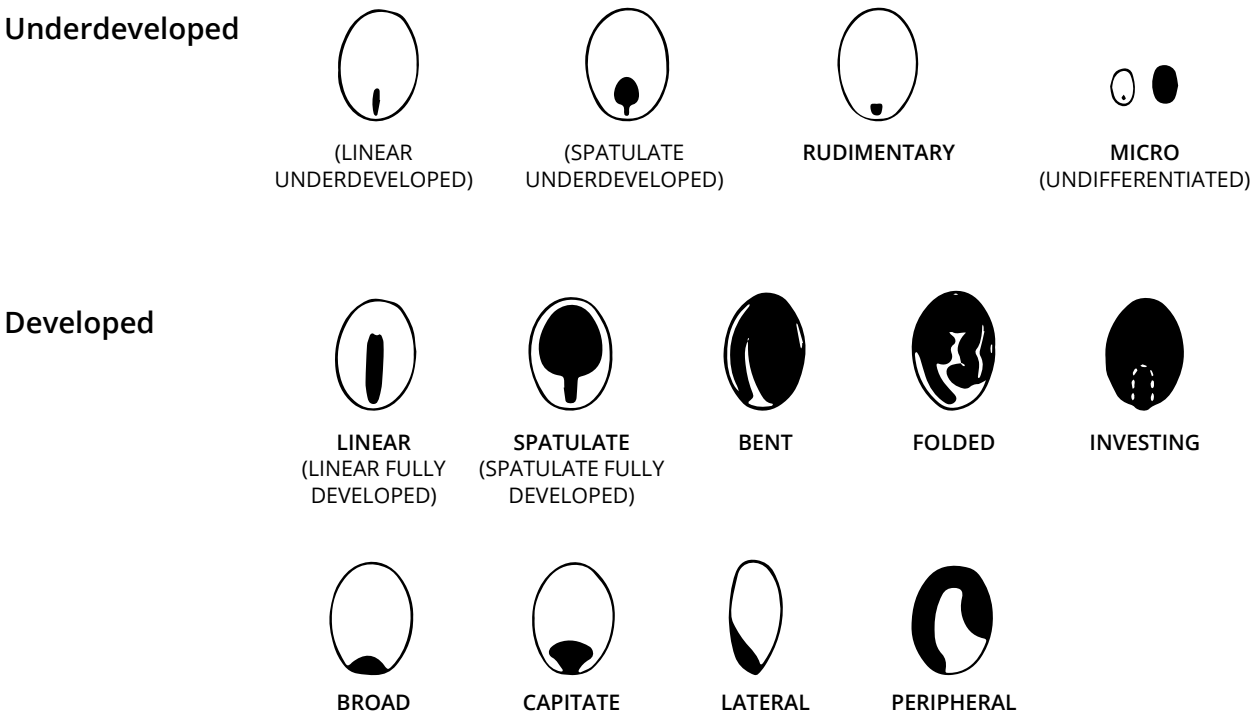


Figure 13. Embryo types (modified from Martin 1946 and Commander 2008). Martin's seed types are hand written and modifications to these seed types by Baskin and Baskin (2007) are typed in brackets. Embryo types are organised according to whether they are underdeveloped or developed. Note that the dwarf type defined by Martin (1946) has been removed by Baskin and Baskin (2007).

Box 1. Formerly used dormancy classes

In the previous edition of these guidelines, the following classes of dormancy were used:

- seed-coat dormancy, in which a physical, chemical or mechanical condition prevents uptake of moisture by the embryo (for example, *Acacia*), or the physical structure of the seed coat or fruit is too strong, preventing the swelling of the embryo (for example, *Owenia vernicosa*); and
- embryo dormancy, in which inhibiting substances, usually within the embryo or surrounding tissue, prevent germination. If removal of the seed coat does not result in germination, this is an indication that at least one dormancy mechanism may be located in the embryo itself (provided the germination conditions are met) (Adkins and Bellairs 1997).

One of the difficulties with classifying seeds into either seed-coat or embryo dormancy is that these classes don't necessarily inform the practitioner of what treatments may be used to overcome dormancy. In both classes, removal of the seed coat is recommended, but for different reasons. Also, 'inhibiting substances' are difficult to detect.

Over the last 20 years, much research into seed dormancy has been done both in Australia and internationally. Most seed scientists now use the classification system first developed by M. Nikolaeva, and modified by Baskin and Baskin (Baskin & Baskin, 2004). The benefits of this system are: (i) dormancy is relatively easily classified, with the use of an imbibition (water uptake) test, a germination test, and inspection of the internal morphology of the seed (i.e. cut the seed open, and take a look at the size and shape of the embryo); and (ii) that once dormancy is classified, pre-treatments known to be successful for that class of dormancy can be used.

Seeds formerly classified as having seed-coat dormancy, may either be those classified as physically dormant (PY) (e.g. *Acacia* spp.) or physiologically dormant (PD) (e.g. *Owenia vernicosa*) under the classification system developed by Baskin and Baskin. These require very different dormancy-breaking treatments - breaking the water-impermeable seed coat for PY species, or alleviating PD by stratification or after-ripening – highlighting the benefit of the Baskins' system. Seeds formerly classified as having embryo dormancy may either have physiological, morphological or morphophysiological dormancy. Removal of the seed coat may or may not enable germination of seeds with physiological dormancy, and is unlikely to enable germination of those with morphological or morphophysiological dormancy.

Treatments which alleviate dormancy

Clues for conditions that overcome dormancy can be sourced from the seed's natural environment. Determine the season of seed dispersal, and the season of seed germination, and examine the environmental conditions that occur in between. For instance, are seeds exposed to hot dry soil over summer, or brief periods of moist soil during summer showers? Do seeds sit in very cold and moist soil over winter before germinating in spring? What are the soil temperatures during germination? Is the region prone to fires or floods? The temperature and moisture conditions in the soil seed bank should lead to protocols for dormancy breaking treatments and incubation conditions. When in doubt, there is an option to place seed in trays of soil and leave at ambient conditions (in the local environment from which seeds are sourced), and observe the germination phenology, i.e. when seeds germinate. Alternatively, place seeds in permeable mesh bags and bury in soil in their local habitat, retrieving periodically to check for in situ germination, and/or incubate ex situ to assess germination (e.g. see Commander et al. 2019, Hoyle et al. 2008b, Martyn Yenson et al. 2021).

Each species may require a slightly different treatment, so consult reports and papers to find out what others have tried. Ensure that treatments are trialled on a small number of seeds first, before treating the whole seed lot. Many treatments potentially risk damaging the seeds, or rendering them non-viable. Thus, it is important to perform a germination test on treated and untreated seeds of each species, to ensure that the treatment is both effective and doesn't negatively impact the seeds.

Physical dormancy – a water impermeable seed or fruit coat

Seeds with physical dormancy are known in 18 families globally, of which 12 are found in Australia, and include Fabaceae, Malvaceae and Sapindaceae. They are characterised by having a thin, water-impermeable layer in their seed or fruit coat. This layer prevents the imbibition of water into the embryo. The seeds (or fruits) have a specialised structure which functions as a **water gap** that is disrupted or dislodged to allow water entry. The water gap has a different structure in different families – in Fabaceae it is a lens, while Malvaceae has a chalazal plug (Baskin et al. 2000). Water can also enter through cracks in the seed coat (Baskin & Baskin 2014). This class of dormancy has been previously referred to as 'hard seededness' (see Box 1), however the term is misleading since many seeds have 'hard' but permeable seed/fruit coats, e.g. *Santalum* (sandalwood) and *Persoonia* (geebung or snottygobble).

To check to see whether or not seeds imbibe water, do an imbibition test. The test is relatively simple to do, provided you have an accurate balance. In the absence of a balance, it may be possible to visually inspect the seeds to determine if they have increased in size due to water uptake. The steps are as follows (see also Turner et al. 2005, Martyn Yenson et al. 2021):

1. Take three or four (or more) subsamples of your seed lot, with a roughly similar weight.
2. Weigh the subsamples and record the weights.
3. Place the seeds on a moist medium, e.g. moist filter paper within a Petri dish. It may be easier to put a small piece of fine mesh on top of the filter paper so that you can lift the seeds in and out of the Petri dish. Label the dishes with the subsample number.

4. Weigh the seeds again after 24 or 48 hours, or at regular intervals, to determine if the weight of the seeds has increased. Remember to blot the seeds dry before weighing, so that the weight of water adhering to the seeds' surface isn't being recorded. Sometimes, seeds can be visually inspected, and those seeds which have imbibed appear to have swelled.

To overcome physical dormancy, the water gap or water impermeable layer can be disrupted using physical scarification, hot water, or acid. The choice of treatment may depend on which treatment is most effective, the number of seeds requiring treatment and the end use of the seeds. It is also useful to perform an imbibition test on both treated and untreated seed to determine if the treatment is effective, and then a germination test on treated and untreated seeds to ensure that the treatment has not adversely affected germination. If optimal treatments are not known, perhaps start with physical scarification, as hot water and acid have potential detrimental effects.

Seeds can be scarified using sandpaper, by making a small cut through the seed coat with a scalpel (also known as nicking or chipping), using a mechanical scarifier (often a rotating drum lined with sandpaper) (Figure 14), or using a vice, hammer or nutcracker to crack the coat. The type of scarification chosen may reflect the scale and also end use of seeds, e.g. for small seed lots, nicking with a scalpel may be selected, but for large seed lots, mechanical scarification may be more time efficient. If cutting the seed coat with a scalpel, firstly dissect a seed to observe the internal morphology, to determine the location of the radicle, then when cutting the seeds, ensure the cut is away from the radicle tip and be careful not to damage other parts of the embryo.

Heat treatments may mimic conditions experienced by seeds in the soil seedbank during fire. A hot water treatment involves seeds being dipped in near-boiling water for a brief period of time. This treatment is relatively inexpensive and easy to employ, and just requires a kettle/urn/hot water bath, cloth or other heat resistant permeable bags, buckets or other containers, and absorbent paper. A hot water bath can be used to ensure that the temperature stays constant, rather than using boiling water from a kettle, which may be too hot when just boiled, and may cool down during the treatment time (Figure 15).

Hot water treatment method:

1. Boil enough water to allow the seeds to be submerged and so the water temperature doesn't drop significantly when the room temperature seeds are added. Allow the water to cool to 90-95°C.
2. Place a quantity of seed to be treated in a porous cloth bag or fine mesh container. Ensure that the seeds can move freely in the bag, and are not packed in tightly, otherwise the water may not reach the seeds in the centre of the bag.
3. Before treating with hot water, prepare somewhere close by to place the seeds when they are taken out of the water.
4. Immerse seed in hot water for time appropriate for the species. If the appropriate time is not known, perform an imbibition and/or germination test on a subsample as described above.
5. When taking the seeds out of the hot water, use appropriate equipment such as metal tongs, and wear heat proof gloves such as oven mitts. If seeds are not contained within a bag, pour them into a sieve over another container.

6. Drain seed and dry on paper towel or other absorbent material, turning often until dry. Seeds may dry faster if they are placed in a single layer. Do not use heat to dry seed as this may damage the seed.

There is a large difference in the response to hot water between physically dormant species, some species may require 30 seconds, others several minutes. Be aware that if the hot water treatment is too long, it may kill the seeds. Also, species differ in their tolerance of high temperatures, and seeds of some species can be damaged by water temperatures of 90-95°C, so lower temperatures should be used. If the treatment is being undertaken for the first time, perform imbibition and germination tests following a variety of treatment times, and possibly also water temperatures, using a small subsample of the seed. This way, the imbibition test will determine which treatment times allow water uptake, and the germination test will ensure that seeds that have imbibed are still viable and can germinate. Ensure that the results of the test are recorded for future reference.

Treating seeds with acid, such as sulphuric acid (H_2SO_4) is also possible (Turner et al. 2009). For instance, *Adansonia gregorii* (boab) has <25% germination when treated with hot water for 1-5 minutes, but 100% germination when treated with acid for 24 hours (Turner et al. 2009). However, it is strongly advised to be a last resort method as it presents numerous risks to the operator and the environment. Read the Material Safety Data Sheet, undertake a risk analysis, develop a safe procedure, use the appropriate personal protective equipment, and dispose of any waste appropriately.

Figure 14. A seed scarifier. (Photo: L. Commander)

Figure 15. A hot water bath used for treating seeds with physical dormancy. (Photo: L. Commander)

Physiological dormancy – a physiologically inhibiting mechanism in the embryo preventing radicle emergence

Seeds with physiological dormancy are unable to germinate due to an inhibiting mechanism in the embryo, which prevents radicle emergence through the covering structures. Unlike seeds with physical dormancy, they can imbibe water and unlike seeds with morphological dormancy, they have fully developed embryos.

Physiological dormancy is often observed in these and many other families:

- Amaranthaceae (Commander et al. 2009b).
- Asteraceae (Hoyle et al. 2008d).
- Goodeniaceae (Hoyle et al. 2008d).
- Gyrostemonaceae (Baker et al. 2005).
- Lamiaceae (Ainsley & Jones 2010).
- Myrtaceae (Commander et al. 2009b; 2017).
- Poaceae (Commander et al. 2017).
- Proteaceae (Baker et al. 2005).
- Rutaceae (Collette and Ooi 2017)
- Solanaceae (Commander et al. 2008).

It is the most common class of dormancy globally (Baskin and Baskin, 2020) including in Australia (Commander et al. in prep), however, in certain ecosystems, other types of dormancy may be more prevalent (Collette and Ooi 2021).

Various treatments to enable germination include:

- after-ripening (warm, dry storage) (Commander et al. 2009a)
- stratification (warm or cold, wet storage, moist storage) (Sommerville et al. 2013; Turner et al. 2006)
- wet/dry cycles (cycling between after-ripening and stratification) (Hoyle et al. 2008a; Chia et al. 2016)
- move-along (similar to stratification, in which seeds placed on a moist substrate and are moved between temperatures corresponding to the seasons which they would experience in the soil seed bank) (Baskin and Baskin 2003; Martyn Yenson et al. 2021)
- 'heat shock', (high temperatures of 50-120°C for a short period of time, e.g. 5 minutes to 3 hours) (Thomas et al. 2003; Tieu et al. 2001)
- Gibberellic Acid (GA_3) (Bell et al. 1995)
- precision nicking (removing the covering structures surrounding the radicle tip, or weakening the seed coat in a specific location, so the embryo does not need to overcome the restraint of the covering structures) (Commander et al. 2008; Liyanage et al. 2020)

To after-ripen seeds, dry them to reduce the seed moisture content (for instance, in a drying room), then store at ambient or warm temperatures, e.g. 20-30°C (Commander et al. 2009; Merritt et al. 2007). If optimum after-ripening conditions are not known, a starting point may

be to store seeds at 50% relative humidity and room temperature, with regular testing used to determine if germination is increasing, and when it is maximised (Baskin and Baskin 2020). As well as increasing the germination percentage, after-ripening can also increase germination rate, and widen the conditions over which seeds can germinate, that is, they may germinate at increased water stress (i.e. lower water potential) and/or warmer temperatures (Baskin and Baskin 2020). Dormancy loss during after-ripening is likely to occur faster at warmer temperatures, but if the combination of seed moisture content and temperature is too high, then viability loss can occur (Figure 16) (Commander et al. 2009).

The time taken dormancy to be alleviated can range from six weeks to 2 years (Merritt et al. 2007). However, ensure that seeds are not stored in after-ripening conditions for too long, as seed viability may decline. Therefore, germination and viability testing during the period of storage is important to monitor both dormancy and viability loss (Baskin and Baskin 2020). Once dormancy is alleviated, seeds may be stored prior to use at low temperatures, to prevent viability loss, but the storage of after-ripened seeds requires further research (Baskin and Baskin 2020).

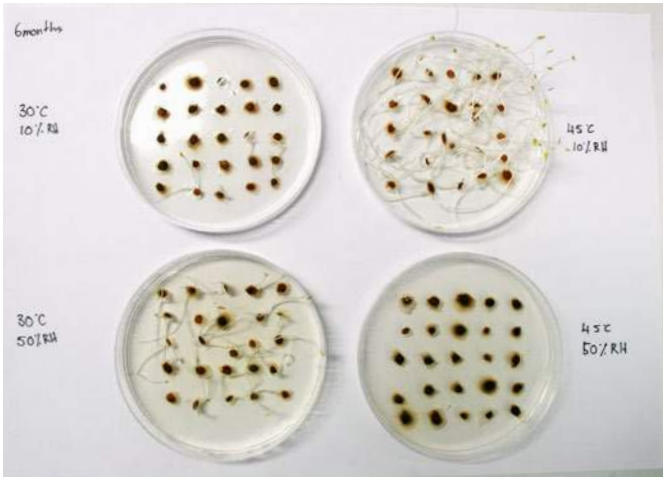


Figure 16. Various combinations of temperature and relative humidity can either alleviate dormancy or lead to viability loss (Commander et al. 2009). (Photo L. Commander)

For stratification, seeds are placed in moist conditions (e.g. water agar or irrigated sand) at cold temperatures (2-10°C for example, in a refrigerator) or warm temperatures (25-35°C) for between a few weeks to several months prior to germination (e.g. see Merritt et al. 2007). Temperature and time of stratification depends on the species, and treatments may be developed based on conditions in their natural environment. Once the stratification period is complete, the seeds are then incubated under conditions appropriate for germination. Stratification increases germination of species such as *Acanthocarpus* preissii (Asparagaceae) (Turner et al. 2006), *Dianella revoluta* (Hemerocallidaceae) (Hodges et al. 2019), *Goodenia fascicularis* (Goodeniaceae) (Hoyle et al. 2008c) and *Viola betonicifolia* (Violaceae) (Sommerville et al. 2013).

Wet/dry cycling involves moving seeds from stratification (wet) conditions to after-ripening (dry) conditions. Wet/dry cycling increases germination of seeds of *Actinobole uliginosum* (Asteraceae), *Goodenia cycloptera* and *Velleia glabrata* (Goodeniaceae) (Hoyle 2008a).

If after-ripening, stratification or wet dry cycles increases germination, the optimum temperature, moisture and time may need to be experimentally determined for each species or seed lot.

In some cases, following dormancy alleviation, a specific set of germination conditions may also be required, such as after-ripening followed by smoke treatment (Turner et al. 2009) or warm stratification followed by fire cues (e.g. *Dianella revoluta*; Hodges et al. 2019). As such, sequential application of multiple treatments may be required.

Manually nicking the seed coat at the distal end (i.e. cotyledon end, so as not to damage the radicle), or side of the seed, with a scalpel, razor blade or nail clippers can also enable germination (Figure 17). Precision nicking involves very carefully removing the part of the seed coat and possibly also the **endosperm** which covers the radicle to remove the mechanical restriction to the embryo growth potential (Figure 18). Nicking increases germination of *Solanum* spp. (Commander et al. 2008), *Grevillea* spp. and *Drosophyllum lusitanicum* (Cross et al. 2017). Removing seeds from an indehiscent fruit (e.g. a woody endocarp) using a scalpel, cracking with a vice or drilling with a small rotary tool (e.g. Dremel) such as for *Persoonia* (Chia et al. 2016) may be effective. These treatments may not be practical on a large scale, but can be useful for small seed lots.

Figure 17. Equipment such as scalpels, razor blades and microscopes are useful to perform precision nicking. (Photos: L. Commander)

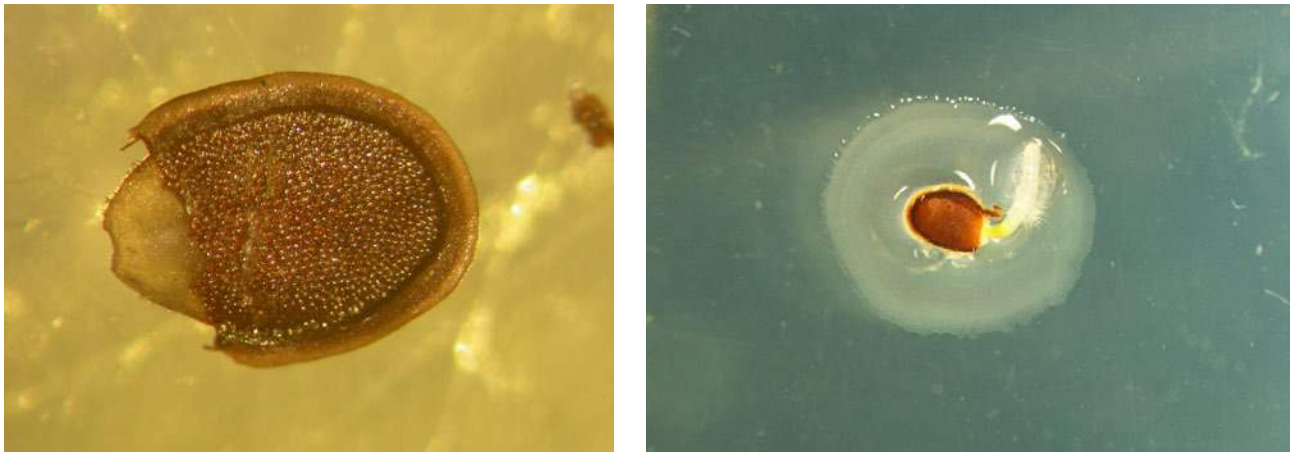


Figure 18. Precision nicking of *Goodenia* sp. (left) and germination of nicked *Goodenia* seed (right). In some cases, the radicle (root) will protrude out of the seed due to water uptake, but the seed will not actually germinate. In the photo on the right, the seed is considered germinated because not only has the radicle protruded, it has elongated and produced root hairs. (Photos: L. Commander)

Combinational dormancy – a combination of physical and physiological dormancy

For seeds with combinational dormancy, firstly physical dormancy must be overcome to allow water into the seed, then physiological dormancy must be alleviated, before seeds can germinate. *Diplopeltis huegelii* (Sapindaceae) has been described to exhibit combinational dormancy (Turner et al. 2006).

Morphological dormancy – an underdeveloped or undifferentiated embryo

Seeds with morphological dormancy have embryos that are undifferentiated – that is, the cells are not differentiated into a radicle and cotyledons, or underdeveloped – the embryo is small in relation to the seed (Baskin and Baskin 2014). The embryos grow within these seeds prior to germination. Seeds will in fact germinate within around 4 weeks if provided with appropriate moisture and temperature conditions. One genus with morphological dormancy is *Burchardia* (Colchicaceae), (Kildisheva et al. 2020).

Morphophysiological dormancy – a combination of morphological and physiological dormancy

Some seeds with morphological dormancy also exhibit physiological dormancy, termed morphophysiological dormancy. In this case, embryos need to grow inside the seed, and physiological dormancy needs to be overcome. These processes may require warm or cold stratification (or both), wet/dry cycles or dry after-ripening. Species/genera with morphophysiological dormancy include *Hibbertia* (Dilleniaceae) (Hidayati et al. 2012), *Aciphylla* (Apiaceae) (Hoyle et al. 2014; Sommerville et al. 2013), *Anigozanthos* (Haemodoraceae) (Downes et al. 2014) and *Leucopogon* (Ericaceae) (Ooi et al. 2006).

Final note

Seeds and seed dormancy types are diverse as are the applications of seed germination testing. Applying a systematic approach to seed germination testing as presented in this chapter will support ecological restoration outcomes and contribute to our understanding of seed ecology, biology and variability.

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Glossary

Agar: a jelly-like substance obtained from algae. Agar is sold as a powder, and when made up with water, is termed water-agar.

Diaspore: the unit of seed dispersal in plants (may be a fruit, mature floret, mericarp, seedling or seed).

Dormancy: a dormant seed (or other germination unit) is one that does not have the capacity to germinate in a specified period of time under any combination of normal physical environmental factors (temperature, light/dark, etc.) that otherwise are favourable for germination, i.e. after the seed becomes non-dormant.

Dormancy class: the classification system developed by Baskin and Baskin (2004) categorises seeds into five classes based on their morphological and physiological properties.

Embryo: develops from the egg cell in the embryo sac after fertilisation, generally consisting of a hypocotyl-root-axis, the radicle, one or two cotyledons and the plumule (shoot apex).

Endosperm: nutritive tissue in developing and mature seeds.

Filled seed: seed that contains an **embryo** (and **endosperm** if applicable).

Germination: begins with water uptake by the seed and ends with the start of elongation by the embryonic axis, usually the radicle. Germination is considered complete when the radicle protrudes through its covering structures.

Germination percentage: the percentage of seeds in the test that germinate.

Germination rate (germination speed): the speed at which germination occurs, and can be described in numerous ways, such as time to 50% germination (days), or mean time to germination.

Laminar flow cabinet: a semi-enclosed cabinet designed to prevent contamination. Air is drawn through a filter and expelled towards the user in a laminar flow, that is, a smooth path, to prevent contaminants from being blown into the cabinet.

Non-viable seed: a seed which is dead, and therefore will not germinate even under optimal conditions or following treatments for alleviating dormancy.

Population: a group of individuals that belong to the same species occupying a particular area.

Seed lot / seed batch: an identifiable consignment of seeds of known weight and source of the same collection of a single species and/or variety, harvested together from a field or stored together in the same storage facility.

Tubestock: plant grown in a pot from seed, or from vegetative propagation. Commonly used term for plants grown in forestry tubes or small containers.

Unfilled seed: seed that is empty and does not contain an **embryo** (and **endosperm**, if applicable).

Viable seed: living seed that can potentially germinate if sown under suitable conditions. Viable seeds include dormant seeds, in which case the dormancy must be alleviated before germination. Thus, a non-viable seed is dead, and therefore will not germinate even under optimal conditions, including following treatments for breaking dormancy.

VAG: Viability Adjusted Germination

Water gap: anatomical structure in the impermeable layer of a seed/fruit coat of a seed with physical dormancy.

Online resources

ANPSA, list of seed suppliers
http://www.anpsa.org.au/seedsupp.html)

Botanic Gardens Australia and New Zealand (BGANZ)
https://www.bganz.org.au/

Bureau of Meterology
http://www.bom.gov.au/

Friends of Kings Park
https://www.bgpa.wa.gov.au/contact-us/16-kings-park-volunteer-master-gardeners

ISTA hand halving
https://www.youtube.com/watch?v=OMevCxMp9FM

ISTA spoon method
https://www.youtube.com/watch?v=90kkVhdBUko

Revegetation Industry Association of Western Australia
https://www.riawa.com.au/

Royal Botanic Gardens, Kew,Seed Information Database (SID)
https://data.kew.org/sid/

Royal Tasmanian Botanic Gardens, germination database
https://gardens.rtbg.tas.gov.au/conservation/tsccgerminationdatabase/

Seeds of South Australia
https://spapps.environment.sa.gov.au/seedsofsa/

Smoke responsive families and genera; instructions for making aerosol smoke and smoke water
Botanic Gardens and Parks Authority - Smoke to sow and grow (bgpa.wa.gov.au)

The Australian Seed Bank
https://asbp.ala.org.au/search#tab_simpleSearch

The Australian Seed Bank Partnership
https://www.seedpartnership.org.au/

Worldclim
https://worldclim.org/

References and further reading

Adkins SW and Bellairs SM (1997) 'Seed dormancy mechanisms in Australian native species', in Bellairs SM and Osborne JM (eds), Proceedings of the Second Australian Workshop on Biology for Revegetation, Newcastle, New South Wales, 11–12 October 1996, Australian Centre for Minesite Environmental Research, Brisbane.

Ainsley P, Jones M (2010) Overcoming physiological seed dormancy in semi-arid *Prostanthera* (Labiatae). *Journal of the Adelaide Botanic Garden* **24,** 1-5.

Baker KS, Steadman KJ, Plummer JA, Dixon KW (2005) Seed dormancy and germination responses of nine Australian fire ephemerals. *Plant and Soil* **277(1/2)**, 345-358.

Baskin CC, Baskin JM (2003) Overview and recommendations for future research priorities on native seed dormancy and germination of Australian plants. *Australasian Plant Conservation* **11**, 2-9.

Baskin CC, Baskin JM (2004) Determining dormancy-breaking and germination requirements from the fewest seeds. In 'Ex situ plant conservation - supporting species survival in the wild. (Eds EO Guerrant Jr, K Havens and M Maunder) pp. 162-179. (Island Press: Washington).

Baskin CC, Baskin JM (2014) 'Seeds. Ecology, Biogeography, and Evolution of Dormancy and Germination.' (Academic Press: San Diego, California, USA).

Baskin CC, Baskin JM (2020) Breaking seed dormancy during dry storage: A useful tool or major problem for successful restoration via direct seeding? *Plants* **9(5)**, 636.

Baskin CC, Thompson K, Baskin JM (2006) Mistakes in germination ecology and how to avoid them. *Seed Science Research* **16,** 165-168.

Baskin JM, Baskin CC (2004) A classification system for seed dormancy. *Seed Science Research* **14(1),** 1-16.

Beardsell D, Mullett J (1984) Seed germination of *Eucalyptus pauciflora* Sieb. ex. Spreng. from low and high altitude populations in Victoria. *Australian Journal of Botany* **32**, 475-480.

Bell DT (1999) The process of germination in Australian species. *Australian Journal of Botany* **47(4)**, 475-517.

Bell DT, Rokich DP, McChesney CJ, Plummer JA (1995) Effects of temperature, light and gibberellic acid on the germination of seeds of 43 species native to Western Australia. *Journal of Vegetation Science* **6(6)**, 797-806.

Bewley JD, Black M (1994) 'Seeds: physiology of development and germination.' (Plenum Press: New York).

Bonney N (1994) What Seed Is That? A Field Guide to the Identification, Collection and Germination of Native Seed in South Australia.

Carvalho FJ, Garcia de Santana D, Borges de Araújo L (2018) Why analyze germination experiments using Generalized Linear Models? *Journal of Seed Science*. **40**, 281-287.

Chapman M, Nelson P, Nicholson H, Dunphy M, McAlpin S (2020) 'Australian Rainforest Seeds: A Guide to Collecting, Processing and Propagation.' (CSIRO Publishing).

Chia KA, Sadler R, Turner SR, Baskin CC (2016) Identification of the seasonal conditions required for dormancy break of *Persoonia longifolia* (Proteaceae), a species with a woody indehiscent endocarp. *Annals of Botany* **118(2)**, 331-346.

Clarke S, French K (2005) Germination response to heat and smoke of 22 Poaceae species from grassy woodlands. *Australian Journal of Botany* **53(5),** 445-454.

Close DC, Wilson SJ (2002) Provenance effects on pre-germination treatments for *Eucalyptus regnans* and *E. delegatensis* seed. *Forest Ecology and Management* **170**, 299-305.

Collette JC, Ooi MKJ (2017) Germination ecology of the endangered species *Asterolasia buxifolia* (Rutaceae): smoke response depends on season and light. *Australian Journal of Botany* **65(3),** 283-291.

Collette JC, Ooi MKJ (2021) Investigation of 18 physiologically dormant Australian native species: germination response, environmental correlations and the implications for conservation. *Seed Science Research* **31(1)**, 30-38.

Collette JC, Ooi MK (2021) Distribution of seed dormancy classes across a fire-prone continent: effects of rainfall seasonality and temperature. *Annals of Botany* **127(5)**, 613-620.

Commander LE (2008) Seed biology and rehabilitation in the arid zone: a study in the Shark Bay World Heritage Area, Western Australia. PhD Thesis, The University of Western Australia, Perth, Western Australia.

Commander LE, Golos PJ, Miller BP, Merritt DJ (2017) Seed germination traits of desert perennials *Plant Ecology* **218**, 1077–1091.

Commander LE, Merritt DJ, Rokich DP, Dixon KW (2009a) The role of after-ripening in promoting germination of arid zone seeds: a study on six Australian species. *Botanical Journal of the Linnean Society* **161(4)**, 411-421.

Commander LE, Merritt DJ, Rokich DP, Dixon KW (2009b) Seed biology of Australian arid zone species: Germination of 18 species used for rehabilitation. *Journal of Arid Environments* **73(6-7),** 617-625.

Commander LE, Merino-Martín L, Elliott CP, Miller BP, Dixon K, Stevens J (2020) Demographic, seed and microsite limitations to seedling recruitment in semi-arid mine site restoration. *Plant and Soil* **457(1-2),** 113-129.

Commander LE, Merritt DJ, Rokich DP, Flematti GR, Dixon KW (2008) Seed germination of *Solanum* spp. (Solanaceae) for use in rehabilitation and commercial industries. *Australian Journal of Botany* **56(4),** 333-341.

Commander LE, Merritt DJ, Turner SR, Ooi MKJ, Guja LK, Sommerville KD, Offord CA (in prep) The ecology, dormancy and germination of Australian native seeds.

Cross AT, Paniw M, Ojeda F, Turner SR, Dixon KW, Merritt DJ (2017) Defining the role of fire in alleviating seed dormancy in a rare Mediterranean endemic subshrub. *AoB PLANTS* **9(5)**.

Davies R, Di Sacco A, Newton R (2015) Germination testing: procedures and evaluation. Technical Information Sheet 13a. Royal Botanic Gardens, Kew.

Downes KS, Light ME, Pošta M, Kohout L, van Staden J (2014) Do fire-related cues, including smoke-water, karrikinolide, glyceronitrile and nitrate, stimulate the germination of 17 *Anigozanthos* taxa and *Blancoa canescens* (Haemodoraceae)? *Australian Journal of Botany* **62(4),** 347-358.

Erickson TE, Barrett RL, Merritt DJ, Dixon KW (Eds) (2016) 'Pilbara Seed Atlas and Field Guide: Plant Restoration in Australia's Arid Northwest.' (CSIRO Publishing: VIC).

Flematti GR, Ghisalberti EL, Dixon KW, Trengove RD (2004) A compound from smoke that promotes seed germination. Science **305(5686),** 977.

Flematti GR, Merritt DJ, Piggott MJ, Trengove RD, Smith SM, Dixon KW, Ghisalberti EL (2011) Burning vegetation produces cyanohydrins that liberate cyanide and stimulate seed germination. *Nature Communications* **2(1),** 360.

Flematti GR, Scaffidi A, Goddard-Borger ED, Heath CH, Nelson DC, Commander LE, Stick RV, Dixon KW, Smith SM, Ghisalberti EL (2010) Structure - Activity Relationship of Karrikin Germination Stimulants. *Journal of Agricultural and Food Chemistry* **58(15),** 8612-8617.

Gianinetti A (2020) Basic features of the analysis of germination data with generalized linear mixed models. *Data* **5,** 6.

Guja LK, Merritt DJ, Dixon KW (2010) Buoyancy, salt tolerance and germination of coastal seeds: implications for oceanic hydrochorous dispersal. *Functional Plant Biology* **37(12),** 1175-1186.

Harrison PA, Bailey TG, Vaillancourt RE, Potts BM (2014) Provenance and seed mass determines the seed germination success of *Eucalyptus ovata* (Myrtaceae). *Seed Science and Technology* **42(3),** 466-472. doi:DOI 10.15258/sst.2014.42.3.14

Hay FR, Mead A, Bloomberg M (2014) Modelling seed germination in response to continuous variables: Use and limitations of probit analysis and alternative approaches. *Seed Science Research,* **24,** 165–186.

Hidayati SN, Walck JL, Merritt DJ, Turner SR, Turner DW, Dixon KW (2012) Sympatric species of *Hibbertia* (Dilleniaceae) vary in dormancy break and germination requirements: implications for classifying morphophysiological dormancy in Mediterranean biomes. *Annals of Botany* **109(6),** 1111-1123.

Hodges J, Price J, Nimmo D, Guja LK (2019) Evidence for direct effects of fire-cues on germination of some perennial forbs common in grassy ecosystems. *Austral Ecology* **44(7)**, 1271-1284. https://doi.org/10.1111/aec.12806

Hoyle GL, Cordiner H, Good RB, Nicotra AB (2014) Effects of reduced winter duration on seed dormancy and germination in six populations of the alpine herb *Aciphyllya glacialis* (Apiaceae), *Conservation Physiology*, **2(1),** cou015, https://doi.org/10.1093/conphys/cou015

Hoyle GL, Daws MI, Steadman KJ and Adkins SW (2008a). Mimicking a semi-arid tropical environment achieves dormancy alleviation for seeds of Australian native Goodeniaceae and Asteraceae. *Annals of Botany* **101,** 701-708.

Hoyle GL, Daws MI, Steadman KJ and Adkins SW (2008b). Pre- and post-harvest influences on seed dormancy status of an Australian Asteraceae species: *Actinobole uliginosum* (A.Gray) H.Eichler. *Seed Science Research* **18**, 191-199.

Hoyle GL, Steadman KJ, Daws MI and Adkins SW (2008c). Pre- and post-harvest influences on seed dormancy status of an Australian Goodeniaceae species: *Goodenia fascicularis* F. Muell. & Tate. *Annals of Botany* **102,** 93-101.

Hoyle GL, Steadman KJ, Daws MI, Adkins SW (2008d) Physiological dormancy in forbs native to south-west Queensland: Diagnosis and classification. *South African Journal of Botany* **74(2)**, 208-213.

ISTA (2020) 'International Rules for Seed Testing 2020.' (The International Seed Testing Association (ISTA): Bassersdorf, Switzerland).

Kildisheva OA, Dixon KW, Silveira FAO, Chapman T, Di Sacco A, Mondoni A, Turner SR, Cross AT (2020) Dormancy and germination: making every seed count in restoration. *Restoration Ecology* **28(S3),** S256-S265.

Langkamp PJ (Ed) (1987) 'Germination of Australian Native Plant Seed'. Inkata Press Pty Ltd, Melbourne.

Liu K, Baskin JM, Baskin CC, Bu H, Du G, Ma M (2013) Effect of diurnal fluctuating versus constant temperatures on germination of 445 species from the eastern Tibet Plateau**.** *PLOS ONE* **8(7)** e69364.

Liyanage GS, Offord CA, Sommerville KD (2020) Techniques for breaking seed dormancy of rainforest species from genus *Acronychia. Seed Science and Technology* **48(2),** 159-165.

Luna B, Pérez B, Fernández-González F, Moreno J (2004) Sensitivity to green safelight of 12 Mediterranean species. *Seed Science and Technology* **32(1)**, 113-117.

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

Mackenzie BDE, Auld TD, Keith DA, Hui FKC, Ooi MKJ (2016) The effect of seasonal ambient temperatures on fire-stimulated germination of species with physiological dormancy: A case study using *Boronia* (Rutaceae). *PLoS One* **11(5)**, e0156142

Martyn Yenson AJ, Offord CA, Meagher PF, Auld T, Bush D, Coates DJ, Commander LE, Guja L, Norton S, Makinson RO, Stanley R, Walsh N, Wrigley D, Broadhurst L (Eds) (2021) 'Plant Germplasm Conservation in Australia: strategies and guidelines for developing, managing and utilising ex situ collections (3rd edn).' (Australian Network for Plant Conservation Inc: Canberra).

Martyn AJ, Seed LU, Ooi MKJ, Offord CA (2009) Seed fill, viability and germination of NSW species in the family Rutaceae. *Cunninghamia* **11**, 203-212.

McNair J, Sunkara A, Frobish D (2012) How to analyse seed germination data using statistical time-to-event analysis: Non-parametric and semi-parametric methods. *Seed Science Research* **22(2),** 77-95. 10.1017/S0960258511000547.

Merritt DJ, Whitehouse KJ, Hoyle GL, Crawford A, Wood JA, Satyanti A, Norton S, Errington G, Martyn Yenson AJ (2021) Seed Banking: Orthodox Seeds. In 'Plant Germplasm Conservation in Australia: strategies and guidelines for developing, managing and utilising ex situ collections (3rd edn.).' (Eds AJ Martyn Yenson et al.) (Australian Network for Plant Conservation Inc: Canberra).

Merritt DJ, Kristiansen M, Flematti GR, Turner SR, Ghisalberti EL, Trengove RD, Dixon KW (2006) Effects of a butenolide present in smoke on light-mediated germination of Australian Asteraceae. *Seed Science Research* **16(01),** 29-35.

Merritt DJ, Turner SR, Clarke S, Dixon KW (2007) Seed dormancy and germination stimulation syndromes for Australian temperate species. *Australian Journal of Botany* **55(3)**, 336-344.

MSB (2014) Millennium Seed Bank Partnership Technical Information Sheets, Board of Trustees of the Royal Botanic Gardens, Kew, UK. http://brahmsonline.kew.org/msbp/Training/Resources

Norman MA, Plummer JA, Koch JM, Mullins GR (2006) Optimising smoke treatments for jarrah (*Eucalyptus marginata*) forest rehabilitation. *Australian Journal of Botany* **54(6),** 571-581.

Offord CA, Meagher PF (Eds) (2009) 'Plant Germplasm Conservation in Australia: strategies and guidelines for developing, managing and utilising ex situ collections (2nd edn.).' (Australian Network for Plant Conservation Inc: Canberra).

Ooi MKJ, Auld TD, Whelan RJ (2006) Dormancy and the fire-centric focus: Germination of three *Leucopogon* Species (Ericaceae) from South-eastern Australia. *Annals of Botany* **98(2),** 421-430.

Ooi MKJ, Denham AJ, Santana VM, Auld TD (2014) Temperature thresholds of physically dormant seeds and plant functional response to fire: variation among species and relative impact of climate change. *Ecology and Evolution* **4(5)**, 656-671.

Plummer JA, Rogers AD, Turner DW, Bell DT (2001) Light, nitrogenous compounds, smoke and GA3 break dormancy and enhance germination in the Australian everlasting daisy, *Schoenia filifolia* subsp. *subulifolia*. *Seed Science and Technology* **29(2),** 321-330.

Ralph M (2003) 'Growing Australian native plants from seed for revegetation, tree planting, and direct seeding (2 edn).' (Bushland Horticulture).

Ralph M, Moorehead G, Ressom J (1993) 'Seed Collection of Australian Native Plants: For Revegetation, Tree Planting and Direct Seeding.' (Murray Ralph).

Rix KD, Gracie AJ, Potts BM, Brown, PH, Gore PL (2015) Genetic control of *Eucalyptus globulus* seed germination. *Annals of Forest Science*, **72(4),** 457-467. doi:10.1007/s13595-014-0450-9

Rix KD, Gracie AJ, Potts BM, Brown PH, Spurr CJ, Gore PL (2012) Paternal and maternal effects on the response of seed germination to high temperatures in *Eucalyptus globulus*. *Annals of Forest Science* **69(6),** 673-679. doi:10.1007/s13595-012-0190-7

Satyanti A, Guja LK, Nicotra AB (2019) Temperature variability drives within-species variation in germination strategy and establishment characteristics of an alpine herb. *Oecologia* **189(2),** 407-419.

Satyanti A, Nicotra AB, Merkling T, Guja LK (2018) Seed mass and elevation explain variation in seed longevity of Australian alpine species. *Seed Science Research* **28(4),** 319-331.

Sommerville KD, Martyn AJ, Offord CA (2013) Can seed characteristics or species distribution be used to predict the stratification requirements of herbs in the Australian Alps? *Botanical Journal of the Linnean Society* **172(2),** 187-204.

Stevens AV, Nicotra AB, Godfree RC, Guja LK (2020) Polyploidy affects the seed, dormancy and seedling characteristics of a perennial grass, conferring an advantage in stressful climates. *Plant Biology* **22(3)**, 500-513.

Stevens J, Rokich D, Newton V, Barrett R, Dixon K (2016) 'Banksia woodlands: a restoration guide for the Swan Coastal Plain.' (UWA Publishing: Perth, Australia).

Sweedman L, Merritt DJ (2006) 'Australian seeds - a guide to their collection, identification and biology.' (CSIRO Publishing: Collingwood).

Thomas PB, Morris EC, Auld TD (2003) Interactive effects of heat shock and smoke on germination of nine species forming soil seed banks within the Sydney region. *Austral Ecology* **28(6),** 674-683.

Tieu A, Dixon KW, Meney KA, Sivasithamparam K (2001) The interaction of heat and smoke in the release of seed dormancy in seven species from southwestern Western Australia. *Annals of Botany* **88(2),** 259-265.

Tieu A, Dixon KA, Sivasithamparam K, Plummer JA, Sieler IM (1999) Germination of four species of native Western Australian plants using plant-derived smoke. *Australian Journal of Botany* **47(2),** 207-219.

Turner SR, Merritt DJ, Baskin CC, Dixon KW, Baskin JM (2005) Physical dormancy in seeds of six genera of Australian Rhamnaceae. *Seed Science Research* **15(1),** 51-58.

Turner SR, Merritt DJ, Baskin JM, Baskin CC, Dixon KW (2006) Combinational dormancy in seeds of the Western Australian endemic species *Diplopeltis huegelii* (Sapindaceae). *Australian Journal of Botany* **54(6)**, 565-570.

Turner SR, Merritt DJ, Renton MS, Dixon KW (2009) Seed moisture content affects afterripening and smoke responsiveness in three sympatric Australian native species from fire-prone environments. *Austral Ecology* **34(8),** 866-877.

Vleeshouwers LM, Bouwmeester HJ, Karssen CM (1995) Redefining seed dormancy: An attempt to integrate physiology and ecology. *Journal of Ecology* **83(6),** 1031-1037.

Willis CG, Baskin CC, Baskin JM, Auld JR, Venable DL, Cavender□Bares J, Donohue K, Rubio de Casas R, Group NGW (2014) The evolution of seed dormancy: environmental cues, evolutionary hubs, and diversification of the seed plants. *New Phytologist* **203(1)**, 300-309.