Florabank Guidelines

Module 10

Seed Quality Testing

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Key points

Seed quality testing involves assessing the purity, seed fill and seed viability of a seed lot.

Seed purity testing determines the proportion of seed to non-seed material in the seed lot.

Seed fill testing determines whether or not the seed contains an embryo.

Seed viability testing determines whether or not the seeds are alive.

Reporting seed quality information is essential for calculating seeding rates and ensuring that only seed lots containing live seeds are used for propagation and restoration.

The data obtained during collection (i.e. location, date) is also an important component of quality information.

Introduction to quality testing

This guideline describes approaches to testing seed quality post-cleaning (see Module 8 – Seed Processing) for those involved in seed-based activities such as seed bank managers, researchers, nurseries, restoration practitioners, seed collectors, community organisations, and seed purchasers. The efficiency and success of seed use depends largely on seed quality, so all must understand the importance of the quality of the seed with which they are working.

Seed should be tested, as the information provided is invaluable and the quality of seed cannot be assessed by eye alone. If seed that is to be used for restoration is not tested and seed quality is poor, significant funds and time can be wasted in the form of site preparation and implementation leading to poor project outcomes. In addition, resources can be wasted storing seed of poor quality. Seed testing can be relatively inexpensive to undertake, compared with the value of the seed lot (in monetary terms, or as a genetic resource), and can be outsourced to a third party (although outsourcing testing can be expensive). If testing is outsourced, it is important for the seed users to know which tests to request, to be able to interpret the results and to know what to do with the information. The laboratory issuing the results should be able to assist in the interpretation of the results.

Seed quality testing can be divided into three parts: (1) identification and assessment of seed characteristics, (2) measurement of seed quality traits such as physical purity, seed fill, seed health (free of diseases and malformations and insect infestation) and viability of a seed lot and (3) seed germination (see Module 11 – Seed Germination and Dormancy). All this information can build a picture of what's in the seed lot (Figure 1).

Seed characteristics

The first section involves determining the characteristics of the seed and its **diaspore** or **dispersal unit** (fruit, cone, nut, berry, pod etc.). The type of **diaspore** will determine how to measure quality. Module 8 – Seed Processing explains the different types of fruits. Some fruits open to release the seeds (**dehiscent** fruits), such as *Acacia, Eucalyptus* and *Grevillea*, and hence, the diaspore that is stored/tested is a seed. For other species, fruits don't open to release the seed (**indehiscent** fruits), and the diaspore that is stored/tested is a fruit. This fruit may contain one seed (e.g. Poaceae, *Atriplex*, some Asteraceae) or many seeds (e.g. *Eremophila*) (see also section X-ray analysis).

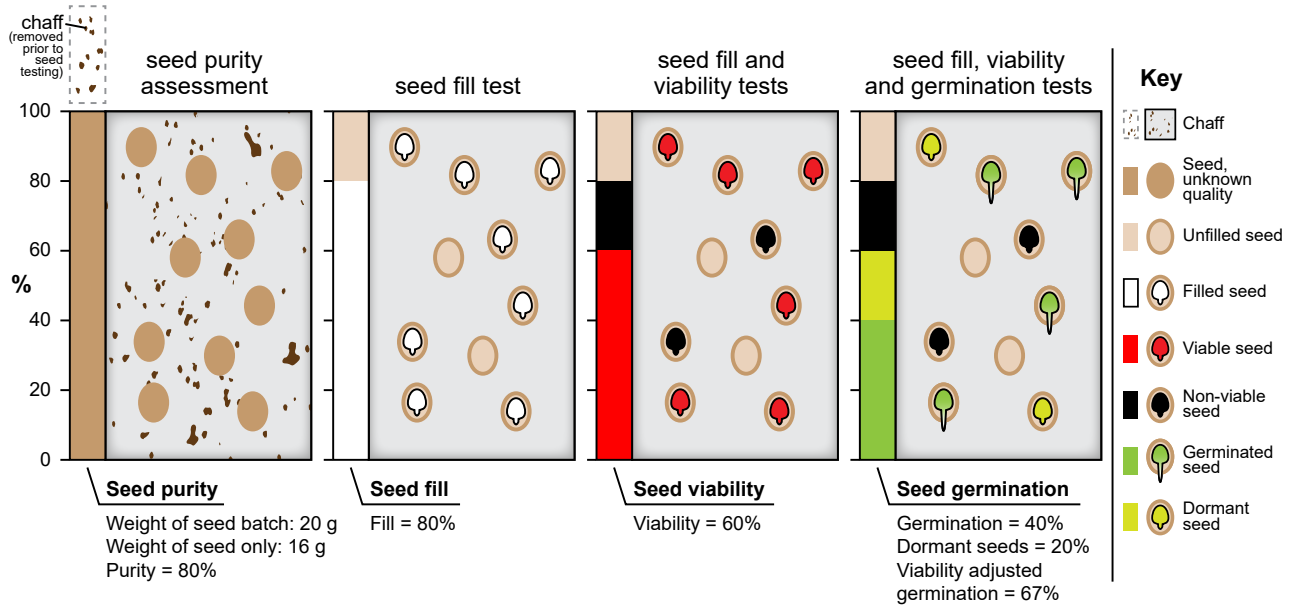


Figure 1. Breakdown of the components of a seed lot, and how different seed tests can identify the different fractions. This hypothetical example shows the breakdown of each component, both as a stacked column graph and as a diagrammatic representation. Different tests (seed purity assessment, seed fill test, seed viability test, seed germination test) elucidate different components. (Figure courtesy of Craig Miskell, CAM Graphics)

Table 1. Sample spreadsheet to record quality information with a hypothetical example based on Figure 1. Information may be kept in multiple spreadsheets. For further information on how to record information, see Module 4 – Record Keeping.

Species	Seed lot number	Collection date (dd/mm/yy)	Total weight (g)	Purity %	Fill %	Viability %	Viability test date (dd/mm/yy)	Germi-nation %	Germi-nation conditions	Germi-nation test date (dd/mm/yy)	Seed per gram	Viable seeds per gram	Total number of seeds	Viability adjusted germi-nation
			a	b	c	d	e	f			g	= d × g /100	= a × g	= (f / d) × 100
Species abc	774513	01/05/20	500	80	80	60	01/12/2020	40	20°C, filter paper in petri dish, dark	01/12/2020	37	22	18500	67

What happens if seed isn't tested?

If seed is not tested, then there is a possibility that non-viable (dead or undeveloped) seed is used, or a sample contains little to no actual seed, leading to propagation and/or restoration failure. It may also lead to potentially false assumptions about the cause of the failure (e.g. inadequate rainfall or poor seed sowing practices) rather than failure of seed to germinate. Ideally, seed should be tested prior to sale, so that the user or purchaser knows the quality of the seed that they are buying, enabling comparison of the cost of seed lots in a standardised way. Purchasers also need quality information so that they can make an informed decision when choosing between batches from different suppliers, as seeds are generally sold by weight not number. Hence, if a seed lot from one supplier costs less per gram but has a lower purity or is older and has lower viability than that from another supplier, it may turn out to be more expensive on a 'cost per viable seed' basis. In a well-structured market, with clear standards around labelling and testing, this is what a buyer could determine from such information. In the absence of such a market, there is little transparency between suppliers and users of seed. See also the RIAWA seed grading system which is used in Western Australia. In addition, the purchaser needs to know the quality of the seed lot to calculate the seeding rate – seed lots with low purity, and/or low viability, will require a higher seeding rate (e.g. kg per ha) than lots with higher purity or viability.

Seed lots should be tested prior to storage, periodically during storage with the frequency dependent on the storage conditions (e.g. every 10 years for seed in long-term storage) and expected seed longevity, as well as when removed from storage, so that the seed bank manager can determine if the storage conditions (e.g. temperature and seed moisture content) are either maintaining or adversely affecting seed viability. If seed quality is not tested prior to storage, but is found to be low following storage, there is no way to determine whether the quality of the seed deteriorated due to suboptimal storage conditions, or whether it was low to begin with. If viability has declined substantially during storage, the manager may then make a decision on changing the storage conditions, or using all of the seed before viability declines further. However, it is the information about seed quality at the point of sale, or use, that is most important to the purchaser or user, and quality at sale should determine the price.

Germination testing determines whether or not the seeds are able to germinate. This test can be used to assess seed viability and also to develop germination protocols. If seeds do not germinate when tested (assuming that they are alive, and that the temperature, moisture and oxygen conditions provided were optimal), then they may require a germination cue, or environmental conditions to alleviate dormancy. Then, further experiments can determine the set of conditions required for germination to develop pre-treatments to enable higher and/or more rapid germination under nursery or field conditions. See also Module 11 – Seed Germination and Dormancy.

Differences in collection sites, collection dates, cleaning methods and storage histories can all result in seed lots with variable quality, so assumptions about seed quality can not necessarily be made from one seed lot to another, even within species.

Seed collection information

The information about a seed lot is also an important aspect of quality. The type of information that should be gathered includes:

- What – the verified name of the species that has been collected.
- Where – the location from which the collection has been made.
- When – the date on which the collection was made.
- How many – how many plants were sampled in making the seed collection and what was the size of the population from which the seed was sourced.

Full details of the data that should be collected is outlined in Module 6 – Seed Collection.

Qualitative seed tests

Seed purity, fill and viability of the seed lot are all measures of seed quality. Seed purity determines the proportion of seed of the target species to non-seed material (and seeds of non-target species) in the lot. Seed fill determines whether the seed has an embryo, whereas viability testing determines whether or not the seed is alive. Viability testing determines the percentage of seeds that are alive with the potential to germinate, whereas a germination test determines the proportion of seeds that can germinate under standardised conditions. Seeds that are non-viable are unable to germinate, but seeds that do not germinate may still be viable but dormant. Seed germination testing is covered in Module 11 – Seed Germination and Dormancy.

Quality tests are conducted on the diaspore of the species. For some species the diaspore is the actual seed e.g. *Acacia* or *Eucalyptus*, whereas for other species the diaspore is a fruit which may contain a single seed e.g. *Calytrix* or multiple seeds e.g. *Eremophila* or *Leucopogon* (see also Module 8 – Seed Processing)

These tests are described in further detail below.

Sampling

Sampling 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 seed lot.

Mix the whole seed lot

Firstly, 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; or
- mixing by mechanical means.

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

Take a primary sample

The next step is to take a sample of seed from the seed lot at random. The amount of seed required is determined by the number of replicate tests to be conducted and the amount of seed required for each test, as well as the total amount of seed and number of containers it is split into (ISTA 2020).

Small seed lots: The simplest method for small seed lots of less than two kilograms is to take a few random spoonfuls of seed from the container (bottom, middle and top).

Large seed lots: For seed lots 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 primary samples can then be mixed together to form a composite sample. Where more than one container is involved, the samples need to be taken from each container.

Extract seeds for the test

Once a sample has been taken, randomly extract the number of seeds required for the test using a method such as the hand halving method or the spoon method.

The hand halving method begins with spreading the sample on a clean, smooth surface, such as a sheet of glass or the shiny side of a sheet of masonite or Laminex, paper or cardboard, or a table. Shape the sample into a square, flattened heap and divide once horizontally and once vertically into four even portions, then divide each portion once again to end up with eight portions. Arrange the portions in two rows of four, combine and retain alternate portions: e.g. combine the first and third portions in the first row with the second and fourth in the second row, remove the remaining four portions. Repeat the procedure to reduce the sample size further until the required sample size is obtained.

The spoon method also begins with pouring the seed evenly over the surface. Remove small portions of seed using a spoon and a spatula from not less than five random places. Combine the small portions together.

For more information on sampling, see Chapter 2 of the International Rules for Seed Testing (ISTA 2020).

Purity

Purity testing determines the percentage composition by weight of pure seed in a sample of the species collected. In addition to the species collected, the sample may contain inert matter and seed of other species different from the species targeted for collection (Figure 2, Figure 3, Figure 4). Inert matter includes broken seeds that are half of less than the size of the original seed, and non-seed material such as twigs, leaves, soil and chaff (ISTA 2020). Pure seed is defined as anything within a sample identifiable as seed from the target species and not only includes mature, undamaged seed but also includes undersised, shrivelled, immature and germinated seed as well as pieces of broken seed larger than half their original size (ISTA 2020). For some species, differentiation between the seed of the target species from other material is possible by a visual assessment, sometimes requiring magnification aids (e.g. magnifying glass or microscope). For other species (e.g. some *Eucalyptus* species), differentiation may be difficult or not even possible visually, however the actual proportion of seed in the 'pure' seed fraction will be calculated in subsequent seed fill and/or germination tests.

To determine purity, a sample is taken from the seed lot at random (Figure 5). The size of the sample will depend on the size of the collection ranging from a sample containing hundreds of seed (or fruit) for smaller collections (Gunn 2001) up to 2500 for large collections (ISTA 2020). This sample is weighed and then the pure seed is separated from non-seed material, or seed from other species. In some instances, it may be important to report on the other species fraction, particularly if the other species are weeds. The pure seed fraction is then weighed and the pure seed percentage (seed purity) can then be calculated according to equation 1 below. It is possible to take three (or more) replicate samples, then calculate an average purity. For species where the diaspore is a fruit, the process is the same as for seed. For simplicity, in the equations below we have used the term seed to mean seed or fruit.

$$Seed\ purity\ (\%) = \frac{weight\ of\ pure\ seed}{weight\ of\ sample} \times 100$$

Equation 1. Seed purity percentage calculation

If the pure seed (or fruit) are counted, the number of seed (or fruit) per gram of sample can be calculated (Equation 2).

$$Seeds\ per\ gram\ (sample) = \frac{number\ of\ pure\ seed\ in\ sample}{weight\ of\ sample\ (g)}$$

Equation 2. Seeds per gram of sample calculation

If the total collection weight is known, an estimate of the total number of seeds in a collection can be made (Equation 3).

$$Seed\ number = total\ collection\ weight\ (g)\ \mathrm{x}\ seeds\ per\ gram\ (sample)$$

Equation 3. Estimated total number of seeds calculation

Recording seed weight (i.e. weight of 1000 seeds) can be useful, and can be calculated from the seeds per gram (Equation 4).

$$1000\ seed\ weight\ (g) = \frac{1}{seeds\ per\ gram} \times 1000$$

Equation 4. 1000 seed weight (g)

Alternatively, to calculate the 1000 seed weight, count out several replicates of 100 seeds, weigh each replicate, calculate the average weight of 100 seeds, and multiply by 10.

If a collection has low purity, it may be improved by further cleaning (see Module 8 – Seed Processing). Increasing seed purity may reduce the volume of seed to be stored, thereby saving on storage costs and reduce the possibility of introducing contamination from the additional matter. However, further cleaning increases processing costs due to extra labour time, so a decision on the cost vs benefit of extra cleaning needs to be made. It may be useful to perform purity tests before and during seed processing to assist with deciding whether or not to do further processing, or to determine the increase in purity due to processing activities.

What level of purity is acceptable? An acceptable purity depends on the end-use of the seed and the cost trade-off (time/expense required to remove impurities) to improve it. For some uses such as nursery tubestock production, high purity can improve the efficiency of seed use and reduce production costs. For other uses such as direct seeding, a lower purity may be tolerable as long as the number of seeds per gram is known so that the seeding rate can be calculated accordingly. Chaff is used in some instances as a bulking agent in seeding machines. If purity is reported, the cost of different collections can be compared (e.g. $ per 1000 seed). Hence, the level of purity depends on the end-use of the seed.

Seed purity stays constant during storage, unlike seed viability, so purity testing does not need to be repeated following storage, unless further processing occurs.

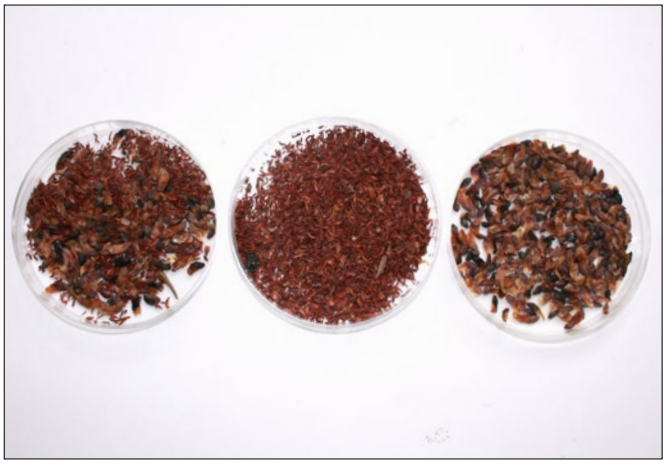


Figure 2. *Corymbia hamersleyana* (from left to right): seed and chaff; chaff only; seed only. (Photo: L. Commander)

Figure 3. Separation of seed (left) from inert matter (right) of *Eucalyptus blakelyi.* (Photo: L. Ruiz-Talonia)

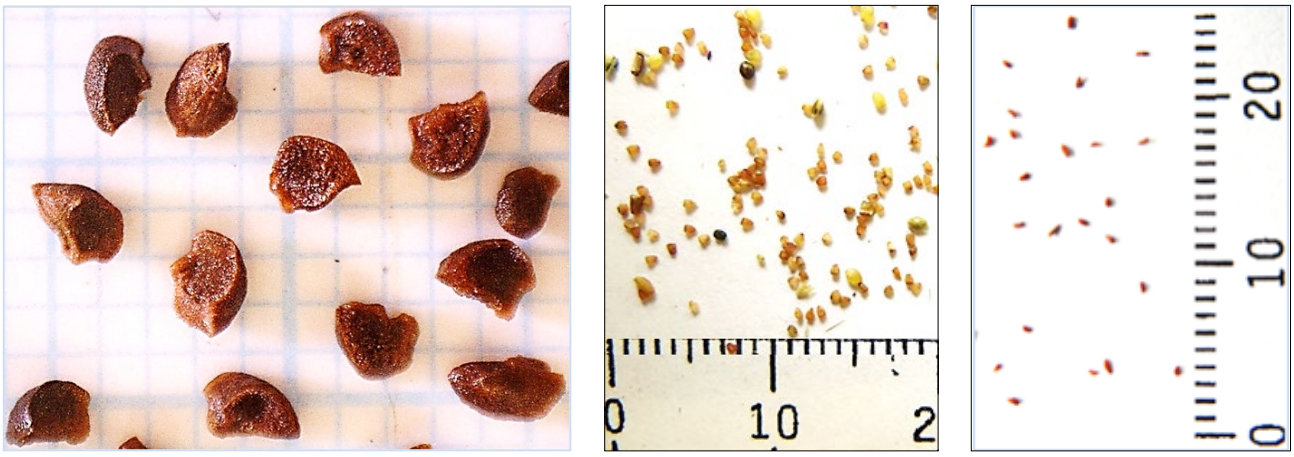


Figure 4. Mixed seed and chaff of (left to right) *Eucalyptus macrorhyncha*, *Sporobolus* spp. and *Melaleuca bracteata* where separation is challenging due to small seed size. (Photo: L. Ruiz-Talonia)

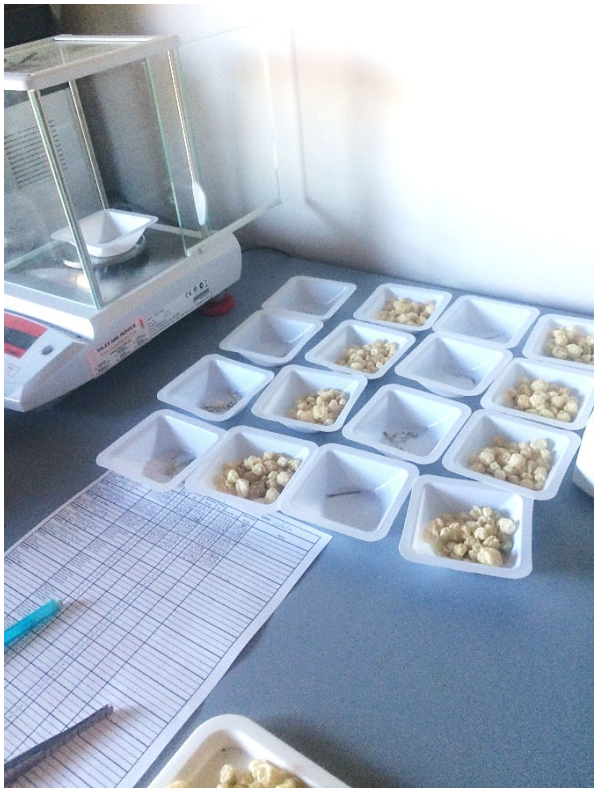


Figure 5. A purity test with 8 replicates divided into inert material and seed. (Photo: A. Quarmby)

Seed fill testing

Where the diaspore of a species is seed, seed fill refers to whether or not a seed contains an apparently viable embryo (and endosperm, for some species) (See Box 1). If the diaspore is a fruit, seed fill will be the number of filled seed contained within the fruit. For seed and single-seeded fruit, seed will be simply filled or empty whereas for multi-seeded fruit, seed fill might be a number > 1. Seed fill can be assessed by cut test or X-ray analysis. There are benefits and drawbacks for each method. In both cases, staff doing cut tests and X-ray analysis need to be trained to do the tests. For some species, a lot of experience is needed to interpret the results.

Box 1. What's inside a seed?

Understanding internal seed morphology, or, what the inside of a seed looks like, is really helpful for testing seed fill and viability (Baskin and Baskin 2007; Martin 1946). In general, seeds consist of a seed coat and an embryo. The seed may also contain storage reserves, usually endosperm (or perisperm). In some species, the embryo is well developed, and the cotyledons (leaves inside the seed) and radicle (root) are differentiated (Figure 8). In other species, the embryo is not differentiated into cotyledons and a radicle (Figure 7). Embryos may have one (monocotyledonous, e.g. grasses (Figure 6)), two (dicotyledonous, e.g. *Acacia*) or many cotyledons (multicotyledonous, e.g. *Persoonia*). Useful photographic records of seed structure and cuttings as well as information can be found in printed and online references, e.g. the Seed Information Database (Royal Botanic Gardens Kew 2021).

Figure 6. *Rytidosperma caespitosum* floret (left), seed containing a lateral embryo (right). (Photos: A. Quarmby)

Figure 7. *Gahnia aspera* (Cyperaceae) whole fruits (left) and sectioned fruits (right) with a visible capitate embryo surrounded by endosperm. (Photos: L. Ruiz-Talonia)

Figure 8. *Petalostigma pubescens* (Picrodendraceae) has a fully developed spatulate embryo. From left to right; whole seed, longitudinal section, cross section. (Photo: L Ruiz-Talonia)

Cut test

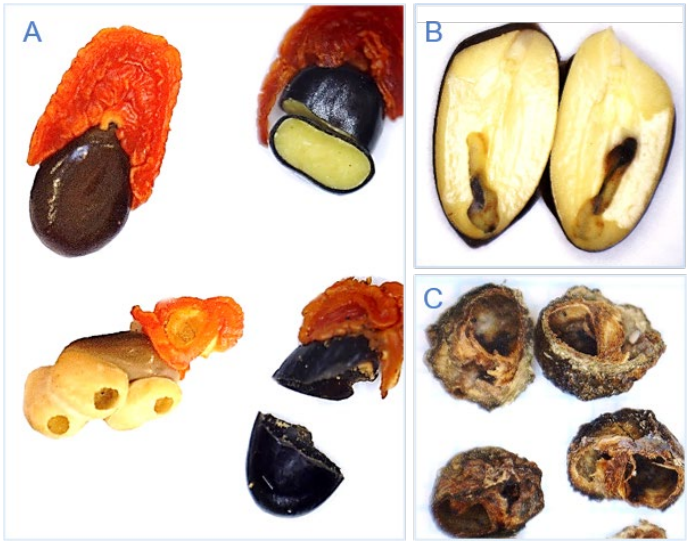


Figure 9. Assessing seed fill of (A) *Acacia salicina* (the two seeds on the top are visually healthy and seeds on the bottom are infested and empty), (B) *A. harpophylla* (damaged) and (C) *Psydrax odorata* (empty). (Photos: L. Ruiz-Talonia)

A cut test involves dissecting the seed (diaspore) or cutting it in half, and inspecting the appearance of the internal structures (endosperm and/or embryo) to distinguish between obviously dead seeds, unfilled seeds, non-seed material, and potentially viable seeds (Figure 9, Figure 10). Seeds containing an embryo (and endosperm) are considered filled, whereas seeds which do not contain an embryo (or endosperm) are considered unfilled (or empty). Generally, a healthy, potentially viable embryo is firm, fresh, healthy in appearance and white to ivory in colour, sometimes ranging to yellow. However, this is not always the case as seeds which have not survived storage may have a similar appearance. A green embryo may be an indication of immaturity, or a **recalcitrant** seed, but may also be (rarely) mature and viable (e.g. *Tetratheca*). A seed which is discoloured inside (i.e. brown, grey, black) is considered non-viable. Cut tests can also ascertain if a seed has been eaten by a granivore.

A cut test is a simple, inexpensive method of determining seed fill. It requires minimal equipment, such as a scalpel or razor blade. A magnifying glass or dissecting microscope can be useful. Disadvantages of cut tests are that they are labour intensive and destructive, are not always feasible for very small seeds, and do not always give an accurate assessment of seed viability. As with the other tests, it does take training and practice to become competent in seed dissection and interpreting the results.

It may be useful to take photos of dissected seeds, to help train staff to differentiate between filled and unfilled seeds of each species. Also, if cut tests are performed before and after storage, photos of dissected fresh seeds can be used for reference.

Figure 10. *Pomaderris* sp. whole seed (left) and dissected seed (right) showing both filled and empty seeds. (Photos: L. Ruiz-Talonia)

X-ray analysis

X-ray cabinets for seed testing usually fit on a bench top and are attached to a computer, which can control the X-ray settings and display the X-ray image (Figure 11). To conduct an X-ray test, place seeds in a petri dish, or separate them individually within a well-plate (Figure 12). Place the petri dish or plate on the shelf within the X-ray cabinet (place the shelf at the top if assessing small seeds, and on a lower shelf if assessing large seeds). Set the time and power and take the image. The shelf position, time and power of the X-ray (measured in kV) can be manipulated until a clear image is taken.

X-ray analysis is quick and non-destructive means by which seed fill can be determined meaning that tested seed can be used for other purposes afterwards. X-ray testing can also provide a photographic record of the test for future reference (Figure 13). Disadvantages of the X-ray test is that the equipment is expensive, requires regular checking (as it is a radiation device) and requires a higher level of training to use the X-ray equipment than the cut test. There are also safety considerations when using X-ray equipment. Also, X-ray testing assesses seed fill, not seed viability. X-ray analysis can in some cases identify seeds which have been eaten by granivores or infested by insect larvae.

Figure 11. An X-ray cabinet used for seed fill testing with an attached computer showing the image. The shelf in the cabinet can be adjusted up or down according to the number and size of the seeds. (Photos: L. Commander)

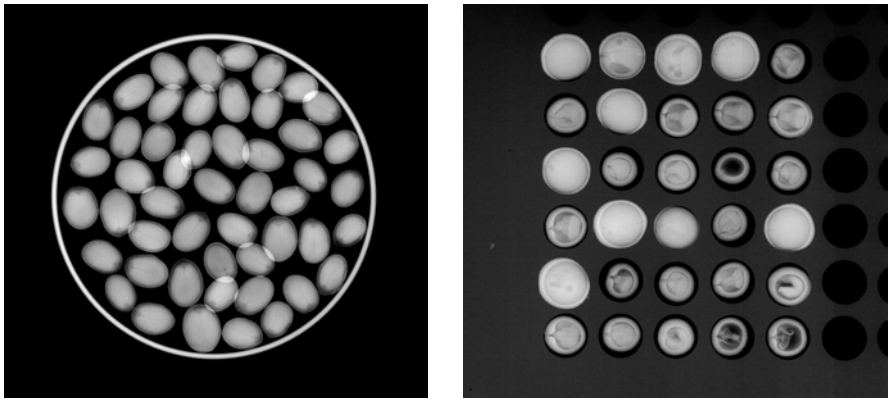


Figure 12. Seeds can be (left) placed in a petri dish, or (right) individually placed in well plates. (Photos: L. Commander)

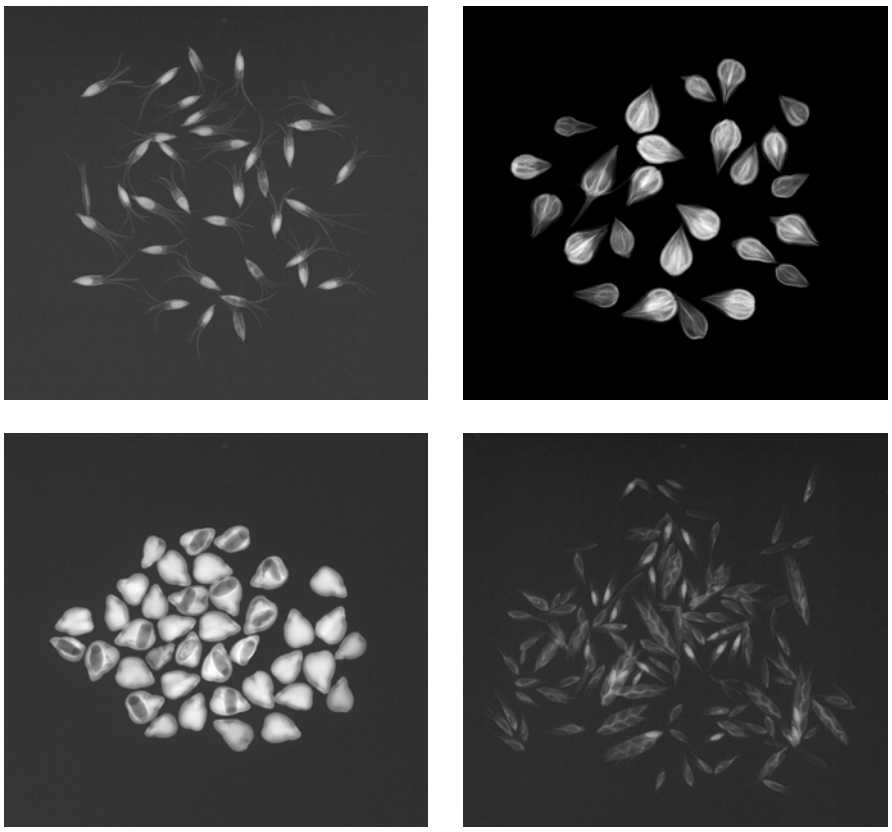


Figure 13. X-ray images of florets of *Amphipogon* sp. (top left), *Eremophila* sp. which are multi locular and contain multiple seeds (top right), *Senna artemisioides* subsp. *oligophylla* of which several have been eaten by predators (bottom left), and *Triodia pungens* florets most of which do not contain seeds (bottom right). (Photos: L. Commander)

Calculating seed fill

To measure seed fill, a sample obtained from the pure seed fraction of the purity test is assessed using either the cut or X-ray test. For seeds, seed fill is calculated by dividing the number of filled seeds by the total number of seeds in the sample (equation 5).

$$Seed\ fill\ (\%) = \frac{number\ of\ filled\ seeds}{total\ number\ of\ seeds\ in\ the\ sample} \times 100$$

Equation 5. Seed fill percentage calculation

For fruit which contain more than one seed, two measures of seed fill can be calculated; number of seeds per fruit (equation 6), proportion of fruit containing at least one seed (equation 7).

$$Seeds\ per\ fruit = \frac{total\ number\ of\ seeds\ in\ a\ fruit}{total\ number\ of\ fruits\ in\ the\ sample}$$

Equation 6. Seed per fruit calculation

$$Percentage\ of\ fruit\ containing\ at\ least\ one\ seed\ (\%) = \frac{number\ of\ fruit\ with\ one\ or\ more\ seeds}{total\ number\ of\ fruits\ in\ the\ sample} \times 100$$

Equation 7. Percentage of fruit containing at least one seed calculation

Like seed purity, seed fill does not change following storage, unlike seed viability. So, seed fill can be assessed once, unless seeds are processed further to attempt to remove the unfilled fraction.

Seed viability testing

Seed viability refers to whether or not the seed is alive and capable of germination. Viable seeds are those which contain a live embryo, whereas non-viable seeds are dead, i.e. they do not contain a live embryo.

Seed viability decreases as seeds age. The rate at which viability is lost will be species and collection specific, and is dependent on storage conditions (see Module 9 – Seed Drying and Storage). A viability test measures viability at a point in time, hence viability tests should be cited along with the collection and test dates, to indicate the age of the seeds when tested and how long ago the test was performed. The two most commonly used tests for calculating seed viability are the germination and tetrazolium tests. Cut tests can be used as a measure of seed viability, but they are less accurate than germination and tetrazolium tests.

Germination test

A germination test involves germinating a sample of a seed from a collection using treatments and conditions conducive to germination (Figure 14). These conditions and treatments should be controlled, well documented and readily repeatable. Germination can be quoted as a percentage (Equation 8) or as a proportion (e.g. 10 out of 40 seeds germinated). The advantage of the germination test is that if seeds germinate then they are obviously viable, however failure to germinate does not necessarily mean that seed is not viable, it may be that the germination conditions are not conducive to seed germination or the seed may be dormant (see Module 11 – Seed Germination and Dormancy for more details). If dormant seed are present in a seed collection, and a standardised way of overcoming that dormancy is not known, then the germination result will be an underestimate of the actual viability of a seed lot. At the conclusion of a germination test, if there are any remaining non-germinated seed, a cut test (see above) can be conducted to distinguish between filled (and potentially viable seed that may be dormant), unfilled seed and obviously dead seed. (Equation 9). Alternatively, a tetrazolium test (see below) could be conducted on any remaining seed to distinguish between viable and non-viable seed. Then, result of the germination test can be combined with a cut test or tetrazolium test after the germination test is finished to estimate viable proportion (Figure 1), and viability adjusted germination % can be calculated (Equation 10). Further information on seed germination testing is covered in Module 11 – Seed Germination and Dormancy.

It is possible to assess the seedlings resulting from the germination test. They can be classified as normal or abnormal, where a normal seedling has all the structures needed for it to develop into a plant.

$$Germination\ (\%) = \frac{total\ number\ of\ seeds\ that\ germinate}{total\ number\ of\ seeds\ in\ the\ sample} \times 100$$

Equation 8. Germination percentage calculation

$$Viability\ (\%) = \frac{number\ of\ seeds\ that\ germinate\ + number\ of\ non-germinated\ \ viable\ seeds}{total\ number\ of\ seeds\ in\ the\ sample} \times 100$$

Equation 9. Viability percentage calculated using a germination test followed by a cut test

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

Equation 10. Viability adjusted germination percentage calculation

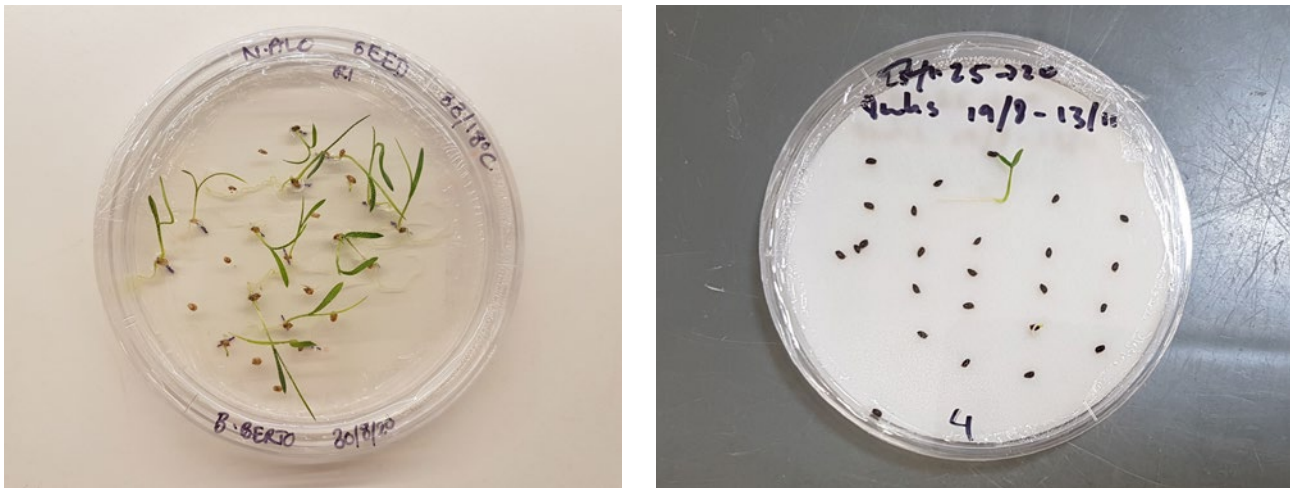


Figure 14. Germination testing in petri dishes on (left) agar and (right) filter paper. (Photos: L. Commander)

Tetrazolium test

Another method to test seed viability is to use a chemical which stains living tissue in the embryo (Figure 15, Figure 16). The most used viability staining technique is the tetrazolium test (TZ) which uses a 1% (w/v) solution of the chemical 2,3,5-Triphenyl tetrazolium chloride. Lower or higher concentrations can be used, but then soaking duration and temperature are modified. Using this technique, seeds are placed in a colourless solution of TZ and through a biochemical process, living tissue is stained red. Procedures for the use of the tetrazolium test, including the preparation of the solution, are detailed in chapter 6 of the ISTA guidelines (ISTA 2020) and in Terry et al. (2003), and is summarised below:

- Firstly, seeds are pre-moistened with water to allow them to imbibe. Seeds with a water impermeable seed coat (see Chapter 9 – Seed germination and dormancy) need to be treated to render the seed coat permeable, for example by nicking or chipping to scarify the seed coat.
- Seeds are then either cut open to expose the internal tissue, the seed coat is removed, or an incision in the seed coat is made, and seeds are placed into the TZ solution.
- The seed are then placed in the dark (e.g. container wrapped in foil) and incubated at 30°C for a period of time dependent upon the species being tested.
- After incubation, the embryo is examined for staining. Seeds with an embryo which stain red are considered viable, and those with an embryo which does not stain are considered non-viable. Sometimes incomplete staining of the embryo may occur. In these instances, a knowledge of the morphology of the embryo is required. Where all embryo structures considered essential for germination have stained, the seed may be considered viable.

Disadvantages of the TZ stain are that it is a destructive test; it requires a high level of training and experience; it is difficult to use and assess staining in very small seed, or for seed with very small or rudimentary embryos; and that the technique needs to be refined for a given species before it can be reliably used. A Material Safety Data Sheet for the chemical should be obtained, and correct safety procedures put in place for storage, use and disposal. Also, there are no standards for staining patterns of most Australian native species, unlike agricultural and horticultural species. Hence, staining patterns may be interpreted differently by different people.

Figure 15. Tetrazolium test of *Maireana georgei* – the red embryos are likely to be viable, the white/yellow embryos are not likely to be viable, and those that are partially stained red, or stained orange may or may not be viable. (Photo: L. Commander)

Figure 16. Tetrazolium test of *Themeda triandra* (Kangaroo Grass) – the embryos are lateral, and seeds contain endosperm. The embryos which have stained red are likely to be viable, and the white embryos are likely to be non-viable. Note that the endosperm does not stain, and has remained white. (Photo: L. Ruiz-Talonia)

Testing very small seeds

Very small seeds such as Orchidaceae, *Juncus* and *Wahlenbergia* may be very challenging to test (Dowling & Jusaitis 2012). In some circumstances, specialised tests will need to be developed for seeds of this type..

Calculating number of viable seeds per gram

The quality measure of viable seed per gram, and to a lesser degree seed per gram, allow for a standardised comparison of seed cost between suppliers on a per viable seed, or per seed basis. This measure can also assist with the calculation of seeding rates (see Module 14 – Direct Seeding).

The number of viable seeds per gram takes into account the viability and weight. To calculate this:

- take one or more replicate samples from the seed lot (sample size depends on the number of seeds and the purity – if the sample is too big the test will be time consuming and use a lot of seed, too small and the results may not be an accurate representation of the seed lot);
- weigh the sample(s) and record the weight(s);
- separate the seed from the chaff;
- count the number of seeds;
- do a viability test on the seeds (either on all the seeds, or a subsample); and
- calculate the percent viability.

The number of viable seeds per gram can be calculated using Equation 11. Then, if several replicate samples are assessed, the mean viable seeds per gram can be calculated using Equation 12.

$$Viable\ seeds\ per\ gram\ per\ sample = \frac{number\ of\ seeds\ in\ the\ sample\ \times viability\ \%}{weight\ of\ sample}$$

Equation 11. Viable seeds per gram of an individual sample

$$Mean\ viable\ seeds\ per\ gram = \frac{Viable\ seeds\ per\ gram\ \ (sample\ 1) + Viable\ seeds\ per\ gram\ \ (sample\ 2) + \ldots}{Total\ number\ of\ samples}$$

Equation 12. Mean viable seeds per gram

Pure Live Seed (PLS) is the percentage of seed (i.e. good viable seed) that has the potential to germinate (i.e. is viable) within a measured one-pound weight of any seed lot (USDA 2009). See Equation 13.

$$Pure\ Live\ Seed\ (PLS)\ \% = \frac{purity\ (\%) \times viability\ (\%)}{100}$$

Equation 13. Pure live seed percentage calculation

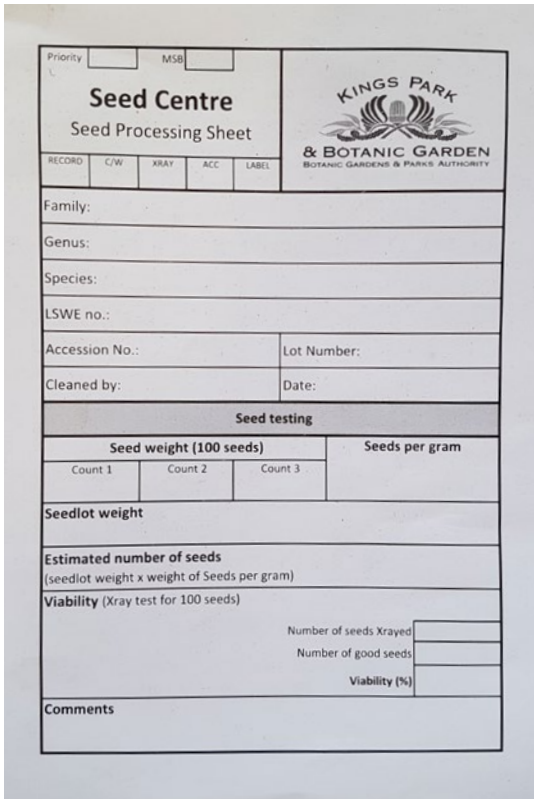
When to test, how often to test

Seed purity can be tested once, as it will not change unless the seed is blended with another material, processed further, or the seed is predated in storage. Seed fill can also be tested once, unless there's a possibility that there are predators in the sample, in which case, they may adversely affect seed fill. If purity and viability are measured more than once and appear to be variable, then sampling techniques may need to be improved.

Seed viability will decline over time, with the rate of decline dependent upon the species and storage conditions. Storage behaviour may be considered orthodox, intermediate or recalcitrant – and this will influence how long and under what conditions seeds can be stored. Also, seed longevity of Australian species can vary significantly (e.g. see Merritt et al. 2014). In general, seed viability should be tested after processing and prior to storage, periodically during storage and when seed is withdrawn from storage for use or sold, and viability tests should always be reported with the seed collection and test date (i.e. seed age / storage time) (see also Module 9 – Seed Drying and Storage and Module 11 – Seed Germination and Dormancy). Testing of seeds in long term storage (-20°C) should occur every ten years, or less if the seed is known to be short-lived or is poor quality. If a significant decline in viability is detected, re-test 5 years thereafter (Davies et al. 2015). For seeds in conditions suitable for short (<5 years) and medium (5-10 years) term storage (see Module 9 – Seed Drying and Storage), re-test schedules are ideally based upon the purpose of the collections, and their expected longevity. Given that seed viability can decline over time, viability test results should always be reported along with the test date. Seed viability at the point of sale or end use is the most critical quality measure for seed users and restoration outcomes.

Reporting / labelling

Results of seed tests can be written on a seed record sheet (Figure 17) and should all be recorded in a database or spreadsheet (Table 1), along with the date of the test (especially for seed viability testing) with a unique seed lot number or accession number linking all records kept for each seed lot. For germination testing, a description of the conditions used for the test (temperature, container, substrate) and a description of any pre-treatments applied should be recorded. Also, keep records of the internal morphology of seeds, photos of cut tests and tetrazolium tests, X-rays etc. to use for training and future reference. Seed lot labels should contain important information that should be passed on to the purchaser or end user (Figure 18). See also Module 4 – Record Keeping.



Priority | MSB

Seed Centre
Seed Processing Sheet

KINGS PARK & BOTANIC GARDEN
Botanic Gardens & Parks Authority

RECORD	C/W	XRAY	ACC	LABEL

Family:

Genus:

Species:

LSWE no.:

Accession No.: | Lot Number:

Cleaned by: | Date:

Seed testing

Seed weight (100 seeds)			Seeds per gram
Count 1	Count 2	Count 3	

Seedlot weight

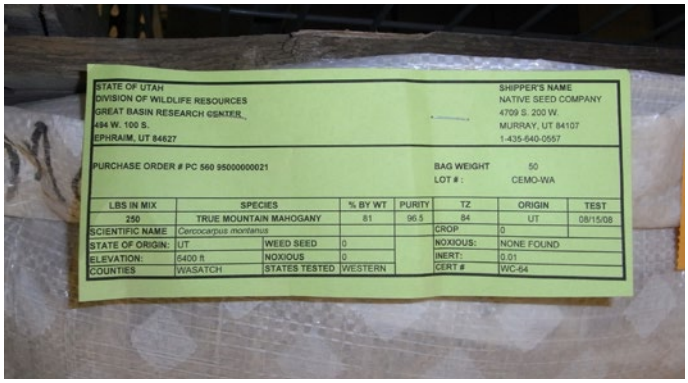
Estimated number of seeds
(seedlot weight x weight of Seeds per gram)

Viability (Xray test for 100 seeds)

Number of seeds Xrayed
Number of good seeds
Viability (%)

Comments

Figure 17. An example of a seed record sheet. (Photo: L. Commander)



STATE OF UTAH
DIVISION OF WILDLIFE RESOURCES
GREAT BASIN RESEARCH CENTER
494 W. 100 S.
EPHRAIM, UT 84627

SHIPPER'S NAME
NATIVE SEED COMPANY
4709 S. 200 W.
MURRAY, UT 84107
1-435-640-0557

PURCHASE ORDER # PC 560 95000000021

BAG WEIGHT 50
LOT # : CEMO-WA

LBS IN MIX	SPECIES	% BY WT	PURITY	TZ	ORIGIN	TEST
250	TRUE MOUNTAIN MAHOGANY	81	96.5	84	UT	08/15/08
SCIENTIFIC NAME	*Cercocarpus montanus*			CROP	0	
STATE OF ORIGIN:	UT	WEED SEED	0	NOXIOUS:	NONE FOUND	
ELEVATION:	5400 ft	NOXIOUS	0	INERT:	0.01	
COUNTIES	WASATCH	STATES TESTED	WESTERN	CERT #	WC-64	

Landmark Native Seed
522 W. RIVERSIDE AVE. STE. 430 / SPOKANE, WA
UNCERTIFIED ALSIKE CLOVER
LOT NO: 7-0011

PURITY:	99.20 %		
OTHER CROP:	0.02 %	TEST DATE:	10/2008
INERT MATTER:	0.21 %	ORIGIN	CAN
WEED SEED:	0.07 %	NET WT:	50 LBS
GERM:	91.00 %		

NO RESTRICTED NOXIOUS WEEDS
PURCHASE ORDER: 560 05000000004
AMS: 3750

Figure 18. Seed labels used in Utah, USA, indicating purity % and other seed lot characteristics. (Photo: L. Commander)

Useful equipment for seed testing

If setting up an area for seed testing, here is a list of useful equipment:

- Dissecting equipment such as forceps (tweezers), scalpels and razor blades of different sizes (Figure 19A).
- Sharps bin for disposing blades.
- Tile, craft cutting mats or plastic kitchen chopping boards on which to cut.
- Trays, sheet of glass, masonite or Laminex, paper or cardboard, cups, spoons, spatulas and rulers for sampling.
- Containers for holding or weighing samples.
- Petri dishes and filter paper for tetrazolium testing.
- Containers (e.g. petri dishes, trays, pots, punnets, plastic food containers) and media. (e.g. agar, filter paper, sand, potting mix, perlite, cotton wool) for germination testing.
- Bench magnifier, desktop magnifying light, hand lens (Figure 19B).
- Dissecting microscope / binocular microscope (Figure 19C).
- Sieves for separating seed from chaff for purity testing (Figure 19D).
- Stand to turn a mobile phone camera into a microscope (or make your own out of plywood and plexiglass (e.g. https://www.instructables.com/10-Smartphone-to-digital-microscope-conversion/).
- Balances, accuracy depending on use, e.g. accurate to 0.01 g (but ideally accurate up to 4 decimal places) for small seed lots or samples (Figure 19E); capable of weighing up to 10 or more kilos for heavy seed lots.
- Recording sheets for recording information about each seed lot (Figure 17).
- 2,3,5-Triphenyl tetrazolium chloride powder.
- Aluminium foil and plastic wrap.
- Zip-lock bags.
- Computer for recording results. Testing results can be recorded in a spreadsheet or database. For more information, see Module 4 – Record Keeping.

More expensive equipment that suits specialist seed testing:

- X-ray cabinet (Figure 11).
- Temperature and light controlled cabinets for germination testing (Figure 19F).
- Controlled temperature cabinet to achieve 30°C for tetrazolium testing.
- Oven for determining seed moisture content (see Module 9 – Seed Drying and Storage).
- Microbalance, accurate to 0.0001g.
- Microscope with camera attached to create a library of images for training, reference and records (Figure 19G).
- Mechanical seed counter (Figure 19H).
- Light box.

Figure 19. (A) Dissecting equipment including scalpels, forceps and razor blades. (B) Bench magnifier with a light. (C) Dissecting microscope. (D) Sieves and containers for separating seed from chaff for purity testing. (E) A balance used for weighing seeds. (F) Temperature and light controlled cabinet for incubating seeds for germination testing. (G) Microscope with a camera attached for taking photos of seeds. (H) Mechanised seed counters. (Photos: L. Commander)

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Glossary

Dehiscent: fruits that open to release seeds.

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

Dispersal unit: see diaspore.

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 is favourable for its germination, i.e. after the seed becomes non-dormant.

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.

Imbibition: absorption of water by seeds. As seeds imbibe and hydrate, water causes turgor pressure (seeds swell). Imbibition is necessary to activate enzymes and transport nutrients to the developing seed embryo, as well as to break down starch into sugars.

Indehiscent: fruits that do not open to release seeds.

Inert matter: non-seed material, including seed-like structures, stem pieces, leaves, sand particles, stone particles, empty glumes, lemmas, paleas, chaff, awns, stalks longer than florets and spikelets, insect parts, pieces of broken or damaged seeds half or less than half the original seed size, fungal bodies like ergot or sclerotia, nematode galls, rodent droppings, flowers.

Intermediate: seeds that display properties between orthodox and recalcitrant.

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

Orthodox: desiccation tolerant seeds, i.e. seeds that survive drying (cf. recalcitrant).

Purity: composition by weight of the pure clean seed of a species (obtained after the elimination of seed of other species and **inert matter**) expressed as a percentage of the mass of the whole sample. Pure seed includes immature, undersised, shrivelled, diseased or germinated seeds. It also includes broken seeds, if the seed is more than half of the size of the original seed, but not pieces of broken or damaged seeds half or less than half the original seed size (which is classed as **inert matter**).

Recalcitrant: desiccation intolerant seeds, i.e. seeds that do not survive drying (c.f. **orthodox**).

Seed lot: a uniquely identified collection of seeds, of a single species and/or variety, sourced from the same location, from a single collection period.

Seed processing: is the preparation of harvested seed for utilisation or storage. It may include drying, threshing, cleaning, and sorting.

Unfilled seed: seed that is empty and does not contain an **embryo** (or **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.

Online resources

Hand halving method
https://www.youtube.com/watch?v=OMevCxMp9FM

International Rules for Seed Testing, Chapter 2
https://www.seedtest.org/upload/cms/user/ISTARules202002Sampling.pdf

Revegetation Industry Association Western Australia, Seed industry standards and accreditation guidelines
https://www.riawa.com.au/assets/documents/01-RIAWA-Seed-Standards-191021.pdf;
https://www.riawa.com.au/accreditation/documents

Royal Botanic Gardens, Kew, Millennium Seed Bank Seed Information Database
https://data.kew.org/sid/

Smartphone to digital microscope
https://www.instructables.com/10-Smartphone-to-digital-microscope-conversion/

South Australian Seed Conservation Centre
https://spapps.environment.sa.gov.au/seedsofsa/

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

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