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Guide to handling of tropical and subtropical forest seed

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Other Chapters of the book Guide to Handling of Tropical and Sub-Tropical Forest Seed by Lars Schmidt soon available on www.dfsc.dk

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11.1 Introduction

Seed testing is used for control of quality parameters during seed handling, and test results are submitted to customers as documentation on seed quality (chapter 14 and 15). Standard parameters such as seed weight, purity, and germination or viability enter as factors in the calculation of seed demand (section 3.4.2, table 3.5). Since seed is sold on a weight basis, these seed quality parameters are essentially also economic parameters. Moisture content in seeds is particularly important during storage (chapter 8).

Quality tests may be carried out at intervals from harvest until the seeds leave storage to be dispatched or sown in the nursery (Yue-Luan 1993). Most tests are simple non-standardized tests serving as practical guidelines during daily seed handling and nursery practice. Such 'simple tests' range from simple cutting tests which are little more than observations, to moisture tests using calibrated field moisture meters. In these cases a quick result is the more important and some inaccuracy will be tolerated.

In order to make data of different seed lots and species comparable, e.g. for marketing and research, tests must be standardized and replicable. The widely adopted methods of standardized seed testing follow the prescriptions of the International Seed Testing Association (ISTA)¹, which were first formulated in 1931 and now are revised and updated every three years. The ISTA rules (ISTA 1996) contain, in addition to standard seed testing procedures, specific guidelines for a number of species. Specific guidelines on testing tropical and subtropical forest species occur in a recently published ISTA handbook (ISTA 1998). In addition ISTA has published a number of more elaborate handbooks on individual seed testing procedures (see list of references).

Standard seed testing requires a fairly advanced seed laboratory, capable of conducting all routine tests according to the international rules. In particular germination tests require a high investment in equipment for germination chambers with control of temperature, light and moisture. Such investments are far beyond the possibility of most field stations to which this guidebook is mainly addressed. Therefore, advanced methods of seed testing will only be superficially described

¹ Several American countries follow the rules of Association of Official Seed Analysts (AOSA) which, however, differ only in minor aspects to the ISTA rules.

11.2 Terminology

in this book. It should, however, be stressed that although it may not be practically possible to perform tests according to the international standards, the ISTA prescriptions contain much information on e.g. pre-treatment and germination conditions which are also useful under less strict test procedures. Although advanced testing procedures are omitted here, a certain basic knowledge is necessary to be able to interpret results from the seed laboratory. Standard parameters such as seed weight, purity and viability are therefore thoroughly explained. A fairly detailed description of sampling is given since the principle of sampling is very important for both simple and advanced testing. In the case of standard testing, seed suppliers are normally the ones to draw samples from seed lots and forwarding them for testing by authorized seed testing laboratories.

For more detailed and elaborate information on how to carry out seed testing, reference is made to above mentioned ISTA publications, publications from ASEAN Tree Seed Centre Project (ASEAN 1991, Yue-Luan 1993, Bhodthipuks *et al.* 1996), and Danida Forest Seed Centre (Poulsen 1993 and 1994)

Seed testing is an analysis of some physical parameters and the physiological quality of a seed lot, based on a small representative sample. The 'quality' (here strictly referring to physiological quality, in contrast to the genetic quality) is the measure of potential performance of a seed lot under optimal conditions. Seed testing includes a number of parameters such as seed weight, purity, viability, germination and moisture content, each with its own test procedure as will be defined and outlined below. In the strict sense, testing always implies a standard procedure, which may be subjected to statistical analysis. In common terms, 'testing' applies to anything with the character of a more thorough examination. In order to emphasize the distinction between the strict and common understanding of the term 'test', this book distinguishes between 'standard testing' and 'simple testing', where the former refers to tests carried out according to ISTA or AOSA rules.

Seed testing is carried out on a **sample** that is a small representative part of the seed lot. The terminology of the various units of sampling is outlined in section 11.4. According to ISTA (1986) a **seed lot** is defined as a 'stated portion of the consignment assumed to be reasonably uniform'. What is 'reasonably uniform' is ultimately the judgement of the seed handler. Normally seeds of the same provenance and the same seed source, collected approximately the same date are bulked before processing and hence typically make up a seed lot. It is impractical to keep too many seed lots separate, and if a reasonable level of uniformity exists, seed lots from different collections may be bulked into one larger lot. Provenances should, however, always be kept separate. It may also be reasonable to split up a large collection into smaller seed lots. This is typically the case for very large quantities of seeds (ISTA rules set an upper limit for the size of seed lots, typically 1000 kg, or 5000 kg for very large seeded species), or if different parts of the seed lot are exposed to different conditions likely to influence uniformity e.g. during processing or storage.

11.3 Timing Seed Testing

A standard test has a certain **design**, which describes how a test is carried out. It always has a number of **replicates** which are similar tests carried out on the same number of seeds from the sample. Five replicates mean that five identical tests are carried out with an equal number of seeds. Replications allow calculation of statistical parameters such as mean and variance, and minimize the chances of an erroneous result.

Preliminary tests to assess maturity and seed quality at harvest are described in chapter 3. It may be relevant to carry out a test on moisture content and possible fungal and insect infestation immediately after harvest to guide preliminary handling before processing e.g. the need for further drying, measures to prevent or reduce fungal damage. If fruits or seeds are temporarily stored for a prolonged period before processing, an interim test may indicate possible deterioration, and whether continuous storage under the given conditions is safe. This test contains the same elements as testing after harvest, i.e. moisture content and infection rate.

If processing is carried out as one continuous procedure, testing during processing is normally not applicable. However, purity tests may be carried out at intervals during seed cleaning to suggest how far cleaning should continue, and moisture content analyses may determine the necessity for further drying (Yue-Luan 1993).

A more thorough test may be carried out between final processing and storage. This test usually contains all the standard elements such as purity, seed weight, moisture content and viability. As these data are the ones normally to be submitted with the seed lot, the tests should as far as possible be carried out by an authorized seed laboratory.

If the seed lot is stored for a prolonged period, or shorter period under conditions where viability is likely to be impaired, viability test(s) during storage may be applicable. Assessment of viability after a certain time of storage may also help to predict the potential storage life of the seed lot (cf. chapter 8). Where seed lots are dispatched to customers, the result of the most recent viability test is obviously the one to accompany the seed lot. Where seeds are stored under ambient conditions, moisture-content analysis during storage is recommended to detect possible increase in moisture content. A seed health test may be carried out before storage to detect possible seed-borne pest and pathogens. Vigour tests may be relevant for seed lots which show high germination capacity under test conditions, but where some deterioration is suspected e.g. after prolonged storage. A guideline on timing of seed testing is summarized in table 11.1.

Table 11.1.
Seed testing during the
period of seed handling.

	Harvest	Processing	Before storage	During storage
Simple tests	Maturity, health (cutting test) Moisture content (moisture meter)	Health (cutting test) Moisture content (moisture meter) Purity (screening)		
Standard tests			Seed weight Purity Moisture content (oven method) Viability/germination (TTZ, X-ray, germination etc.)	Viability/germin-ability Moisture content
Special tests			Health test (Vigour)	Health test Vigour

11.4 Sampling

Whether a sample is to be submitted for standard testing or is used for more simple tests, it must comply with the basic rule of being representative of the whole seed lot in any aspect to be tested. A sample should thus have the same average seed size, moisture content, viability etc. as the whole seed lot. The higher the degree to which a sample is representative of the seed lot from which it was taken, the better the test results can be valid for the whole lot. The importance of proper sampling becomes more evident when expressed negatively: if a sample is not representative, then the quality of the seed lot cannot be concluded from the test results and the whole exercise is wasted. A practical way of testing representativeness is to carry out test on two individual samples: if sampling is ideal, the results of two individual samples should give the same result with regard to all tested aspects within the magnitude of statistical error. Thorough theoretical background and practical guidelines on sampling are found in 'ISTA handbook on seed sampling' (ISTA 1986).

Sampling is subject to a number of practical difficulties because a seed lot is never fully homogeneous. For example, seeds stored in bags or containers tend to stratify themselves according to gravity and any other physical features during handling (ISTA 1986). Further, the environment of the immediate vicinity of the seed influences seed characters. The external environment will have a higher influence on seeds located at an outer or upper position of the seed lot than those located in the interior. Hence, a sample taken from the top of a container may contain seeds which are on average smaller, lighter, drier or have different viability than the average seed. Also impurities tend to be stratified by the impact of mechanical handling. If a seed lot contains a lot of inert matter, purity percentage taken from the top and the bottom of containers or bags may be very different (Peterson 1987). The characters of individual seeds vary in many aspects that may influence their being representative, e.g. size, maturity and infestation. Therefore a sample must be large enough to cover

the full variation within the seed lot. Yet the individual test rarely comprises more than 5 replications of 25-100 seeds.

All techniques applied in sampling aim at obtaining samples which are representative of the lot from which they were taken. Two main pre-conditions help to assure homogeneity:

1. Variations of seed characters within the seed lot should be as small as possible. Therefore, apparently different seed lots should be kept separate and tested individually. Variations typically occur between different provenances, growth sites and degrees of maturity (cf. the concept of 'seed lot').
2. Homogeneity within the seed lot should be assured by thorough mixing. For large seed lots sub-samples should be taken from several locations within the seed lot. Where a large seed lot is stored in several individual containers, sub-samples are taken from each of these containers and according to their relative size, if their size varies. However, for seed lots stored in more than six containers, special rules apply for sampling frequency; samples may be taken from a smaller number of containers than the total, but the containers from which the samples are taken should be selected in an unbiased way.

11.4.1 Drawing samples

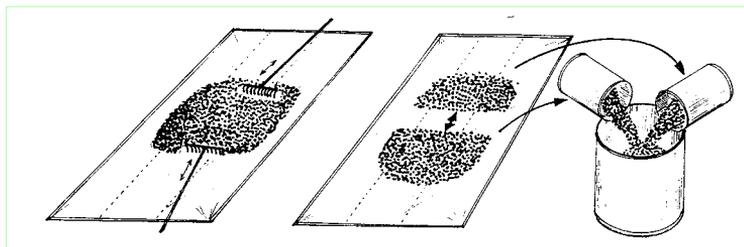
In principle, there are two ways of drawing test samples: (1) by subsequent divisions after mixing, and (2) by triers (fig. 11.2) taking out samples from different parts of the seed lot and then mixing them into a larger sample.

Mixing and division

Small seed lots of e.g. few kilos can usually achieve a high degree of uniformity by hand mixing. For larger lots, both hand and mechanical mixing can be quite unreliable. Especially when mechanical mixing is employed, both seed and impurities tend to distribute themselves in the lot according to physical features such as size and weight. The risk of stratification depends both on mixing procedure, seed and debris. Stratification is, obviously, a higher risk for seeds with a high variation in morphological characters. A seed lot of pines where only part of the seeds have lost their wings will for instance typically be distributed with the winged seeds on top and the de-winged seeds at the bottom.

Proper mixing is most easily assured manually: the seed lot is poured onto the floor or other smooth surface. Seeds are mixed by manually shovelling or raking from side to side. When the lot has been manually mixed, it is divided into equal parts. Each part is put into a container and the lots are then simultaneously poured into a larger container. The procedure may be repeated once or twice (Willan 1985). It is necessary to be at least two persons to carry out the mixing, and the quantity is limited to what can be easily lifted by the persons.

Figure 11.1.
Manual mixing of a
seed lot prior to sampling.



Larger seed lots are initially mixed as above and then shovelled into a pile. The pile is then divided into four parts. One part is taken out and mixed as above, or, if still too large to handle: mixed, piled and divided until it achieves a reasonable size. The procedure of division is employed because seed and debris tend to stratify while being piled up. Relatively small lots or manually divided lots may be divided into the required size by one to several successive divisions in a mechanical divider. Several types are available. They are normally used for subdividing submitted samples during seed testing (see below), but can also be used for drawing samples from seed lots. The principle of mechanical dividers appears from fig. 11.5. Some dividers separate seed lots into halves. The size of the sample may then be further reduced by several successive divisions. Other types of dividers are capable of separating the seed into several equal sections in one procedure. One part is then used as a sample for testing.

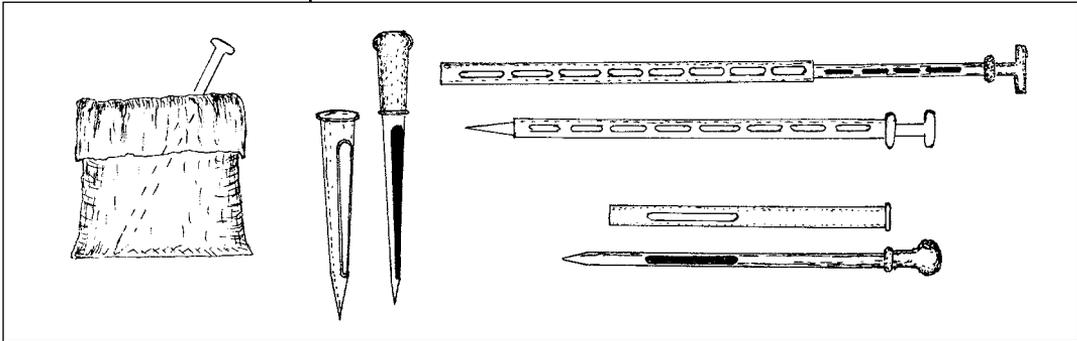
If seeds are stored in several containers and bags, yet belonging to the same seed lot, each part of the seed lot should contribute with a proportionally equal amount of seeds to the sample, i.e. a container with 20 kg of seeds should contribute twice as much to the sample as one containing 10 kg.

Triers

Thorough mixing of large seed lots prior to sampling is laborious and time consuming. A less thorough mixing (although a certain level of homogeneity should be achieved) may be allowed if sub-samples are drawn from different positions in the seed lot. In practice these sub-samples (in the ISTA terminology called 'primary samples') may be taken directly from containers or bags by the help of 'triers'. The design of commonly used trier types is illustrated in fig. 11.2. A trier consists of two tubes, one fitting outside the other like a sleeve. The two tubes have equal size rectangular slots along their side to allow seeds to flow in. The inner tube may be a continuous tube or transversely separated into several compartments or pockets by partings under each hole. The latter type is preferred since it gives a more even sampling, especially where seeds are not freely flowing (e.g. rough, angular or winged seeds). In the open tube type, seeds that enter into the top slots may tend to fall through the tube into the lower parts if these have not already been filled with rapidly inflowing seeds (Edwards and Wang 1995). This error may be reduced by inserting the trier into the container in a slightly slanting position (ISTA 1986). Opening and closing the holes or pockets of the trier is carried out by turning or sliding the inner tube with a handle. Where

the inner tube has no partings between the pockets (i.e. a continuous tube), the trier can be emptied through an upper opening. The end of the trier is tapered or pointed to allow its easy insertion into the seed lot. Several sizes of triers are available, the diameter and length vary according to seed size.

Figure 11.2.
Various types of triers used for
sampling in large seed lots.



Sampling by the help of a trier follows five steps.

1. Close the holes of the trier by sliding or turning the outer sleeve.
2. Insert the trier right down to the bottom of the bag, container or pile of seeds while keeping the holes closed.
3. Open the holes by sliding or turning the outer sleeve; move the trier slightly to assure that the seeds flow into the holes freely and fill the trier.
4. Close the holes by turning into closed position and pull out the trier.
5. Empty the trier into a pail. Trier types with no partings between the holes can be emptied through the top holes. If the trier has separate pockets, it must be emptied through the holes.

If the seeds flow freely into the holes or pockets, a representative sample will be taken from each level of the container. If the containers or bags contain the same amount of seed, each sample will take out the same percentage of the total. If the content of containers varies, the sample size should be adjusted accordingly. With containers of say 25 and 50 kg, one sample may be taken from the 25 kg container every time two samples are taken from each 50 kg container. Each sub-sample taken with the trier is put into a container for further handling as described below.

Triers are quick and convenient tools for sampling large seed lots. They can be used for all free-flowing seeds of small or intermediate size. However, large, rough, winged, angular and other seed types which are too big or do not easily enter into the trier must be sampled by other methods, e.g. by removing portions at different levels of the seed lot by hand, or by the above mentioned mixing and dividing method.

11.4.2 Reduction of sample size for testing

Several terms apply to the different types of samples during the sampling process (fig. 11.3): a primary sample is a small quantity of seed taken out from a single position in the seed lot, e.g. by the help of a trier. When a number of primary samples taken from different parts of the lot are bulked, they make up a composite sample. Usually this sample is several times larger than the sample actually needed for testing. For official seed testing, ISTA (1995) has issued prescriptions for the quantity of seeds needed by the seed laboratory to carry out standard tests. The quantity, which varies with species, is called the submitted sample. This sample is further reduced in the laboratory to a working sample according to the quantity required for the individual test (ISTA 1986, ISTA 1998).

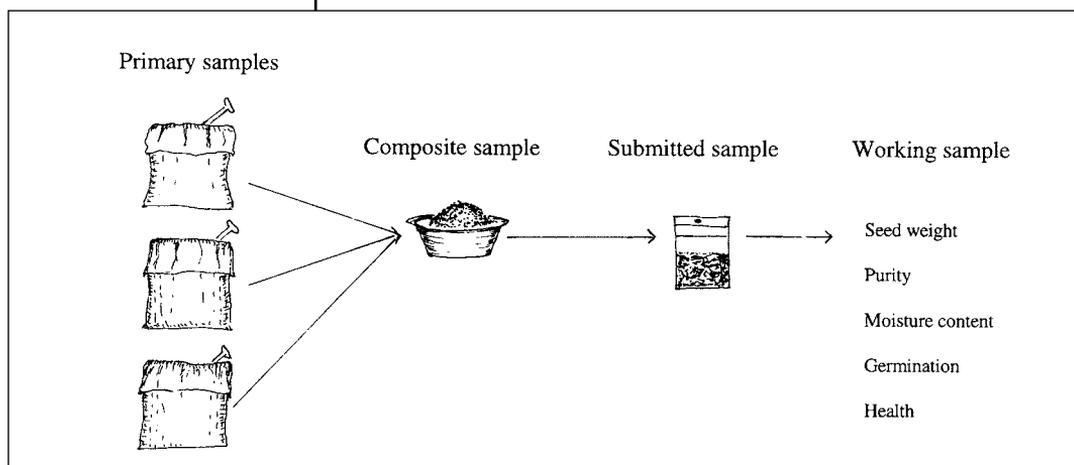


Figure 11.3.

Procedure of sampling. Primary samples are drawn from the seed lot and mixed into a composite sample. The composite sample is reduced to a submitted sample to be forwarded to the seed laboratory for testing. In the seed laboratory working samples are drawn for the individual test. The same working sample may be used for more than one test if the test is not destructive e.g. purity test → germination or moisture content.

Reduction of sample size, whether composite sample to submitted sample, or submitted sample to working sample, must be conducted under observation of the same strict rules of maintaining representativeness and being unbiased as in the previous steps of sampling. Because the quantity of the composite sample is relatively small, it requires little effort to maintain the samples in a homogeneous state. The methods of sample reduction are similar to those employed for sampling from small seed lots. In its simplest form, seeds are spread on a plane table in an even layer. The sample can then be divided in halves once or several times by a ruler or other straight tool (Peterson 1987) (fig. 11.4). Mechanical dividers are convenient accessories for unbiased reduction of sampling size (fig. 11.5). They can be used for most seed types, although very large, rough or winged seeds may cause some problems. Such seeds are most easily separated by the above mentioned 'ruler parting'.

ISTA (1996 and 1998) provides rules for minimum working samples for seed analysis. To carry out basic tests of purity, seed weight, moisture content and viability/germination analysis roughly 2500-5000 seeds are needed, depending on seed size. However, for very small

seeded species, a sample size of less than 1-5 g is impractical, although it may contain much more seed in number than actually required. For large seeded species, reduction of sample size to a minimum of 500 seeds is acceptable. ISTA (1995) proposes submitted samples to the laboratory being twice the size of the total required working samples. Examples of the weight of some submitted samples are listed below.

Figure 11.4.
A seed sample can be reduced to a working sample by repeated halving (Robbins).

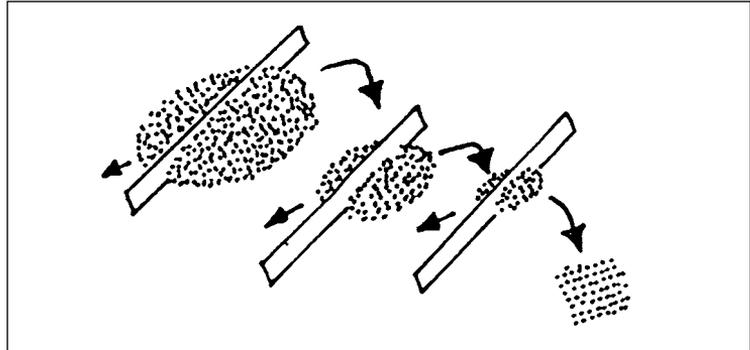
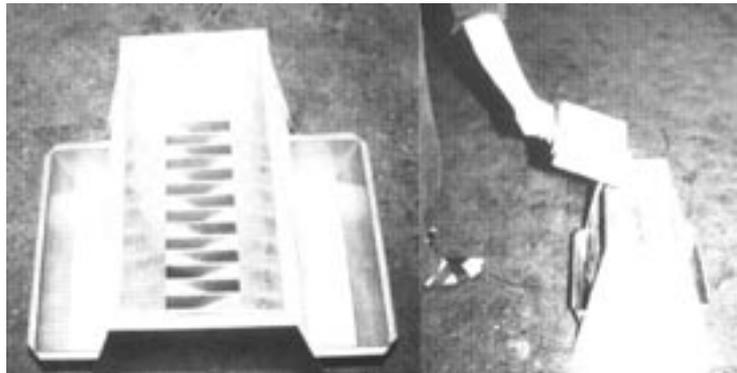


Figure 11.5.
Some mechanical dividers used for accurate and unbiased reduction of sample size. The conical divider (left) divides the sample into two or more equal size parts according to number of spouts at the bottom, the riffle type divider (right) divides the sample into two parts of equal size.



Species	Submitted sample	Species	Submitted sample
<i>Acacia nilotica</i>	1100 g	<i>Dryobalanops oblongifolia</i>	24000 g
<i>Acacia senegal</i>	550 g	<i>Gliricidia sepium</i>	835 g
<i>Acacia tortilis</i>	420 g	<i>Khaya nyasica</i>	2500 g
<i>Azelia quarzensis</i>	25 kg	<i>Khaya senegalensis</i>	1600 g
<i>Cedrela odorata</i>	165 g	<i>Swietenia macrophylla</i>	2400 g
<i>Ceiba pentandra</i>	500 g	<i>Tamarindus indica</i>	3600 g
<i>Dalbergia melanoxylon</i>	840 g	<i>Ziziphus mauritiana</i>	3500 g

Table 11.2.
Examples of weight of submitted samples for seed testing, given that each submitted sample contains 2x minimum weight of working sample (2500 seeds) (from ISTA 1998).

11.5 Simple Seed Testing

Where seeds are to be sown locally and immediately after collection or processing, expensive testing rarely makes sense (Yue-Luan 1993). Yet, information on seed quality is still very useful for nursery operation. Further, regular simple tests often serve as a valid guideline during seed handling. Simple tests do not follow the strict conditions prescribed for standard testing. Therefore comparison of test results becomes less valid. Yet simple tests often suffice when information is only required for an individual seed lot, e.g. to determine the need for further cleaning or drying, or to state the physiological quality. Simple testing encompasses the same parameters as those used in standard tests, which will be described more detailed in section 11.6.

Seed weight. There is no short cut as compared to the standard method; number of seeds and their weight must be known to calculate number of seeds per kg. An electric digit scale is preferred. If such equipment is not available, weighing may be carried out on a balance. There are two methods:

1. Put a known number of seeds on one side of the balance and weights on the other until it balances.
2. Put a known weight on one side and add seeds on the other until it balances.

Count the seeds and calculate seed weight as described in section 11.6.1.

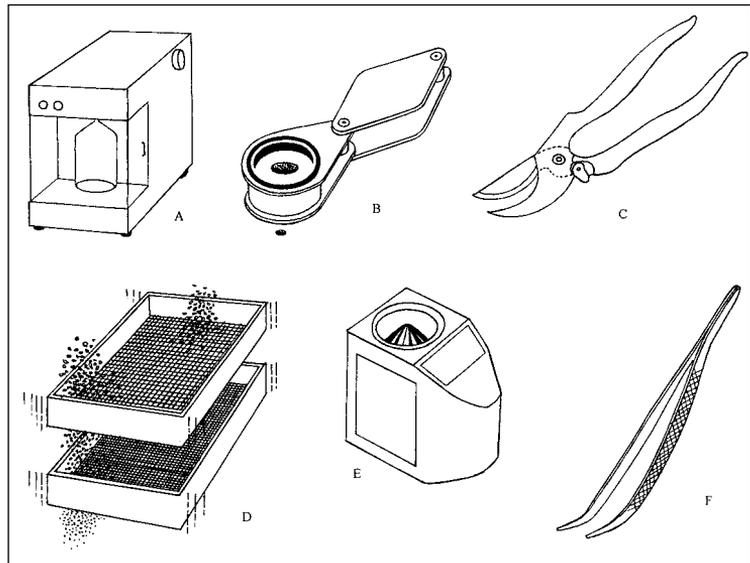
Purity. We are interested in knowing the fraction of pure seed, not the composition of other matter. Therefore we weigh a sample of seeds with impurities, then separate the two fractions and weigh one of them (which in practice should be the seeds as it is usually the larger fraction and as such gives a more exact weight).

1. Pour the sample into one bowl of the balance against a known weight e.g. 250 g. Remove all the entire pure seeds manually with tweezers. Throw the impurities out and weigh the seed fraction.
2. As above but sorting is done by spreading the sample on a table cloth or the like. Impurities may also be removed by blowing, sifting or letting the seeds roll down a slanting cloth frame as described in chapter 6. However, care should be taken during sorting not to lose 'pure seeds' because the sample is small.

Moisture content. Quick measurement of moisture content may be carried out with the aid of a moisture meter as described in appendix A5.3. It is important that the moisture meter is initially calibrated by several measurements on samples where moisture content has been measured by the standard method (see below). Microwave drying is a quick method of moisture measurement for large seeds for which moisture meters are less applicable. The seeds are cut into small pieces before drying in the micro-oven for 5-6 minutes (ISTA 1991, Bonner *et al.* 1994). The sample is weighed before and after drying and moisture content calculated as after the oven drying method (see below).

Germination and viability. For discussion of the two terms, reference is made to section 11.6.4. The simplest viability test is the cutting test. Seeds are cut longitudinally through the embryo. For some seeds soaking in water facilitates cutting. Seeds with firm, white or light green, healthy looking embryo are deemed viable (Bonner *et al.* 1994). Viability tested by the tetrazolium test (TZ) is described in appendix A11.1. It can be carried out at ambient temperature and does not require sophisticated laboratory equipment. Germination trials in nursery plots give valuable information on germination under field conditions and are often the only method for recalcitrant seed. Because such trials are essentially strongly influenced by the environment, comparison of different seed lots are normally subject to large errors. Seedbeds or pots are prepared as during normal nursery operation. Where dormancy is known or suspected, appropriate pretreatment should be carried out prior to sowing. It is advisable to sow the seeds in a design that permits easy counting of germinants.

Figure 11.6.
Some equipment for simple seed testing. A balance, B magnifying glass, C secateurs, D screen, E moisture meter, F tweezers.



11.6 Standard Seed Testing Parameters

As stated above, this book will not cover the practical methods of standard seed testing, but rather explain the background of information provided by seed testing. Detailed information on methods of analysis, prescriptions, definitions etc. should be sought in specific seed testing handbooks, in particular the newest version of ISTA international rules for seed testing (ISTA 1996 or later). Reference is also made to ISTA 1998 and several papers from the ASEAN Forest Tree Seed Centre Project (Yue-Luan 1993, Bhodthipuks *et al.* 1996, and ASEAN 1991). Generally, standard seed testing is carried out whenever seeds are to be stored for a prolonged period, or whenever seeds are to be traded by authorized seed suppliers.

11.6.1 Seed weight

Seed orders are given by weight, seedlings planted by numbers. Seed weight is therefore, together with purity and germination percentage, important when calculating seed demand for a given planting programme (table 3.5). Further, seed size may be correlated with

vigour and hence be an indirect measure of potential performance (see further below).

There are two ways of indicating seed weight: either in number of seeds per kg (or for small seeds occasionally per 100 grams), or in weight in grams for 1,000 seeds. Because it is not always clear whether the former figure refers to a pure sample, seed testing always indicate the 1000 (pure) seed weight (tpsw). The figure can easily be transformed to number of pure seeds per kg.

Examples:

- a. 1,000-seed weight of *Eucalyptus camaldulensis* is 1.5g. Number of seeds per kg is: $1000\text{seeds}/1.5\text{g} \times 1000\text{g} = 666,000$ seeds.
- b. *Pinus caribaea* contains 3500 pure seeds per kg. 1,000-seed weight is: $1000\text{g}/3.5 = 285\text{g}$.

Seed weight is usually calculated on replications of samples of 100 seeds. For very large seeds, calculation is conveniently based on a smaller number. In official seed analysis, variance analyses are carried out based on several replicates of 100 seeds. The figure expresses the variation in seed weight within the sample. Seed weight analysis uses the same criteria as purity test for what may be included as 'seed' in the calculation (see below).

Seed weight varies both with seed size and density, and there are reasons to be observant on factors that may influence these, especially when comparing figures. For example, the term 'seed' is in some cases subject to some confusion. Clear indications of what is the tested unit are sometimes necessary e.g. with or without wings, arils or pericarp. In *Swietenia macrophylla* seed weight of winged seeds is about 2100 seeds per kg, while for de-winged seeds it is about 2300 seeds per kg. Moisture content influences density and thereby seed weight. Seed weight of de-winged *S. macrophylla* roughly increases from above 2400 per kg at 5% m.c. to 2300 seeds per kg at 9% m.c.

In the species list of multipurpose trees and shrubs, Carlowitz (1991) indicates variation in seed weight of several species, in some as large as 5-10 times. Although some variation may be ascribed to different units used (winged/de-winged, seed, pyrene etc.) and possibly moisture content, seed weight is known to be one of the very variable seed characters within species. It is influenced by genetic, developmental and environmental factors (chapter 2).

Relatively high seed weight is often desirable since it is often correlated with rapid germination and good seedling establishment (Griffin 1972, Sorensen and Campbell 1993). It should, however, be noted that direct comparison between provenances is rarely valid, since variation caused by genetic differences may overshadow possible vigour differences.

In common terms purity is an expression of how 'clean' the seed lot is. A purity analysis at a certain stage of processing may serve as a guideline for the necessity of further cleaning. However, as explained in chapter 6, it is in practice never possible to achieve a completely clean or pure seed lot by mechanical processing, because the physical characters of other seed or inert matter may be so similar to those of the seeds in question that separation is impossible. Yet information on the actual composition of the seed lot is important for the seed handler, both in relation to the factors of seed price and seed demand as mentioned above, and because any type of impurity may hamper practical sowing. Certain types of impurity may also harbour infective fungi, which in turn could hamper seed quality, especially when associated with high moisture content (chapter 6 and 8).

Purity of a seed lot indicates in percentage how large a fraction is made up of pure seeds of the species in question, and how much is made up of inert matter and other seeds. Impurities may be any non-seed material (leaf, flower, fruit fractions, soil etc.), small fractions of seeds of the actual species, as well as seeds of other species. ISTA (1996) specifies the pure seed fraction to contain:

1. Intact seeds of the actual species as well as dead, shrivelled, diseased, immature and pre-germinated seeds.
2. Achenes and similar fruits e.g. samaras, with or without perianth and regardless of whether they contain a true seed, unless it is apparent that no true seed is contained.
3. Fractions of broken seeds, achenes etc. which are more than half of the original size. However, seeds of e.g. legumes and pines that have the entire seed-coat removed are regarded as inert matter.

During purity analysis each 'pure seed' fraction (1-3) is separated from the working sample. Purity is expressed as the weight percentage of pure seed fraction over the total weight of the working sample:

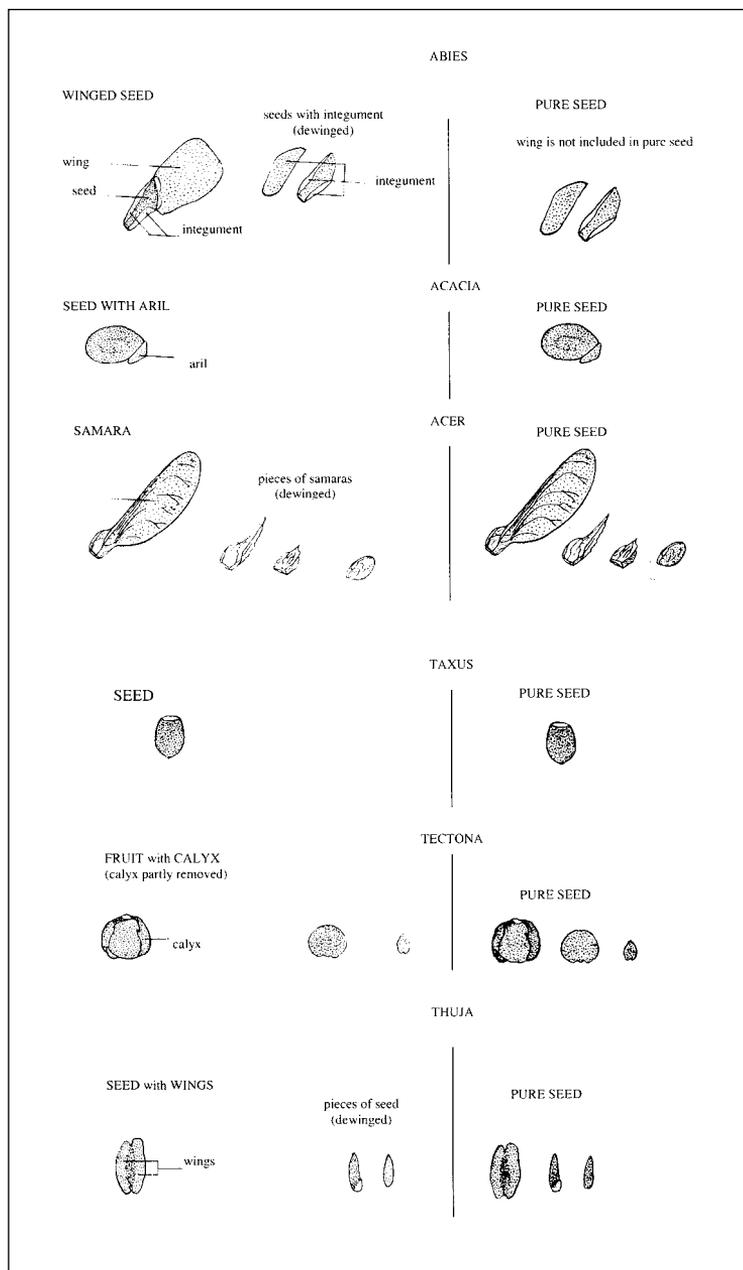
$$\text{Purity} = \frac{\text{Weight of pure seed (g)} \times 100}{\text{Total weight of working sample (g)}} \%$$

Since 'pure seed' may include both dead and empty seed, plus damaged seed, purity does not tell anything about viability. Forest seeds are often collected by hand either by harvesting directly from the tree or, for large seeds, by picking from the ground. In both methods the risk of contamination with foreign seed is small. Large seeds are generally easy to clean and purity analyses are therefore often omitted (Yue-Luan 1993). However, for smaller seeds contamination with other seeds may occur e.g. during processing. Therefore impurities are normally separated into 'inert matter' and 'other seed'. Each component is reported as a percentage of total weight (ISTA 1998).

Table 11.3.
Example of fractions
indicated in a purity
test for *Pinus merkusii*.

	Weight	Percentage
Working sample	60 g	100 %
Pure seed	54 g	90 %
Other seed	1 g	1.7 %
Inert matter	5 g	8.3 %

Figure 11.7.
Examples of 'pure seed' defini-
tions according to ISTA (1991).



11.6.3

Moisture
content

Moisture content (m.c.) is crucial in connection with storage and longevity. Since moisture content of seeds tends to vary with atmospheric humidity (chapter 5 and 6), it is important that exposure to varying humidity is minimized before testing. Therefore, seeds should be packed in waterproof material as quickly as possible after sampling. In order to avoid the possibility of water condensing on the seed when removed from cold store, seed should be allowed to reach ambient temperature before the container is opened.

Under laboratory testing, seed moisture is measured by the oven-drying method, which is the **direct** method, prescribed by ISTA and described below. This method can also be used for calibrating moisture meters for indirect measurement of moisture content (appendix A5.3). The **indirect** methods provide very quick results, which can be used as a guide during seed handling, e.g. to determine the necessity for further drying.

Moisture content of a sample is the loss of weight when it is dried in accordance with the prescribed rules. It is expressed as a percentage of the weight of the original sample (ISTA 1996). This is the fresh weight basis. Moisture content measurement contains the following components:

1. Container (heat resistant) including cover is weighed (M1).
2. Seeds are ground or cut into smaller fractions before drying to assure that moisture can escape from the interior.
3. The seeds are placed in the container and weighed together with the container (M2).
4. Seeds are placed in an oven at 103 +/- 3°C for 17 +/- 1 hour.
5. After drying, the seeds are placed in a desiccation chamber while cooling (to avoid reabsorption of moisture from the atmosphere).
6. After cooling, the seeds plus container are weighed again (M3).

The moisture content (fresh weight basis) is calculated: m.c. =

$$\frac{(M2-M3) \times 100}{(M2-M1)} \%$$

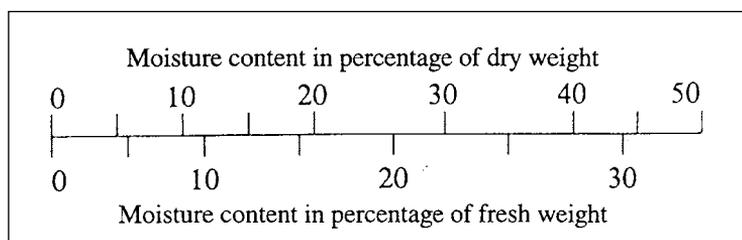
Seeds typically contain far less moisture in the seed-coat and possibly pericarp than in the embryo and endosperm. Hence, processing may influence moisture content both directly in terms of drying rate, and indirectly in connection with extraction and possible de-winging. For example, moisture content of a seed sample of *Swietenia macrophylla* would normally be smaller if the seeds are not dewinged before treatment, since in that case the entire dry wing contributes to the seed weight, yet little to the total moisture content.

The method anticipates that the total loss of weight is caused by evaporation of water. In practice other volatile compounds such as

oil and resin are also lost during drying which, in seeds rich in these compounds, contribute to an overestimation of the moisture content. Despite this potential source of error, the oven-drying method is still used as a standard for these seeds, but the seed handler should be aware of the likely overestimation of the real moisture content when testing oil or resin rich seeds (Poulsen 1994).

The above methods all refer to calculation of moisture content on fresh weight basis. Moisture content expressed as loss of moisture in percentage of dry weight (dry weight basis) is little used but it still occasionally appears in the literature. A conversion scale is shown in fig. 11.8. It should be noted that percentages above 100 may occur when calculated on dry weight basis while such figures are obviously impossible when calculated on fresh weight basis.

Figure 11.8.
Approximate conversion scale
of moisture content calculated
on dry weight converted to
moisture content on fresh
weight basis (Willan 1985).



11.6.4 Viability and germination

A high germination percentage is obviously desirable for the nurseryman, anything other than pure germinable seed is waste. Therefore a germination or viability test should indicate the potential germinability which, with proper handling, should reflect expected germination in the nursery. Germination potential is most directly determined in a germination test: under the appropriate conditions everything that can germinate should germinate. Germination tests are widely used in both standard seed testing and more informal simple nursery tests. However, the tests have several limitations, some of which may either over-estimate or under-estimate the actual germination potential of a seed lot. Three situations where germination tests are less applicable are the following:

- Where seeds have a very short viability. Duration of a germination test is typically 3-5 weeks. For short-lived recalcitrant seed significant loss of viability may take place during the test period. Hence, the germination percentage obtained by the test may not be valid for the seed lot from which it was taken because the viability of the seed lot has declined during the test period.
- Where germination is delayed or suppressed by deep dormancy. If pretreatment has been insufficient to overcome dormancy, germination may be low even if seeds are viable.
- Where fast test results are required. Especially for slow germinating species (some species take several months to germinate) the duration of a germination test may be inconvenient. Where a seed lot is to be dispatched soon after collection, there is often not enough time for a germination test.

Where germination tests for some reasons are inconvenient or unreliable, or where shortage of standard germination facilities limits the use of germination tests, germination potential may be tested by indirect methods viz. viability tests. These tests do not prove that seeds are germinable, only that they are (most likely) alive. It is essential to distinguish between the two terms 'viability' (percentage) and 'germination' (percentage); the two terms refer to different types of test result, and they are not synonymous. Although prescribed test procedures aim at creating concurrence between the two types of tests, some divergence may occur. Seeds deemed viable may not be germinable because of an advanced stage of deterioration (reduced vigour) or dead tissue in vital parts of the embryo. Another example is tetrazolium staining which indicate live tissue (see below). Young (immature) seed may stain normally by this procedure although they have not achieved germinability. On the other hand, viability tests are not always inferior to germination tests. In the three situations listed above a viability test is preferred. Viability tests are also used as a supplement to germination tests in order to examine the character or quality of seeds that have not germinated during the standard test.

Several types of viability tests are available. The most common ones are cutting test, tetrazolium, X-ray, excised embryo, and hydrogen peroxide test, which are described below. Of these methods only tetrazolium, hydrogen peroxide and excised embryo tests actually prove a life manifestation, in the first case as the activity of a metabolic enzyme complex, in the latter as a directly observable embryo development. It should be emphasized that all types of viability test are subject to some subjectivity in the interpretation of results. Viability tests are generally less applicable to very small seeds such as eucalypts, and for excised embryos, the method is practically impossible (Boland *et al.* 1980).

Cutting test

Cutting tests are never used as the sole viability test in standard testing, but rather to examine the conditions of non-germinated seeds in a germination test. The method is, however, widely used in simple seed testing, both during collection and processing. It is described in section 11.5 in connection with simple tests. In a cutting test, viable and dead seeds are distinguished visually, which in practice means that seeds that are empty, insect-damaged, under-developed or showing other distinct signs of damage are deemed non-viable, and the remaining portion viable (although actual life manifestations are not proven).

Tetrazolium

The tetrazolium (TZ) test is the most widely adopted biochemical method to examine seed viability. The method is also called topographical tetrazolium test (TTZ) to emphasize that specific areas of the seed are examined rather than just general evidence of viability (Enescu 1991). A thorough description of the theory and practice of tetrazolium testing is given by Moore (1985), Yu and Wang (1996), and Enescu (1991). The practical method is summarized in appendix

A11.1. TZ test is especially useful as an alternative to germination test for species that require long periods of pretreatment to overcome dormancy (e.g. several temperate species), but the test is also widely used as a quick test for species with less complex dormancy. Although the method is in principle applicable to all seed types, interpretation of results becomes extremely difficult for very small seeds.

The principle of TZ test is as follows: dehydrogenases are a group of metabolic enzymes in living cells. During the reduction processes in the metabolically active cells dehydrogenases release hydrogen. The hydrogen is able to reduce an applied pale yellow solution of 2,3,5-triphenyl tetrazolium chloride or bromide (TZ) to a stable, bright red triphenylformazan. Hence, the formation of red formazan is an indication of dehydrogenase activity, which is in turn an indication of viability. Because staining of tissue is local, it is possible to distinguish living (red-coloured) and dead (colourless) parts of the seed. Where dead (necrotic) tissue occurs only superficially in cotyledons, while the radicle stains normally, the seeds may still be viable. On the other hand, even small patches of necrotic tissue in the vital part of the embryo normally means that the seed would not be able to germinate. The exact evaluation of these partly stained seeds requires a fair amount of experience. Moore (1985), Yu and Wang (1996) and Enescu (1991) contain examples of TZ staining pictures of a number of seeds, which may serve as guidelines for interpretation of the results of TZ staining.

Seed embryos are likely to stain whether they are dormant or not, and damaged but not necrotic tissue may stain normally. Therefore the result of the TZ test is likely to include the three classes in the germination test: normal seedlings, abnormal seedlings, and live but not germinated seeds (including hard seeds) (ISTA 1998). Yu and Wang (1996) found good concordance between TZ test and germination in comparative studies of different viability tests on several tropical tree species. A precondition for application of the TZ test is that the seeds are mature i.e. physiologically germinable. Immature seeds may stain normally because they contain live cells, but would give poor results in a germination test. Another source of error is that seeds infected by fungi may stain because of the metabolic activity of the fungi and not the plant cells. However, such fungal cells generally stain dark brownish-red, not bright red as live sound plant cells do (Mittal 1997, pers. comm.).

X-radiography

X-radiography is a quick test to differentiate empty, under-developed, insect or physically damaged seeds from morphologically intact and healthy seeds by the aid of X-rays (ISTA 1996). A thorough description of principle and practice in X-radiography in tropical tree seeds is found in Simak (1991) and Saelim *et al.* (1996). X-rays are electromagnetic waves with wavelengths of 0.05-100Å² (visible light approx. 4000 - 8000 Å). The seeds are placed between the X-ray source and a photosensitive film or paper. When the seeds are exposed to X-rays of low energy (longer wavelength, approx. 1 nanometre), an image (radiograph) is created on the film/paper. Photographic processing

² Wavelengths of light are usually indicated in Å (angstrom) or nm (nanometre). 1 Å = 0.1nm, or 1 nm = 10Å.

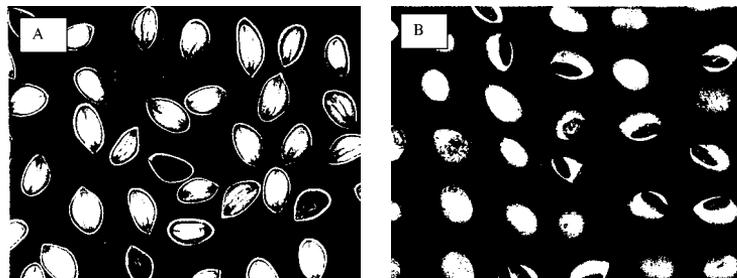
converts the radiograph into a visible picture. Since X-rays are non-destructive, seeds examined by the X-radiographic method may also be used in direct germination tests.

Because normal X-radiography is unable to distinguish aged or physiologically damaged seeds from sound seeds, the best correlation of the X-ray and germination test is found where empty seeds, insect damage or other physical damage contribute largely to possibly reduced seed quality. The method is especially useful where such damage or lack of development are not apparent on the exterior of the seed, e.g.:

1. Empty seeds in pines, eucalypts and others, where the seed develops into full size even if it contains no embryo.
2. Insect infested seeds where no entry hole is visible, e.g. legume seeds infested by bruchids or conifers or eucalypts infested with chalcids (e.g. *Megastigmus* spp.).
3. Seeds enclosed by a hard fruit structure, e.g. drupes or samaras, where the pericarp or endocarp bears no sign of presence or condition of the enclosed seed(s). X-radiography may reveal both number of seeds in such fruits and their condition.
4. Seeds where internal mechanical damage to the embryo may have occurred e.g. during processing.
5. Seeds with shrunken or underdeveloped embryos e.g. immature seeds.

X-radiography is especially useful for estimating viability of recalcitrant seeds because they are short-lived and their germination potential has to be determined quickly (Saelim *et al.* 1996). Chaichanasuwat *et al.* (1990) found good conformity between X-radiography and germination test for *Peltophorum pterocarpum*. In comparative studies of different viability tests, Bhodthipuks *et al.* (1996) found that X-radiography generally over-estimated viability as compared to germination tests. Laedem *et al.* (1995) found good correlation between X-radiography and germination test in *Dalbergia cochinchinensis* and *Pinus kesiya*, both of which had a high seed quality, while there was low correlation for *Pinus merkusii* in which the germination percentage was low. It may from these observations be generalized that the method is less applicable to seed lots of low physiological quality.

Figure 11.9.
X-radiographs used for seed quality analysis.
A) *Pinus kesiya*, the picture reveals some seeds with rudimentary embryos and some empty seeds.
B) *Albizia procera*, the picture reveals seeds infected with bruchid beetle.
From Saelim *et al.* 1996.



Application of specific contrast chemicals e.g. BaCl₂, AgNO₃, NaI, or KBr to the seed before X-ray enhances the possibility of evaluating viability of tissue. Because these chemicals stain differently in live and dead tissue, the X-ray contrast (XC) method gives a different image of live and dead seed (or seed tissue) similar to the TZ test (Saelim *et al.* 1996, Simak 1990). However, since X-radiographs are black and white, interpretation of the results requires even more experience than the TZ test.

Excised embryo test

This method is used where seeds germinate very slowly or where the seeds are deeply dormant and require long pretreatment. It may also be used where the nature of dormancy and hence pretreatment is not known. The principle of the test is that the embryo is manually excised from the seed-coat and possible endosperm under aseptic conditions, placed on filter or blotting paper and incubated in germination cabinets at 20-25°C. The result of the excised embryo test is germination percentage under incubation. It should be noticed that in order to be statistically valid, also seeds with damaged, deformed, discoloured or lacking embryos must be included in the final calculation (ISTA 1996). Because the embryo is surgically excised from the seed during the operation, it requires a certain minimum size of seed and embryo before it is practically possible. The method is thus not applicable to very small seeds.

The excised embryo test is a transition form to a true germination test, since the embryos are evaluated on radicle development that is essentially an early germination event. However, the germination process is concluded before the seeds develop into seedlings that could be evaluated for normal growth, as is done during normal germination tests.

Hydrogen peroxide test

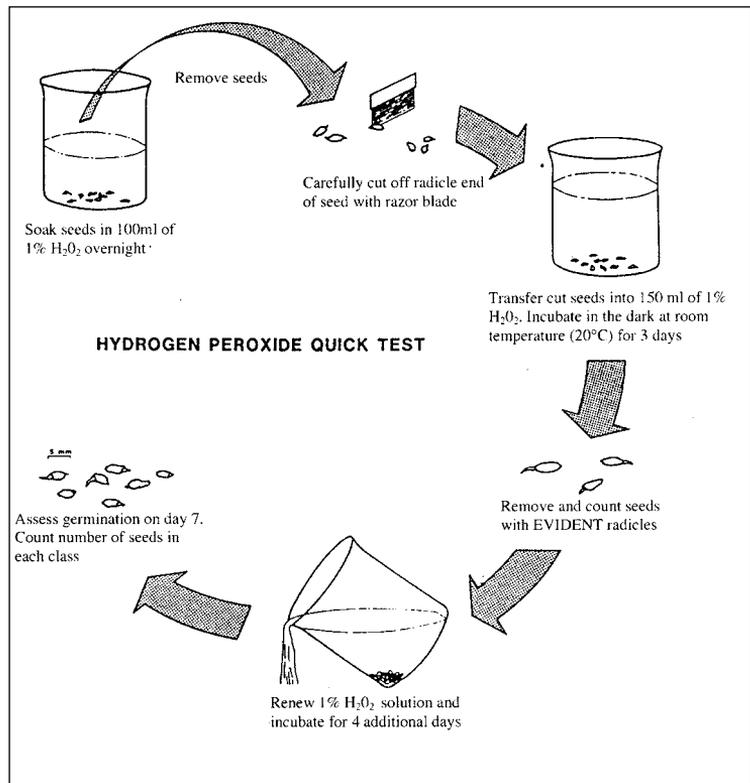
This test is another viability test, which forms a transition to a germination test. It is described in detail in Bhodthipuks *et al.* (1996). During the test, initial germination is evaluated after application of the chemical hydrogen peroxide (H₂O₂). The purpose of applying H₂O₂ is to increase the supply of oxygen to speed up initiation of germination. The application is sometimes used as a pretreatment (chapter 9). The H₂O₂ test method is illustrated in fig. 11.10. Seeds to be tested are initially soaked in a 1% solution of the chemical for 8-12 hours. They then have a small piece of their seed-coat removed at the radicle end and are incubated for a period of about 7 days. The solution is changed after about 3 days. Incubation is conducted under dark conditions as the chemical is very light sensitive. Seeds are considered viable when radicles emerge from the cut end.

Germination

During germination tests, seed quality is measured directly as the ability of the seed to germinate under **optimal** germination conditions of temperature, moisture and light. It is anticipated that germination is not impeded or delayed by possible dormancy. Therefore seeds should be pretreated before a germination test. Germination under the ISTA standard test is subject to strict prescriptions to pretreatment methods and germination conditions (ISTA 1996). Germination is normally carried out in germination cabinets under controlled environment. The conditions prescribed by ISTA include the following variables:

- temperature (level and regime, e.g. constant day and night or fluctuating)
- light (+/- light or period of day/night cycles)
- substrate (sand (S), top of sand (TS), top of paper (TP), between paper (BT) and pleated paper (PP) (parentheses correspond to abbreviations used in ISTA prescriptions)).

Figure 11.10.
Procedure for hydrogen peroxide viability test. From Laedem 1984.



The ISTA rules also indicate days of first and last count in order to standardize the duration of the test period. Although these rules are standardized for laboratory tests, they may also serve as guidelines for more informal nursery tests. Germination is carried out on the 'pure seed' fraction (see section 11.6.2), which on one hand excludes all 'other seeds', on the other hand includes large, damaged seeds.

Germination is defined as 'the emergence and development of the seedling to a stage where the aspects of its essential structures indicate

whether or not it is able to develop further into a plant under favourable conditions in the soil' (ISTA 1996). This means for tree seeds a root system, shoot axis, cotyledons, and terminal bud. The exact criteria of evaluation vary slightly between species, e.g. in eucalypts a seed is considered to have germinated when the radicle has developed normally and the cotyledons have emerged from the seedcoat and have unfolded (Boland *et al.* 1980).

Germinated seeds are counted regularly during the prescribed germination period from the indicated 'first count' to 'final count'. Counting once per week is usually sufficient, but species with rapid germination may be counted and removed every two days. Removal of germinants is done in order to facilitate subsequent countings and to avoid possible fungal spread. Both 'normal' and 'abnormal' germinants are counted, registered and removed during the period. At the end of the period all ungerminated seeds are examined. The final test result is grouped into the following classes:

1. Normal germinants. The cumulative number of seeds which have developed into seedlings of normal and healthy appearance with all essential structures of a seedling. This also includes seedlings where possible damage is caused by secondary infection.
2. Abnormal germinants. The cumulative number of seeds which have germinated during the test period but in which the seedlings show abnormal or unhealthy appearance e.g. lacking essential structures such as cotyledons, or being discoloured or infected by seed-borne pathogens (primary infection).
3. Ungerminated seeds. Seeds which have not germinated by the end of the test period. These are grouped into the following sub-classes:
 - a. Hard seeds, which are seeds that remain hard because they have not imbibed (normally because of insufficient pretreatment).
 - b. Fresh seeds, which are seeds that have not germinated although they appear firm and healthy.
 - c. Dead seeds, which are seeds that are soft, or showing other signs of decomposition.
 - d. Other seeds, e.g. empty seeds.

Category a. and b. may be germinable but dormant. Their correct status may be further determined by viability test. If the number of viable but not germinated seeds is high, a new germination test following new pretreatment may be appropriate.

The final evaluation of the germination test is reported as germination percentage or germination capacity, which counts 'normal germinants'.

11.7 Other Seed Testing

Two additional seed quality tests shall be mentioned viz. vigour and seed health tests. Their rationale is discussed below. Neither of the methods are carried out as routine tests by seed laboratories. Unlike the above tests there are no strict adopted standard procedures for conducting the tests and evaluating the results. However, in some cases results from germination or viability tests can be used for evaluating both seed vigour and health.

11.7.1 Vigour test

The main limitation of the germination test is its inability to detect quality differences among seed lots with high germination percentages. Vigour test is a more sensitive test, which aims at detecting such differences. Several definitions of seed vigour exist, the two most common ones are formulated by ISTA and Association of Official Seed Analysts (AOSA):

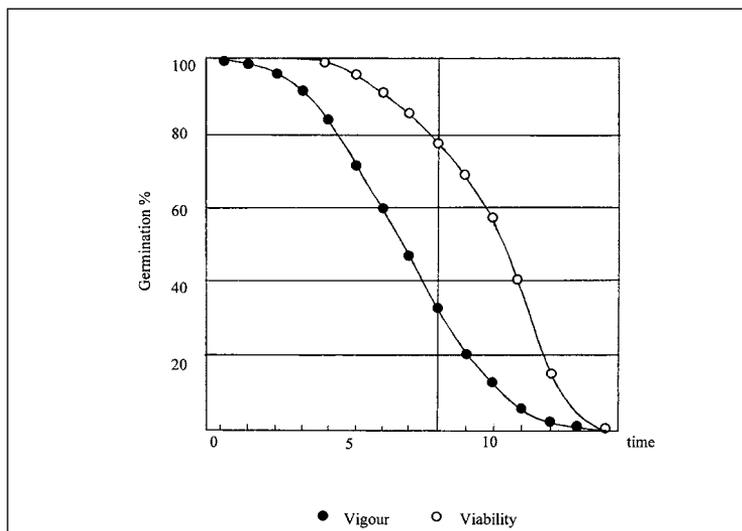
1. Those seed properties, which determine the potential for rapid, uniform emergence, and development of normal seedlings under a wide range of field conditions (AOSA 1983).
2. The sum of the properties which determine the potential level of activity and performance of the seed or seedlot during germination and seedling emergence. Seeds which perform well are termed 'high vigour seeds' (ISTA 1995 cit. Perry 1981).

Practical application of a vigour test is mainly related to field performance and storability. Since germination tests are carried out under optimal germination conditions, the test results express the germination potential, i.e. likely germination under ideal conditions of temperature, light and humidity. This figure may be quite different from the performance under stressed field conditions. As discussed in chapter 8.5, seeds undergoing natural ageing during storage are likely to lose vigour at a faster rate than they lose viability. For the individual seed, decline of vigour may be manifested in reduced germination capacity under suboptimal conditions. Hence, a standard germination test of a low-vigour seed lot may show relatively high germination, while a test conducted under stressed conditions (which probably in most cases would better reflect real field conditions) may show comparatively poor germination (ISTA 1995). Further, a low vigour seed lot has in itself comparatively short storability, whereas a high vigour seed lot will produce a high number of normal seedlings under a wide range of environmental conditions and it will store better (i.e. lose viability at a lower rate). As can be seen from fig. 11.11, there is high conformity between viability and vigour for high vigour seed lots.

Since seed vigour is a concept describing several characteristics associated with seed performance, and not a single measurable character, there is no adopted standard procedure to measure vigour, but rather a number of options. Vigour tests are described thoroughly in ISTA (1995) and AOSA (1983). Some of the methods are summarized below.

Figure 11.11.

Relation between viability and vigour over a period of time. The relation may be expressed as germination under a certain set of conditions after a certain ageing period. The viability curve represents germination under optimal conditions while the vigour curve expresses germination under stressed conditions. Example: after 8 months' storage, germination under optimal conditions is about 80% while under stressed condition it is only about 30%.



Velocity of germination

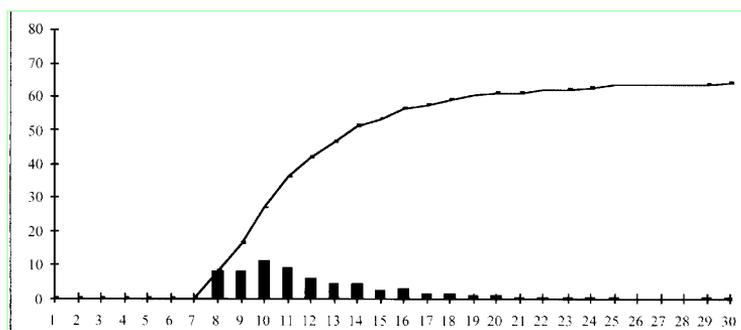
Germination percentage only states the percentage of the seeds that have germinated during the test period (germination capacity), not whether germination occurred during the first or last part of the test period. Under field conditions rapid germination is obviously an advantage for seedling establishment. Speed of germination is an expression of seed vigour. It is anticipated that high-vigour seeds germinate faster than low-vigour seeds under any conditions. Speed of germination can be calculated from the daily germination records. An example of records of a daily count of a four-week germination test is presented in fig. 11.12. The velocity of germination, termed 'germination energy' can be expressed in various ways from the germination results (cit. Willan 1985).

1. As the percentage of tested seeds that germinate within a given period, shorter than the total test period, e.g. 7, 14 or 21 days, depending on species. In the example in fig. 11.12, 48% have germinated after 14 days, 60% after 21 days.
2. As the percentage of tested seeds that germinate up to the time of peak germination, which is the highest number of germinants appearing in a given 24 hour period. The peak of the records in fig. 11.12 occurs at day 10, up to which 26% have germinated.
3. As the number of days required to reach 50% of the final germination percentage. It is 11 days in fig. 11.12.
4. As the average germination speed over the full test period, based on daily counts. This is calculated by the following formula:

$$\frac{\Sigma \text{daily germination speed (DGS)}}{\text{total test period (days)}} = \frac{\text{Cumulative daily germination percentage}}{\text{number of test days}} \div \frac{\text{total test period (days)}}{\text{number of test days}}$$

Some attempts have been made to combine germination capacity and germination energy into a single figure, expressing both total germination and germination speed. The term 'germination value' was introduced by Czabator (1962) and later evaluated and modified by Djavanshir and Pourbeik (1976). However, the term is not much used, since it is usually preferred to report germination capacity and germination energy in separate figures.

Figure 11.12.
Daily recorded germination in a germination test of *Pinus caribaea*. Columns indicate the average percentage of germinants registered each test day during the full test period. Curve indicates the cumulative percentage of germinants over the period (Data from Paul 1972, quoted in Willan 1985).



Conductivity test

The conductivity test is based on the assumption that a disintegration of cell membranes in the seed takes place during seed deterioration. During the early stages of imbibition the cell membranes reorganize and repair possible damages. Delayed repair or failure to overcome such membrane damages causes leakage of electrolytes from the imbibing seeds. A conductivity meter can measure the leakage of electrolytes into the water in which seeds are imbibed. Since high-vigour seeds are able to reorganize their membranes more rapidly and repair any damage to a greater extent than low-vigour seeds, electrolyte leakage can indicate level of vigour. Hence, low conductivity indicates low electrolyte leakage and thus high vigour, high conductivity accordingly indicates low vigour (ISTA 1995).

Accelerated ageing

Accelerated ageing (AA) is a stress test with two main applications in practical seed handling: (1) to predict the potential storage life of seed, and (2) to assess vigour of a seed lot. The first application is discussed in connection with storage in appendix A8.1. AA is based on the assumption that if seeds deteriorate at a certain predictable rate under a given set of storage conditions (mainly as a function of temperature and humidity), then deterioration will occur much faster under sub-optimal conditions of increased temperature and/or humidity. The basic assumption is that the same process of deterioration which takes place during a natural (slow) ageing period will occur during a short period when seeds are exposed to unfavourable conditions (Delouche and Baskin 1973). In other words: natural deterioration is simulated and compressed into a short convenient test period. Under such conditions high-vigour seed lots will show only slight decline in germination while low-vigour seed lots will decline markedly after exposure to AA (Elam and Blanche 1989).

11.7.2 Seed health testing

AA has proven a useful method for comparison of parameters related to seed deterioration. However, it is quite evident that at least some factors have a larger influence in AA than during natural ageing mainly because of higher humidity. Micro-flora (fungi) and repair mechanism of cell organelles are two factors apparently more prevailing under AA conditions than under natural ageing (Priestley 1986).

Stress test

A number of methods have been used to evaluate seed and seedling performance under stressed conditions, which are all attempted to simulate single stress factors occurring under field condition. These tests are germination tests carried out under sub-optimal conditions and hence differing from normal germination tests. The type of suitable stress factor depends on species and, except for the exhaustion test, the factor most likely to be encountered in the field. The methods of conducting stress tests are described thoroughly in ISTA (1995) and AOSA (1983). The methods are mentioned only briefly here.

During the Hiltner test the ability to overcome physical stress is evaluated by germinating the seeds under a 3-4 cm thick layer of crushed brick stone or gravel. Cold test evaluates the ability to germinate and grow under low temperatures. This test is frequently used for temperate species but is also suitable for tropical and subtropical highland species. High temperature and water-stress are other variable factors likely to reflect difference in vigour.

The exhaustion test is based on the principle that seeds germinated in darkness do not carry out photosynthesis but rely entirely on nutrients derived from the seed. The germinants become etiolated, and after a specified test period the dry weight of the seedlings is measured. Seedlings derived from high vigour seeds have the highest dry weight (Poulsen 1993). Obviously this method is not applicable to seeds that require light for germination (cf. sections 9.5.5 and 10.5.3).

Seedling growth

The standard germination test only distinguishes between normal and abnormal germinants. Variations in seedling size and vigour are likely to occur within the category 'normal germinants'. Since initial growth is highly influenced by the seed, evaluation of seedling vigour, expressed e.g. as dry weight or evaluated in size classes, is in turn an expression of seed vigour. Comparison of different seed lots must obviously be carried out under strict observation of standard germination conditions and duration of test period. The latter implies that seedlings must not be removed during the test as is customary during normal germination evaluation.

Seed health is indirectly revealed during viability, germination or vigour tests since infected seeds are often unable to germinate or appear non-viable when examined by e.g. X-ray, TZ test or other

viability test methods, or they germinate slowly and produce poor seedlings. However, in some instances a more thorough examination of the presence, and type of seed-borne pests and pathogens is relevant. Especially in international transfer of seed, where there is a risk of introducing seed borne organisms together with seeds, a special health test may be required. Methods of seed health testing are described in Richardson (1990), and the ISTA rules (ISTA 1996) provide general guidelines on health testing.

The level of seed health testing varies from simple assessment of infection rate by visual examination of the seed sample under a stereo microscope, to thorough examination and species identification after incubation.

Assessment of insect infestation rate may be carried out as part of X-radiography or a cutting test as described under viability. Where identification of insects is required, it is often necessary to acquire adult specimens. Since the insects present inside the seeds are often in the larva or pupal stage, incubation under conditions that promote their development may be necessary.

Fungal spores present on the surface of the seed may be detected by microscopical examination of an aqueous suspension after washing the seeds in a small quantity of water (Desai *et al.* 1997). However, most fungal examination requires pre-incubation under warm moist conditions. Incubation is normally conducted on blotter paper, sand or agar plates. Sand and blotter paper are used where germination is desired. After a few days' incubation fungal growth may be visible on seed-coats or as symptoms appearing on the seedlings. It should be stressed that certain pre-treatment methods e.g. sulphuric acid or hot water used for breaking physical dormancy should not be used in seed health testing as they may kill possible fungi and hence interfere with the result. Where pretreatment of such seeds is necessary, they should be mechanically scarified. During the agar method seeds are placed on the surface of a sterile nutrient agar gel during incubation. The fungi will grow and form a colony on the agar plate, which may be identified by its colour and type of growth (Desai *et al.* 1997).

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Appendix A11.1 Procedure for tetrazolium testing

This appendix gives practical guidelines for conducting viability tests with tetrazolium. The procedure as described here has been compiled from Yu and Wang (1996), Moore (1985) and ISTA (1996). For detailed information reference is made to these sources.

Since TZ measures the activity of metabolic enzymes (dehydrogenases) in living cells, it is necessary that seeds are imbibed and incubated at a temperature allowing active metabolism during the test. For most species it is necessary to pre-moisten the seeds, either slowly (between or on top of moist paper) or fast (soaking in water) until fully imbibed. Seeds with a hard seed-coat must be scarified (punctured) to allow imbibition. Seeds enclosed within a hard pericarp must be scarified or extracted prior to imbibition.

Tetrazolium chloride or bromide is available as a salt which is dissolved in water before use. The salt as well as the solution are light sensitive and must be stored in a dark bottle and/or in darkness. To avoid light exposure the bottles may be wrapped in light-proof material such as alu-foil. Solutions stored cold may retain their strength for several months. The chemical is normally not re-used but discharged after completion of the test. Tap water (clean) may be used for the solution provided the pH is within the range 6.5 - 7.5. If the pH is higher or lower, a buffer solution prepared with KH_2PO_4 and Na_2HPO_4 should be used. A buffer solution is prescribed by ISTA (1996):

Solution 1: Dissolve 9.078 g of KH_2PO_4 in 1 litre of water

Solution 2: Dissolve 9.472 g of Na_2HPO_4 in 1 litre of water,
or 11.876 g of $\text{Na}_2\text{HPO}_4 \times 2\text{H}_2\text{O}$ in 1 litre of water

Mix two parts of solution 1 with three parts of solution 2. If the solution is not clear, add a drop of alcohol to clear it. Check the pH (6.5-7.5).

For most species a 1% TZ solution is used. This concentration is achieved by dissolving 1 g TZ salt in 1 litre of water or buffer. The practical steps of the TZ test are as follows:

1. Prepare hard seeds for imbibition by scarification, puncturing or extraction.
2. Pre-moisten seeds by soaking, or between or on moist paper at approx. 20°C for 3-48 hours (depending on species, cf. ISTA prescriptions); drain off water.
3. Immerse seeds in the TZ solution. The seeds should be completely covered.
4. Incubate seeds in the TZ solution in darkness at 30-35°C for 1-24 hours (depending on species, cf. ISTA prescriptions).
5. Wash seeds in distilled water and place them on moist filter paper until evaluation.
6. Evaluate staining.

The duration of incubation in TZ should as far as possible follow the guidelines as listed by e.g. ISTA (1996). For species where no

information is available, duration must be determined by experience. Too short incubation implies insufficient staining. Too long incubation makes evaluation difficult since the tissue tends to be very dark coloured and dead tissue dissolves. Evaluation should be done shortly after termination of incubation; the seeds are meanwhile stored in the dark. A few hours' exposure to light, e.g. in connection with evaluation is, however, not critical (Nydam 1997, pers. comm.). Seeds fully stained are classified as viable; those not stained as non-viable. Seeds only partly stained are thoroughly examined. Only those with fully stained embryos, or with only minor portions of the cotyledons unstained, are classified as viable. Detailed guidelines on the evaluation are given in the above references.

Tetrazolium chemicals have been under suspicion for having carcinogenic effects. Though there is at present no confirmation to that, it is advised to handle the chemical with care i.e. using mask and ventilation during handling of the powder and rubber gloves during handling of dissolved chemical.