FloraBank

GUIDELINE 8

BASIC GERMINATION AND VIABILITY TESTS FOR NATIVE PLANT SEED

Australian National Botanic Gardens

Natural Heritage Trust
Helping Communities Helping Australia
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CSIRO
FORESTRY AND FOREST PRODUCTS
Australian Tree Seed Centre

The efficiency and success of raising plants in a nursery or by direct seeding depends to a great extent on the physical quality of the seed. This quality can be guaged by the seed's viability and germinability. It is not possible to determine whether a seed is alive by simply looking at it. It needs to be tested to establish whether it is viable and germinable. For anyone involved in a revegetation program – those who collect and supply seeds as well as those who plant them – at least a basic understanding of viability and germinability is required to be able to collect and store native seeds that will remain alive and may be germinated or sown with a reasonable expectation that they will grow. It is also important to know how much seed to sow – too little seed will result in insufficient plants while too much seed could be wasting a scarce resource.

This guideline describes basic, low-cost approaches to testing native seed for people involved in revegetation. If you know how, you can determine a seed's viability quickly and easily. There are also simple, standardised germination tests that should be routine procedure when entering seed into storage.

In many cases, those collecting and storing seed do not have the resources or need for equipment typically used by official seed testing laboratories. However, this should not deter people from conducting routine germination tests on all seedlots prior to, and during storage. Small community seed programs can make an important contribution. Given that there are many thousands of native species and only a limited number have had their germination requirements determined, any information that can be provided by community seed programs on germination techniques and results is invaluable.

Some community-based seed suppliers are able to obtain access to test equipment as the scale of their operation increases, however they may find that they do not have the time or technical know-how to operate the equipment at anything other than a basic level. It is important that seedbanks in this situation adopt the approach outlined in this guideline so that a minimum standard of seed quality is assured. It is more important and cost-effective to routinely use the best standard method within your reach than to over-commit to complex equipment and methods, follow non-standard test procedures or fail to routinely apply the tests. Alternatively, seed can be sent to a reputable seed laboratory that conducts routine germination tests.

Whatever method of testing is used, the results should be representative of the seedlot and should be reliable determinants of sowing rates, requirements for seed and storage needs.

Background information is provided in this guideline to assist the reader in understanding how to conduct germination and viability tests. The procedures described are based on those used by the Australian Tree Seed Centre. These are based on the rules of the International Seed Testing Association, recognised worldwide for agricultural crops and some tree and shrub species. For more information see Boland *et al.* (1980), Gordon *et al.* (1991), International Seed Testing Association (1996), Langkamp (1987), Morse (1997), the Australian Tree Seed Centre website (www.ffp.csiro.au/tigr/atscmain/index.htm) and other references listed at the end of this guideline.

While these publications cover a limited range of Australian native species, for the most part they describe testing procedures for orthodox seeds and seeds with dormancy induced by hard seed coats (included are *Acacia, Atriplex, Bothriochloa, Dichanthium* and *Eucalyptus*). This is a starting point which establishes principles that underpin testing of Australian native species. A review of Australian literature about seed testing is included in 'Seed testing and storage of Australian native species' by GJ Morse. Minimum seed quality and labeling standards for some native species are set out in some of the State Agricultural Standards Regulations.

What happens if you don't test seed?

If you turn over seed quickly and use the best possible collection and storage practices then you may take the view that you are doing all you can to guarantee quality. Some seedbanks do not do any testing and rely on in-field germination rates and the feedback of seed users as their sole means of assessing seed quality. There are some problems with this approach.

When determining seed quality on the basis of user feedback, a seedbank manager is relying on the user's capability and expertise. It is possible that the user is not using the correct germination procedure for that species or seedlot and that this, rather than any lack of viability, is the real cause of poor germination. Alternatively, the user may have stored the seed inappropriately (for example, under hot, humid conditions in direct sunlight) allowing the seed to lose viability after dispatch from the seedbank. In-house testing provides a basis for assisting users if they have problems.

If the seed does have poor viability and is not part of a routine testing program, a seedbank manager will not know if the seed had poor viability when it was collected or if storage practice is to blame. Indeed for unfamiliar species a seedbank manager may not be aware of the baseline viability and the variation that naturally occurs in the species. Be aware that many Australian species have naturally low seed viability. In addition, perhaps one-third of native species require some form of pre-treatment for their seed to germinate. Perhaps the same numbers again are extremely difficult to germinate.

First, inspect the seedlot

A good visual inspection will help to determine whether a seedlot is worth accepting into your seedbank. Seedbank managers must be satisfied that the seed is sufficiently clean and of the correct species before it is accepted. There are many ways, which may not be plain to the eye, in which quality can be impaired. Sometimes mistakes are made in species identification, handling after collection, or packaging for transport. Clues to a seed's quality could be who collected it, where it was collected, and when and how it was packaged. So ask lots of questions about how the seed came to you.

When making a visual inspection, you will need a sample of the seedlot (about a spoonful). Ensure the sample is representative of the seedlot, as transport and storage will cause many impurities to settle in the bottom of the container. This can be useful if you need to clean the seed (again), but be aware that obtaining a sample by skimming the surface of a seedlot can lead to a biased (better) assessment if most of the impurities are at the bottom.

Place the sample on a flat surface (for example, a table) and *look* at it. Look for impurities such as leaves, sticks, dirt and other foreign matter, the presence of insects, species identity, and so on. With very small seed, it may be necessary to use a small hand lens. Separate out some seeds: what colour and shape are they and how similar are the seeds? Sometimes parts of the fruit may be present in the seedlot (for example, dispersal units); are they still carrying seed?

In some seedlots it is difficult to find any seed at all and this may be a reflection of their worth. Some seedlots may consist solely of chaff and non-viable seed material due to poor pollination, selfing or immaturity. Seed colour is sometimes an indication of viability, but often some form of germination testing will be required for this to be confirmed.

In some native grasses it is difficult (and not always desirable) to separate the caryopsis ('seed') from other structures. It may also be difficult (for example, in the case of chaffy grasses such as *Astrebla, Themeda*, and *Dichanthium*) to establish if a seed is present. In many instances the seed set may

be naturally very low. In this case it is best to inspect a larger sample of the seedlot and count those with a caryopsis present. If the proportion is low, it may be due to seasonal conditions, immature seed harvesting, or even rough handling. Germination counts may be related to the proportion of viable seed based on this inspection.

Acting on results

If there is doubt about the purity and quality of the seedlot, a standardised approach is to conduct purity and viability tests. This will determine whether the seedlot should be rejected (see below).

In practice, you need to determine whether the seed is acceptable, given its species and ease of cleaning. If the seed is relatively easy to clean using rapid cleaning methods (for example, using sieves) then it is expected that the seed be almost free of impurities. However, where mechanical methods are not effective or available it is necessary to accept impurities in the seedlot (for example, in the case of many native grasses).

Just how clean a seedlot should be is a matter of experience, for there is no standard for most native seed. It is therefore up to individuals to set their own standards or guidelines. These would normally be based on the nursery or direct seeding equipment requirements, or on commercial considerations (for example, payment by weight or purity).

If it is determined that the seed contains excessive impurities or a mixture of foreign seed, then it should either be cleaned or rejected and returned to the collector.

Essential information about the seedlot must be supplied by collectors, including:

- their name
- the collection date
- plant scientific name
- location details
- number of plants sampled
- likely origins of the population, and
- whether a voucher specimen was collected.

(See FloraBank Guideline 4: *Keeping records on native seed.*)

This information is important in any consideration of quality. Seed should not be used in revegetation (or paid for) unless this information is supplied.

Seed testing

There are two different types of tests used to determine the quality of seed. A **viability test** assesses whether a seed is dead or alive and therefore indicates the *potential* of a seedlot to germinate, but not the number of seeds *able* to germinate. The **germination test** assesses the portion of seeds within a sample that are *able* to germinate. This gives an indication of how much one needs to sow and how the seed should best be germinated. Very often native seed has some form of dormancy that requires treatment in order to prepare the seed for germination. The term 'seed dormancy' refers to a condition in a living seed that prevents it from germinating even when provided with optimum conditions of temperature, moisture, light and air.

Recommended test program

Following drying, cleaning and accession to storage, there are four components to the recommended testing program for all seedlots (except those that will be sown immediately):

- taking a representative sample of the seedlot
- germination testing of all seedlots entering storage
- viability testing of some seedlots, and
- re-testing of all seedlots in storage every five years (or less for short-lived seed, such as rainforest species).

In addition, the following optional tests can be carried out if they are specifically required and the resources for the tests are available:

- a purity test, for a standardised assessment of the cleanliness of a seedlot
- a vigour test, particularly on seed stored for some time (over 10 years), and
- a moisture content test, to better understand seed storage conditions.

FloraBank recommends that, wherever possible, a germination test be carried out on all seedlots, either as they enter storage or are distributed for planting. Properly conducted germination tests are a reliable way to determine what results may be achieved in the field, especially for seed with dormancy problems.

Germination tests can determine not only how much seed to use, but also what the best method for germination is (for example, what pre-treatment methods to include for overcoming seed dormancy). Monitoring the germination of seed that has been in storage provides information on the effects on seed longevity of storage conditions. It also provides information on the quality of seed held in storage, and whether it is still viable and vigorous enough to send to users.

Viability tests are a rapid way to determine seed quality but they are less reliable and more subjective than germination tests. They do not take into account dormancy problems and tend to overestimate the likely germination. Viability tests should be undertaken on seedlots that will not be tested for germination. In some cases they should be undertaken in conjunction with germination tests. Viability tests are useful when the quality of seed is in doubt after a visual inspection. The seed of some species may lack viable embryos, and there is no point in progressing to germination tests if the seedlot contains mostly empty seed coats, or is otherwise not viable (for example, due to insect attack or immaturity). Viability testing is faster than germination testing, so it can be useful if an assessment of physiological performance is required quickly (for example, when seed will be sown immediately following collection). As a result, viability tests are especially useful for recalcitrant seeds (fleshy seeds often associated with rainforest trees) known to have a very short storage life; or for seeds that are known to have dormancy; or for seeds that germinate slowly and will take more than six weeks to complete that germination.

Viability tests are also useful in verifying the result of a germination test, particularly one that results in poor germination because of seed dormancy or unfavourable moisture, temperature and light conditions. They are therefore frequently used where the germination technique or pre-treatment method for a new species is unknown. Germination and viability test results should be similar. However if germination is considerably lower than the viability result, then conditions for germination may not be adequate.

Representative sampling

This 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 but is also representative of the whole seedlot.

To achieve this you must first uniformly mix the seedlot. This can be done by:

- shaking up the seedlot in its container, or
- emptying the seedlot from one container to another, or
- mixing by hand, or
- mixing by mechanical means.

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

The next step is to take a sample of seed from the seedlot at random. The amount of seed required is determined by the number of tests to be conducted and the amount of seed required for each test.

Small seedlots

The simplest method for small seedlots of less than two kilograms is to take a few random spoonfuls of seed from the container (bottom and top). Spread the seed on a clean surface (for example, a table), mix thoroughly and randomly pull out the number of seeds required for the test.

Large seedlots

For seedlots larger than two kilograms a series of samples may be taken at random by using your hand or a standard measure such as a half-cup. These samples can then be mixed together to form a primary sample. Where more than one container is involved, the samples need to be taken from each container.

The primary sample may still be too large for a test and is therefore further reduced to provide a final sample for testing. There are a number of methods used to achieve this final test sample. The easiest method begins with spreading the primary sample on a clean flat surface, such as a sheet of glass or the shiny side of a sheet of masonite or laminex. Shape the sample into a square flat heap and divide once lengthwise and once across into four even portions. Combine two diagonally opposite portions and return the rest of the seed to the seedlot. Repeat the procedure to reduce the sample size further until you are left with approximately twice the amount of seed needed for the test (for more information on sampling, see International Seed Testing Association 1996 and Langkamp 1987).

Germination tests

Basic germination tests can be performed with very few resources if a standard approach is consistently used. The basic methods described below are carried out indoors, where temperature, moisture and light conditions for germination can be controlled to a greater degree than is possible in an outdoor environment. This may include the use of an airconditioner for temperature control. Elliot and Jones (1980) and Wrigley and Fagg (1979) provide illustrations of a germination unit, comprising an automatic watering source and a heat source (in the form of a light globe), that could be adapted for germination testing. They require at least the same conditions as are present when seed is sown in a nursery, in the backyard or directly in the field.

Nursery facilities can also be used for conducting germination and vigour tests. They are especially suited to large seed (rainforest) species which can take more than six weeks to germinate. Nursery facilities are useful where indoor methods result in serious fungal problems. It is important to ensure the test area is rodent-proof, that moisture levels are maintained and that germinated seeds are counted regularly.

The more control you have over growing conditions the better, but it is not essential to use (or own) growth cabinets and other expensive laboratory equipment. The most important aspects are to standardise your methods and keep accurate records (of the number or weight of seed used in each test replicate and of the germination results over time).

Our recommended standard approach relies on:

- testing by replicates
- doing the best job possible to control temperature, moisture and light conditions throughout the test
- monitoring germination, temperature, light and moisture conditions regularly
- conducting tests over a longer period than may be the case using a growth cabinet.

Germination test samples

A germination test should be carried out on four replicates with a known number or weight of seeds per replicate. A replicate is an identical sub-sample of the test sample Yes, you split it up again! The four replicates are each tested separately and the results for each compared. The closer the results (less variation between the replicates in a test) the more reliable is the test and its findings. When counting out seeds they should, again, be selected randomly, and not biased towards any particular seeds. The replicates must be labeled and kept separate throughout the test.

For species where seed can be counted, replicates should comprise a minimum of 25 seeds. It is a good idea to weigh the counted seed so that a germination rate per unit of weight (per 100 grams) may eventually be determined. Knowing the germination rate per unit of seed weight is useful in the nursery and in direct seeding.

It may not be practical or possible to count out the required number of seeds if they are very fine, such as those from eucalypts and melaleucas. Instead, testing is carried out on replicates of equal weight for such seedlots. When weighing the replicates, the sample will include impurities as well as seed. After being weighed, the impurities can be removed before the seed is placed on the growing medium. The Australian Tree Seed Centre website (www.ffp.csiro.au/tigr/atscmain/index.htm) contains germination standards for a wide range of forest tree species and includes recommended replicate weights for seed of eucalypts and other fine-seeded species. Weights are generally between 0.1 and 1 gram.

For very small seedlots, say less than 10 grams, a germination test using four replicates may be excessive. In such cases, a single replicate will be sufficient.

Germination test equipment and conditions

Germination tests can be set up in a similar way to normal sowing, taking into account the need for moisture, warmth, oxygen and light.

Container

A wide range of containers and substrates is used for germinating seed. All should be large enough to allow for unrestricted growth of germinants to a stage at which they can be assessed. Containers should have a clear lid that is close fitting to prevent moisture loss. Commonly used containers include nine-centimetre glass petrie dishes (for fine seed), plastic take-away food containers or containers in which alfalfa and bean sprouts are sold. A number of laboratories roll large seeds in moist absorbent paper toweling, which is then placed inside a thin, open-necked plastic bag. All containers should be clearly labeled, identifying the seedlot and replicate, and using the same system as on the test record sheet so that seed counts are tallied for the correct replicate. Record the type of container used.

Sowing medium

The substrate must be able to hold moisture and be free of chemicals, pests or diseases. Commonly used substrates include absorbent paper, sterile sand, vermiculite and agar. The thickness of the substrate should be approximately twice the thickness of the seed. The seed is placed on top of this medium. In the case of grasses where the whole dispersal unit is used for the germination test, one method used by the Australian National Botanic Gardens is to place the sample in the container on moist paper over terry towelling (which can hold a lot more moisture).

Record what sowing medium is used.

Moisture level

As general rule of thumb, the sowing medium should be moist without having free water collecting in the container; however, seed of certain species may have specific requirements, which will need to be worked out through experience.

Temperature

Temperatures for germination of most species are in the range of 15–35°C: tropical species tend to germinate better in the

range of 25–35°C, temperate species in the range of 15–25°C. Constant versus alternating day/night temperatures can also have an effect on germination. The Australian Tree Seed Centre tests species at a constant temperature. Bonney (1994) has reported that alternating day/night temperatures (when germinating seeds from semi-arid and arid areas of South Australia) plays a large role in softening hard seed. When testing a number of eucalypts, Grose (1962) found little difference in germination between constant and alternating temperatures, however the rate of germination was slower under alternating temperatures.

Above a certain minimum, seed is fairly tolerant of a range of temperatures. It is therefore recommended that if facilities are not available to control the temperature (for example, you do not have an airconditioner) then you can simply place the seed tests in a well-lit part of a room (for example, close to a window, so long as it is not too hot). If possible, record the minimum and maximum temperatures each day (see Guideline 2 for information on inexpensive digital temperature meters).

Light

Light is required for the successful germination of many species, especially when seedling assessment is based on appearance. Seedlings grown in the dark will not produce chlorophyll and will have a very pale appearance. The light can come from natural or artificial sources.

Controlling fungi

Fungal contamination is usually associated with immature, damaged or old seed. It can also arise through overly-severe seed pre-treatment (such as immersion in boiling water) or hygiene problems in the testing area. A number of preventative measures can be taken to reduce the risk of fungal contamination.

- Cleaning and disinfecting the test area between batches helps limit the spread and severity of fungal attack.
- You should ensure that individual seeds in the test do not touch each other, that there is good aeration and that contaminated (decayed) seed is removed (but counted) from the test to prevent the spread of contamination.
- A practical way to treat external contamination is to soak seed for 10 minutes in a one per cent solution of sodium hypochlorite (domestic bleach), then rinse the seed and allow it to surface dry before sowing. When soaking, it is important that each seed is thoroughly wet (no air bubbles), which can be difficult with some (hairy) seeds. A couple of drops of detergent will help. Be aware that the concentration of household bleach is usually five per cent sodium hypochlorite, so you should dilute the bleach further, to achieve one per cent. Replace your bleach annually, as it goes off, and freshly dilute each batch for use. Bonner *et al.* (1994) recommends soaking for 20 minutes in a 10 per cent sodium hypochlorite solution or a 30 per cent solution of hydrogen peroxide (H_2O_2).

Seed dormancy

Numerous native species are known to have some kind of dormancy. As many of these species are not fully understood, finding successful methods to artificially overcome dormancy and prepare for germination will often have to be done by trial and error. Environmental conditions during seed maturation and time of collection can influence the degree of dormancy.

There are basically two types of dormancy:

- 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).

In seeds of some species that are dormant at harvest, dormancy breaks down naturally over time (for example, *Themeda triandra*). Other species require some form of pre-treatment in order to remove the dormancy.

The biggest problems with dormancy in seed testing are:

- knowing which of the many recognised dormancy-breaking method(s) to apply to which species
- determining which method is most successful, and
- identifying those species for which there is as yet no successful procedure to break seed dormancy.

In recent years there has been valuable research into methods of dormancy breaking, and their application to species (many of these methods are specific to genera). There is a great need to share information about new and successful methods and expand the range of species for which dormancy can be successfully overcome. The FloraBank website has a forum for posting messages and questions about germination of native species. The typical methods in current use are listed under the heading Seed Dormancy Breaking Methods, and are based on Australian Tree Seed Centre practices.

Eventually, FloraBank hopes to provide a digest of referenced information about pre-treatment for commonly revegetated native species. For additional information on seed dormancy see Adkins and Bellairs 1997, Boland *et al.* 1980, Bonner *et al.* 1994, Bonney 1994, Doran *et al.* 1983, International Seed Testing Association 1996, Langkamp 1987, Murphy and Dalton 1996 and Willan 1985. There are also websites with information on propagation and germination techniques for native plants. These include the Australian Tree Seed Centre (www.ffp.csiro.au/tigr/atscmain/index.htm.), the Australian National Botanic Gardens (www.anbg.gov.au/PROPGATE/plant01.htm), Kings Park and Botanic Garden (www.kpbg.wa.gov.au) and the Society for Growing Australian Plants (www.farrer.riv.csu.edu.au/ASGAP/).

Germination counts

A count of the seedlings germinated (germinants) should be carried out at regular intervals, especially once the radicle and cotyledon(s) have emerged on the seedlings. The number of germinants is recorded for all replicates in the test and all seeds must be counted as having either germinated or not. Only those seedlings that are 'normal' (that is, those that demonstrate a capacity for sustained development under suitable conditions) are considered to have germinated (Peterson 1987). However, where time and other resources are limited, a seed may be counted as having germinated once the radicle is at least three times the length of the seed. Abnormal seedlings should not be considered to have germinated. This includes damaged, deformed, decayed or mouldy seedlings, albinos or those with abnormal cotyledon, radicles or hypocotyl.

Once they have been counted as germinated, the seedlings in each replicate are removed from the test dish and a record to that effect made on the test record sheet. The test should continue until all the seeds have germinated or until no further germination has occurred after two consecutive counts. Make sure that the number of germinants counted is recorded against the appropriate replicate.

Ungerminated seed

At this stage, ungerminated seed should be counted, inspected and a record made against the appropriate replicate. In the case of hard-seeded species, record whether the ungerminated seeds are soft or hard. A tally of hard seeds indicates that the pre-treatment was not sufficient to cause imbibing of the seed. A record should also be made of insect-attacked or mouldy seed, as these conditions indicate injury or death.

If the number of hard, injured or mouldy seeds is in excess of 25 per cent, re-test the seedlot using a different germination procedure. With eucalypts and other small seed, an inspection is made after squashing ungerminated seed with a pair of tweezers or forceps (known as the 'squash test') to expose the embryo. For acacias, forceps can be used for soft seed, otherwise the seed is subject to a cut test. Any ungerminated seed found to have a firm white embryo is considered to be potentially viable.

A measure of viability is obtained by combining the count of potentially viable ungerminated seed with the germination count for each replicate (for example, 80 per cent germinated seed plus five per cent viable ungerminated seed equals 85 per cent viability).

On completion of the germination test

A record is kept of each test of the seedlot, covering;

- test method and conditions (for example, minimum and maximum temperatures)
- replication weight or number of seeds
- dates of commencement and germination count
- number of germinants, and
- the person who did the test.

(Examples of germination test sheets are provided in attachments four and six of Florabank Guideline 4: *Keeping records on native seed.*)

Acting on results

The number of germinants (normal seedlings) in each replicate is tallied and the mean of the four replicates calculated and converted to a germination figure per 100 grams of seed. Where the number of seeds is known, the percentage of germinated seeds in each replicate is also calculated and the mean of the four replicates provides a germination percentage.

The variation in germination counts between replicates should be within standard limits. As a rule of thumb, the difference between the germinant count for the highest and lowest replicate should be no more than one-third of the total germinant count for the four replicates (for a statistically accurate determination, refer to the tolerance tables contained in International Seed Testing Association 1996).

If germination in the most recent test is significantly lower than in a previous test, re-test the seedlot to confirm this result. Where germination falls below 50 per cent of that found in a previous test, the seedlot might not be suitable for further storage or use. Reduced germination (over earlier test results) is a widely accepted indicator of seed deterioration in storage. Also look for signs such as a much-delayed germination or a greater proportion of abnormal seedlings as these may also be taken into account and can influence the vigour of the seedlings.

Re-testing

Re-tests are carried out on seedlots when a germination test gives unsatisfactory results, when there have been changes in the composition of the seedlot (for example, re-cleaning) or after a period of time in storage (each five-year period).

Viability tests

There are a number of different methods for testing viability. These include cutting open the seed or squashing it to reveal the embryo, doing a chemical staining test and even using X-ray techniques. We recommend you use either the 'cut test' or the 'squash test' (both are described below). Tests not covered here include the 'excised embryo test' in which the embryo is removed from the seed coat and inspected, and the 'Tetrazolium chloride (2,3,5-triphenyl tetrazolium chloride) stain test', which differentiates living from dead tissue by staining live tissue red (for specific instruction on the procedures to follow, refer to International Seed Testing Association 1996).

Cut test

A cut test can provide a 'quick and dirty' method of assessing the quality of a seedlot without undertaking a germination test. Following a visual inspection, randomly select a minimum of 25 seeds from the seedlot. For more reliable tests, four replicates of 25 seeds should be used.

Use a sharp blade (scalpel, knife or sharp secateurs) to cut right through the length of each seed. Visually inspect the seeds (using a small hand lens if necessary). Count the number of seeds that are firm and creamy-white in colour (these are considered viable) and record the results as a percentage of the sample (for example, if 10 seeds were counted from a sample of 25, the result would be 40 per cent).

Squeeze test

The approach is similar to a cut test, but is more suitable for fine seeds (eucalypts, melaleucas) which cannot be cut in half. The standard four replicates of 25 seeds are taken at random (or from a weighted sample) and soaked in water for two to four days. The water is drained (through a very fine sieve or nylon stocking) and the individual seeds gently squeezed using a pair of tweezers. The results are visually inspected and the number of viable seeds counted.

An alternative method, which can be effective for oily soft seeds, is to spread the samples between two pieces of absorbent (brown) paper. Roll a glass bottle or rolling pin over the seeds with enough pressure to crush them against the paper. Viable seeds will leave an oil stain against the paper, whereas dead seed and chaff will not stain. Count the oil stains to determine the number of viable seeds per unit weight.

Optional tests

Purity test

This is an optional test used to determine whether the seed is clean enough to accept into storage and to send to users. It provides a more standardised approach than the visual inspection methods described above. As defined by the International Seed Testing Association (1996), the object of the purity test is to determine the composition by weight of the pure seed as a percentage of seed of other species and inert matter (such as twigs, leaf matter, fruit particles, soil, foreign seed and so on). Determination of physical purity follows the principle rules of the International Seed Testing Associaton (1996) but with sample size reduced to take into account the comparatively small size of the seedlots likely to be handled.

The test is conducted on one gram of seed or 1000 seeds (whichever is the larger amount). After the sample is weighed, the pure seed is removed and weighed separately. The term 'pure seed' refers to seed of the species under consideration and includes:

- mature seed
- undamaged seed
- undersized, shrivelled, immature and germinated seed, and

- broken pieces of seed that are more than half their original size (International Seed Testing Associatioin 1996).

The seed of other species and the types of other matter present in the batch should be identified as far as is possible.

The percentage of pure seed is calculated as follows:

$$\text{Purity (\%)} = \frac{\text{weight of pure seed fraction} \times 100}{\text{total weight of sample}}$$

The smaller the seed, the more difficult the purity test will be. The distinction between true seeds of the species under investigation and trash can be ambiguous for some seeds. For example, in the case of eucalypts, no distinction is made between the pure seed and chaff components.

Vigour test

This is an optional test used to determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions. Old seeds, or seeds stored under adverse conditions, can decline in vigour. Seed vigour normally declines faster than a seed's ability to germinate. This test would not normally be carried out in the course of revegetation work because seed is not stored for very long, and it can be difficult to accurately assess vigour; however, you should be aware of the test.

The rate of germination is possibly the simplest indicator of vigour. This may be expressed as the number of days it takes a seed to reach 50 per cent germination, or the number of days it takes to complete germination (when 90 per cent of the sample has germinated). The approach is the same as for a germination test but the germinants should be counted every one or two days and the time period for germination may need to be extended. Compare the results with previous tests on the same seedlot or with other seedlots of the same species. If the time taken for germination to be completed is 25 per cent longer (or more) than for other tests, either the seedlot is considered to have an unacceptable lack of vigour, or the germination conditions are unfavourable. Inspect the germinated seedlings, checking for stunted growth or abnormalities and whether the seedlings have sufficient vigour to shed the seed coat.

Another approach is to raise 25–50 seedlings in a nursery and compare their growth and health with seedlings of the same species from fresh seed. Again, compare times taken for germination and then growth rates.

(For more information refer to International Seed Testing Association 1996 and Peterson 1987).

Seed dormancy-breaking methods

The following treatments for seed dormancy are based on current practices at the Australian Tree Seed Centre. The Centre handles seed from across Australia but primarily from the eastern States. There are generally no recognised standards for dormancy-breaking methods applicable to one species across Australia, and the approach taken for species within a genus may vary greatly. In addition, by visiting seedbanks, FloraBank has found that when confronted with a new (dormant) species, people in different parts of Australia choose different methods to try to overcome the problem, based on their experience. For example, some in Western Australia try smoke water first, whereas few would do so in eastern States (although they have assessed its use).

When choosing a treatment for seed dormancy, either refer to the references listed below, have a look at the web sites listed (if you have access to the Internet) or talk to others who may have tackled the species before.

Try the least severe method first to avoid damaging your seeds. Increasingly severe methods can then be tried if necessary. Fresher or slightly immature seed may not require as severe a treatment as fully mature seed. If you get stuck, try the FloraBank

website Germination Forum page where you can put a question on the bulletin board for others to answer.

Boiling-water and hot-water treatments

Boiling water; immersion for one, two or five minutes

Seed is immersed in boiling water (100ºC) for a nominated time. It is then removed from the heat source and placed either directly into a germination container or into room-temperature water, and left to soak for approximately 24 hours before sowing.

Boiling water; pour and soak

Seed is placed in a container and boiling water (approximately 10 times the volume of the seed) is added. The seed is then left to soak in room-temperature water for approximately 24 hours, before sowing. The soaking process allows seed to imbibe water, which hastens germination.

Hot-water treatments

Whilst seed of most Australian acacias requires some form of boiling water pre-treatment in order to promote germination, there are a number of species or specific seedlots which respond better to a hot-water treatment (90ºC for one minute). These include *A. mearnsii, A. stenophylla, A. synchronicia, A. pachycarpa, A. pendula,* and *A. tephrina*.

Scarification or cracking of the seed coat

Scarification abrades the seed coat, permitting water absorption. Scarification may be done by hand, especially for germination test purposes, or by mechanical scarifiers which rotate the seed in a drum against a rough surface (normally sand paper). The coarseness of the surface, duration of scarification, amount of seed and thickness of seed need to be taken into consideration when using this method.

Mechanical scarification of seed is infrequent, due to the ease and success of boiling-water treatments.

Manual nicking

Manual nicking is often used to determine optimum germination of a seedlot, especially after boiling-water treatments have proved unsuccessful. Secateurs, nail clippers or a scalpel blade can be used to remove a small section of the seed coat at the distal (cotyledon) end of the seed. A vice can be used to split very thick seed coats (such as, *Macadamia*). Manual nicking is a slow process, so is not recommended for large numbers of seeds. It is useful as a research tool for small numbers of seeds or to compare the results of other pre-treatment techniques. Manual nicking usually gives the best germination result.

Acid scarification

Acid scarification is seldom used on seed of Australian species, as there are easier and safer methods to use. However, it is recommended as an alternative treatment for seed of species with very thick seed coats. The method simulates what happens to seeds when they are eaten by animals prior to germination. If animal consumption is a known precursor to germination, then the acid treatment may be warranted.

Seed is soaked in concentrated sulphuric acid (95 per cent, 36N) at room temperature for a nominated time (30–120 minutes), depending on the species. The seed is then removed from the acid and rinsed under running water for at least 10 minutes. This can be done by placing the seed in a perforated steel tea infuser.

CAUTION: Extreme care is required when handling concentrated acids. Only trained staff should administer this procedure within a fume cupboard. **Never pour water into undiluted acid**; rather, pour a small quantity of acid into running water. Beware of the gases given off by this procedure. Protective clothing, including glasses and gloves, must be worn. If accidental splashes

occur, a concentrated solution of potassium or sodium bicarbonate may be used as an antidote. Alternatively or additionally, wash the affected area in running water or use an eye-wash bottle.

Cold moist stratification

Cold moist stratification is used to overcome embryo dormancy in a number of cool temperate eucalypts (*Bursaria occidentalis, Nothofagus* spp.) as well as a number of cool temperate acacia species (for example, *A. mearnsii, A. kybeanensis*). Seed is placed in moist conditions at 3–5°C (for example, in a refrigerator) for between three and nine weeks (depending on the species) prior to germination. This can be done after a germination test is set up. Once the stratification period is complete, the containers are removed and seeds are allowed to germinate under appropriate conditions. Cold stratification is often useful for obtaining even germination (that is, all the seed germinating together).

Chemical treatments

Whilst not a prescribed treatment for overcoming physiological dormancy, some problem species may respond to chemical treatments.

Hydrogen peroxide – Seed coats are cut to expose the radicle. The seeds are then incubated in a solution of one per cent hydrogen peroxide (H_2O_2) for 48 hours. This is done in darkness with alternating temperatures of 20°C and 30°C. Radicle growth is measured after three to four days, and the seeds are placed in fresh hydrogen peroxide (Bonner *et al.* 1994). The method is not practical for very small seeds and may take seven to eight days to get a result.

Citric acid – Soak seed for 48 hours in a one per cent citric acid solution, or in combination with stratification (Bonner *et al.* 1994).

Potassium nitrate – A 0.2 per cent solution of potassium nitrate (KNO_3) is prepared by dissolving two grams of KNO_3 in one litre of water. This solution is used to saturate the germination substrate at the beginning of the test. This method is used for a number of agricultural and vegetable seeds, as indicated by the International Seed Testing Association (1996).

Gibberellic acid – Bachelard (1967) found that the germination of dormant seeds of *Eucalyptus delegatensis, E. fastigata* and *E. regnans* could be improved by 24 hours' immersion in gibberellic acid (GA_3) at concentrations of 50 and 100 milligrams per litre, and germinated at 21°C. The International Seed Testing Association (1996) recommends that the germination substrate is moistened with a 0.05 per cent solution of GA_3, prepared by dissolving 500 milligrams GA_3 in one litre of water.

Procedures for removing inhibitory substances

Seed of many Australian species contain inhibitors in the seed coat that prevent or delay germination. In these instances the inhibitor can be leached out by placing the seed under running water for several hours (or even days) or soaking the seed in a large volume of water that is changed at frequent intervals (every six to 12 hours). It has been reported by McKintyre and Veitch (1972) that seed of *Eriostemon australiaus* germinated after the radicle end of their seed coats were chipped and they were leached in running water for two weeks. Seed of the *Correa* species are also reported to improve their germination substantially after being soaked in running water for one to two weeks (Elliott and Jones 1980). Bonney (1994) reported that ripened seed of *Boronia* and *Eriostemon* need to be placed in moving water for many hours to help leach out inhibitors. This can be achieved by suspending the bag of seed in the cistern of a flushing toilet. Alkaline solutions have also been used as a leaching method. Seed of *Themeda triandra* (syn. *T. australis*) which is reported to be dormant at maturity, responded to gibberellic acid and removal of the glumes and/or palea and lemma. It is also reported that dormancy in this species is naturally overcome after six to 10 months in storage (Groves *et al.* 1982).

Recent research into the treatment of certain species (particularly from Western Australia) using varying degrees of smoke

(normally in the form of 'cool smoke water') has shown promising results (Dixon *et al.* 1995). The method entails the pre-treatment of seed by soaking for approximately six to 24 hours in a 10:1 water:smoke solution. Smoke water is available from Kings Park, Perth, Western Australia or under the trade name of Regen 2000. Other suppliers are listed at the FloraBank website's Seed Equipment page.

Fermentation of seed (such as *Eremophila, Santalum, Nitraria*) can also be helpful (Bonney 1994). Peeling or slitting the seed coat of *Grevillea* and *Dianella* species has been known to give improved germination (Bonney 1994).

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