Cutting Tests

Cutting tests are 'seed anatomy tests' used to estimate germination and characterize a seedlot through visual inspection. Seed are dissected and classified into categories displaying similar characteristics. Cutting tests are essential as a decision tool in seed processing and upgrading, but are useful in any situation where an estimate of germination is desirable. Cutting tests can also reveal the presence and extent of problems (i.e., % and degree of immature or deteriorated seed) within a seedlot.

It is not always possible, based on seed anatomy, to predict whether a seed will germinate, but for most seedlots, cutting tests provide a good approximation of the proportion of viable seed. Although cutting tests can be performed on dry seed, the imbibed seed usually provides a better illustration of seed condition and a better prediction of germination. The moisture status, presence of deterioration, insects and fungi can all be viewed within the tree seed – take a look.

Cutting tests are most commonly performed on 50 seeds, but this can vary depending on seed condition and precision desired. Seed are generally cut **longitudinally** (lengthwise) and the best view of internal structures is obtained if one dissects through the thinnest dimension of the seed (Figure 18). Various means can be used to hold the seed in this unstable position: forceps, tape, fingers [be careful] or even a small template for the larger seed. It is safest to cut the seed so that its narrowest edge is placed on a firm surface

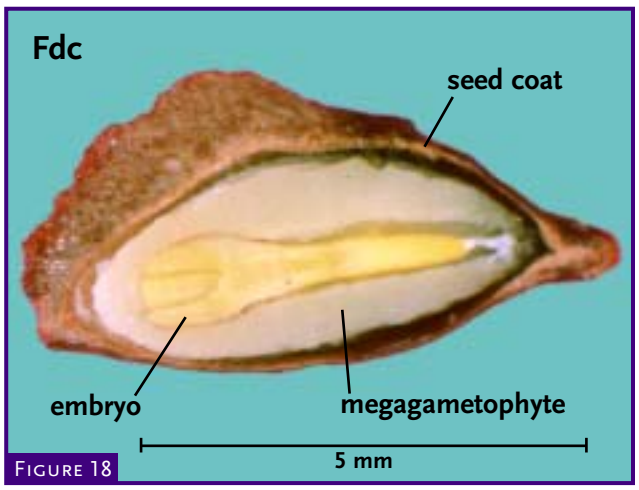

Figure 18

A longitudinal section of a 'dry' Douglas-fir seed.

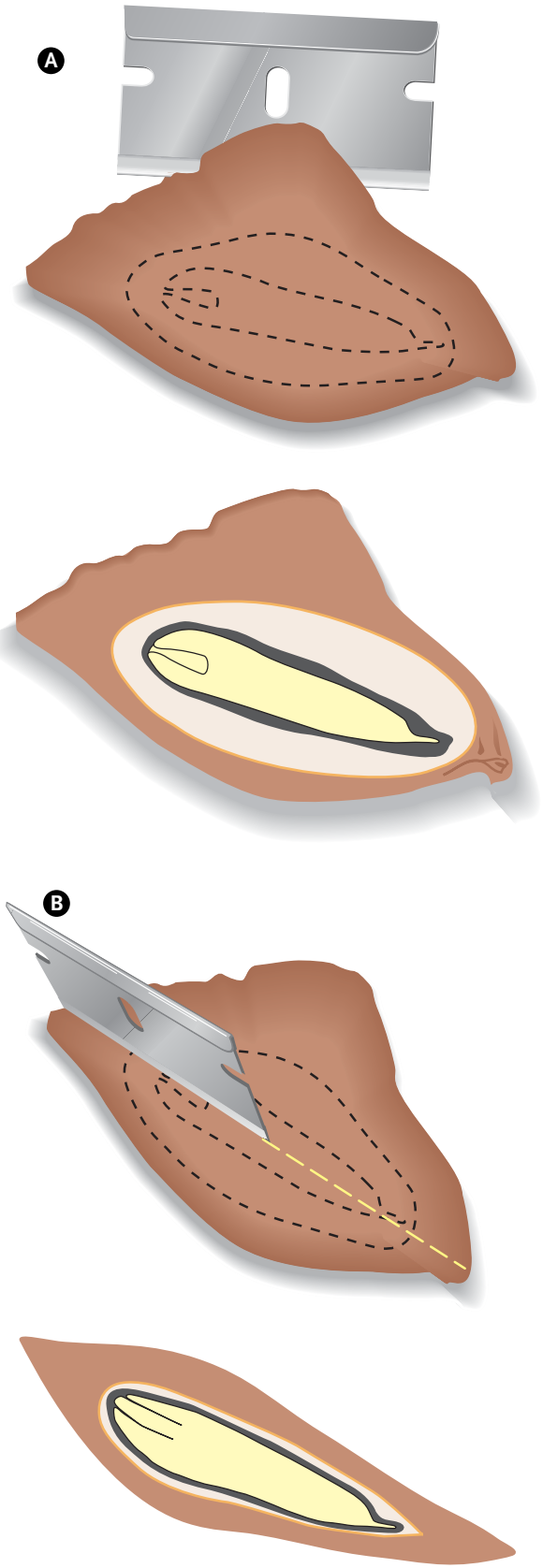

FIGURE 19

Methods and results of performing cutting tests (A) through the thinnest axis or (B) with the seed placed on a flat surface.

and a clean, sharp razor blade or scalpel pushed downwards through the middle of the seed with constant pressure (FIGURE 19A). With care, holding seed between your fingers and slicing can be a quick and effective method. Practise with larger seed first.

Seed can also be cut with the widest dimension resting on a firm surface making the seed much more stable, but resulting in a less illustrative view of the internal components, especially the megagametophyte (FIGURE 19B). It is more difficult to consistently cut the **embryo** in half with this method. Some experimentation may be required for each species. An aid in classification and counting is to place seed on masking tape and then cut and separate the seed halves allowing for examination of both halves. This eliminates seed moving during the assessment and allows for a direct comparison between adjacent seed. Employ the most comfortable method for you that produces longitudinal sections enabling you to assess seed quality.

Seed Problems

This section illustrates some problems causing seed mortality (inability of a seed to germinate under any conditions) or reduced seed quality that can be observed in cutting tests. These illustrations will assist in the classification of seed required in cutting tests. Some seed morphologies are easily interpreted as being incapable of producing a germinant, but it is not always possible to determine if a seed will or will not germinate based solely on its anatomy and morphology. The best one can do is point out the important characteristics to look for in a cutting test.

The two most easily recognized reasons for failure of seed germination are: lack of an embryo (resulting in an empty seed) and the complete deterioration of all seed contents (generally called dead-filled seed). These two morphologies are illustrated with Amabilis fir in FIGURE 20. Empty seed are a common feature in most conifers as the seed coat and megagametophyte can still develop without fertilization. The megagametophyte of the unfertilized seed usually degenerates leaving only

the megaspore cell wall. This results in an empty seed that appears flat from the outside. In most species, empty seed are easy to remove during seed processing due to their lower **specific gravity** and are therefore not common in processed seedlots. If one is performing cutting tests on unprocessed seed include an empty seed category in your cutting tests.

The megagametophyte will not develop in species of *Pinus* if the ovule is unpollinated. This is the reason that the crush test is used by some to evaluate lodgepole pine seed. In the crush test, you simply apply pressure to the seed and if the exudate appears white, indicating the presence of the megagametophyte, you can assume an embryo is present. This may be suitable to some situations, but it gives no indication of embryo development or health. The longitudinal seed cut is recommended.

It has been observed that empty seed of *Abies* spp. often appear to have thicker seed coats than seed that develop with a fertilized embryo. One often knows that contents are absent in *Abies* spp. by the difficulty in cutting the seed. In Figure 21a, a thickened seed coat of a subalpine fir seed with a deteriorating megagametophyte is shown. With continued deterioration all that will remain within the seed coat is the megaspore cell wall (Figure 21b).

Empty seed can also be the result of insect damage as shown in Figure 22 with the complete destruction of seed contents by the *Megastigmus* seed chalcid in coastal Douglas-fir. There are no morphological clues to the presence of this pest in a seedlot, but they can be detected by x-ray radiographs. More detailed coverage of insect problems of cones and seed can be found in the suggested readings.

Fully deteriorated or dead-filled seed often contain a brown tar-like material, but no embryo or megagametophyte can be recognized. These seed have specific gravities similar to viable, filled seed and can be difficult to remove during the processing of some species. Figure 23 illustrates one hypothetical pathway for this type of deterioration. In Figure 23a, the embryo and

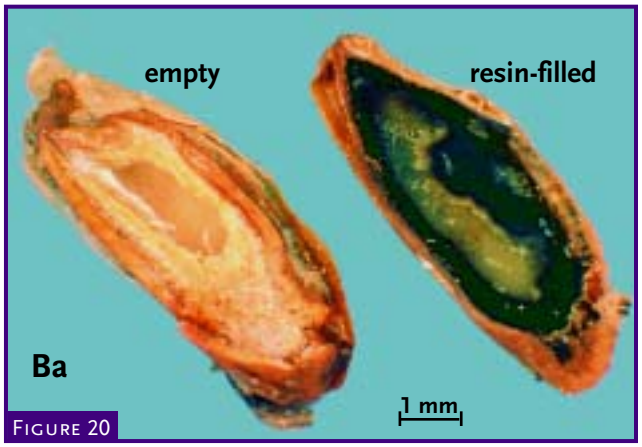

Figure 20

Comparative morphology between an empty and resin-filled Amabilis fir seed.

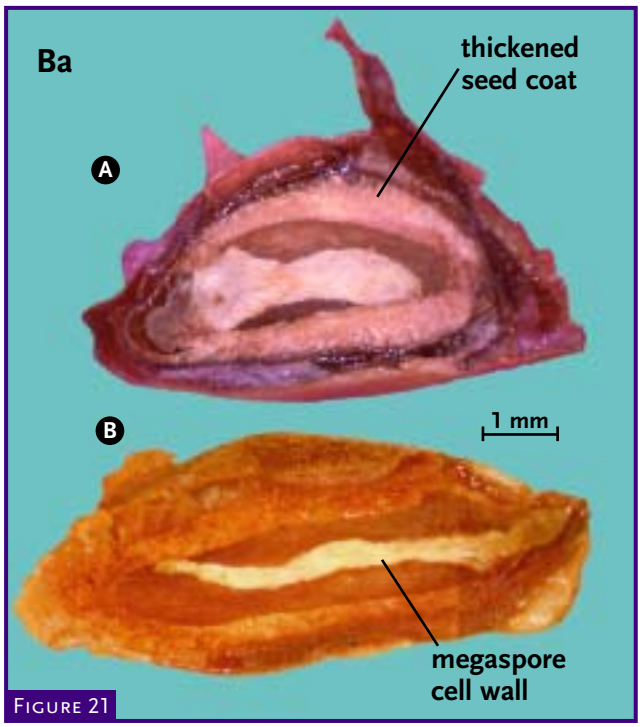

Figure 21

Empty seed of Amabilis fir displaying (a) a thickened seed coat and deteriorating megagametophyte and (b) completely deteriorated megagametophyte.

Figure 22

Destruction of a Douglas-fir seed by a Megastigmus *larva.*

megagametophyte appear translucent, have an uncharacteristic colour and a rubbery texture. In Figure 23b, the seed is undergoing a transition to a solid resinous consistency, but the outline of the embryo can still be recognized. Fungal mycelia are present between the megagametophyte and the embryo. It is not common to see seed at this intermediate stage of deterioration. In Figure 23c, the tissue is completely transformed into what is referred to as resin-filled, dead-filled, or 'woody' seed. This problem is quite common in *Abies* spp. but has also been observed in spruce, hemlock, western redcedar and yellow-cedar.

In Figure 24, an interior spruce seed displays deteriorated contents that are interspersed by fungal hyphae. This type of marbled seed is not common in seed cuts and is probably another intermediate stage in deterioration similar to Figure 23b. Many fungi can be found on the seed

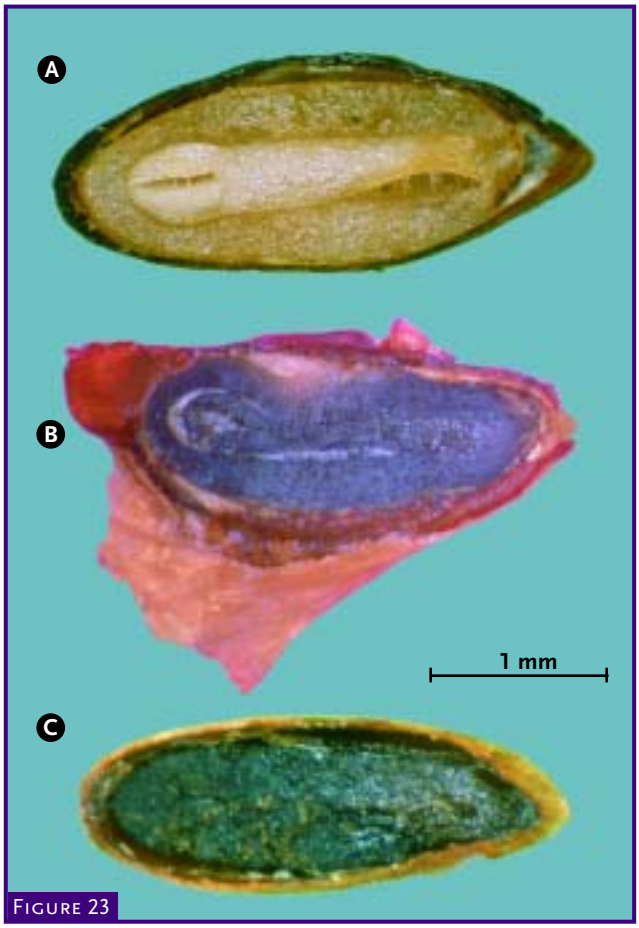

Figure 23

A possible deterioration pathway for resin-filled seed from (a) gummy tissues with structures distinct, to (b) solidified contents with structures barely visible, and (c) solidified contents with no structures evident.

Figure 24

Fungi inside a deteriorated seed of interior spruce.

coats of conifers and are usually a combination of pathogenic and non-pathogenic fungi. Three seed-borne fungi have been identified as problematic in B.C. conifer nurseries: *Fusarium* sp., *Caloscypha fulgens*, and *Sirococcus strobili*. This manual will not go into the biology of these species, but growers should be aware that fungal assays are being performed for these pathogens on seedlots in storage. For further details on disease problems consult the 'Suggested Readings' section.

A common type of deterioration is the discoloration of tissues or apparent 'bruising.' In Figure 25, a healthy seed is compared to seed with a completely deteriorated and partially deteriorated megagametophyte. The reserves of the partially discoloured megagametophyte have been depleted and it is uncertain whether enough remains to supply the energy for germination to occur. The cause of the discoloration is not known and it is probable that several factors may produce similar results. Seed deterioration can be minimized by storing seed at low temperatures and low moisture contents. Seed quality at time of initial storage is another factor influencing seed longevity. Gentle handling of seed during seed processing, seed preparation, and sowing is important to maintain the seedlots potential.

Another sign of deterioration that has been observed in some seedlots, particularly spruce, is the presence of a translucent embryo as shown in Figure 26. This morphology is abnormal and it was anticipated that these seeds would not germinate. Comparison between proportions of these seed in a seedlot and final germination clearly indicated, however, that at least some seed

with this morphology do germinate. It was found that this translucency was a transient character in many seed and upon imbibition and stratification many seed returned to a morphology more representative of spruce. Cause of this morphology is unclear, but heat damage is a possibility. It is exceptions like this that make it difficult to prescribe strict guidelines for determination of germinable seed; there will always be exceptions.

Seed reach their maximum viability and peak maturity at the time of natural seed shed. Variation in seed maturity has been noted between stands, between trees within a stand, and between cones on the same tree[57]. Seed maturity is usually based on the presence of a fully developed embryo. In B.C. the primary, but not sole, criterion used is that the length of the embryo should be at least 90% of the corrosion cavity. Immature seed also possess a megagametophyte that is soft, milky, but generally not firm as those found in mature seed. By cutting seed longitudinally and examining the embryo length you can determine when seed maturity is achieved. Seed not achieving this degree of maturation may still germinate, but seedling vigour and storability will probably be reduced[13].

In Figure 26a, the embryo has elongated to about 33% of the corrosion cavity, the cotyledons are not very apparent, making it unlikely that this seed will germinate. In Figure 26b, the embryo is about 50% of the corrosion cavity, the cotyledons are distinct, but not fully elongated. This seed may germinate, but it will likely be slow to develop and will probably not produce a sellable seedling.

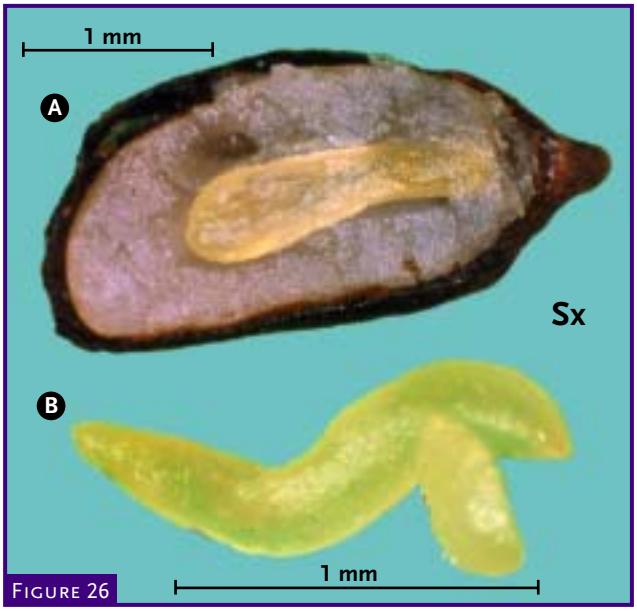

Figure 26

Interior spruce with a translucent embryo (a) within the seed and (b) excised from the seed.

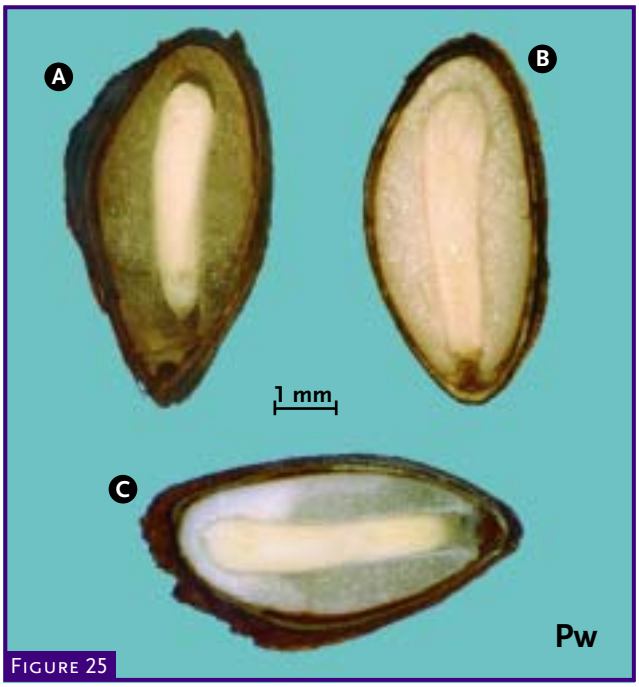

Figure 25

Western white pine seed showing (a) healthy seed, (b) total deterioration of the megagametophyte tissue, and (c) incomplete or ongoing deterioration of the megagametophyte.

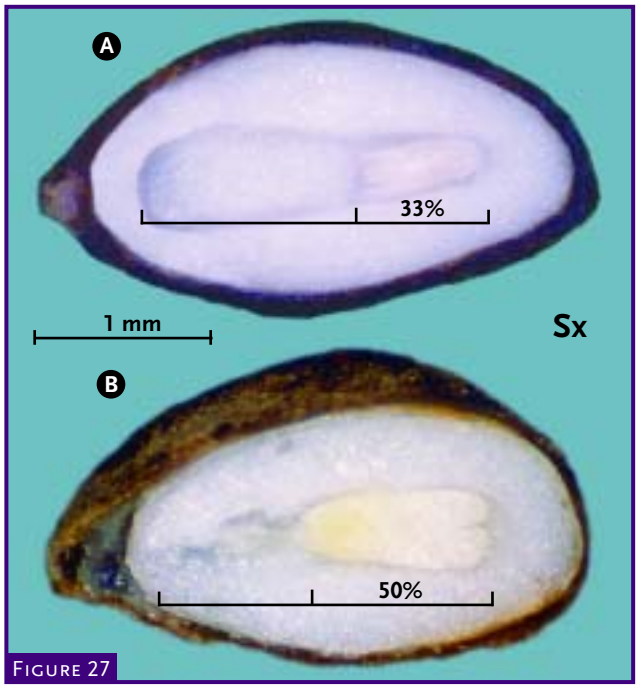

Figure 27

Immaturity of spruce seed displayed by (a) 30% embryo development and (b) 50% embryo development.

Seed Classification

The first step in classifying a seed sample is to examine the external morphology. Are cracked or decorticated (seed coat removed) seed present? Does the seed show high variability or uniformity in size or shape? Is resin vesicle damage evident as a dark grey colour, sticky feel, and distinct aroma? Are fungi present on the seed coat? Many external characteristics are useful in alerting one to problems inherent in a seedlot (i.e., decorticated or cracked seeds sink quickly when placed in water allowing for relatively easy upgrading).

The act of cutting the seed also provides information related to seed quality. A viable seed can be cut fairly easily with a crisp, clean cut. Ease of cutting varies by species with yellow pine and Amabilis fir being the most difficult to cut due to their relatively thick seed coats. Seed that cut very easily are probably empty or immature. The 'feel' to cutting seeds will come with experience (repetition).

Once seeds are cut they need to be classified to provide an assessment of seedlot quality. Seed should be categorized as soon as possible after cutting as exposure to room conditions will result in dehydration, discolouration and changes in morphology. The heat from microscope lights can also change seed morphology and exposure should be kept to a minimum.

Many methods of classifying cut seed can be constructed. Depending on species and seed quality, the categories can vary greatly and one classification will not be suitable to all situations. Some characteristics to look for are the colour, **opacity**, consistency, texture, and degree of development of the seed components. Cutting tests are subjective and although most people will obtain similar results for good quality seedlots, interpretations may vary greatly for 'problem' seedlots (i.e., seedlots with a large proportion of seed marginal in appearance).

One of the simplest and most common classifications is to divide the cut seed into three categories : i) viable seed, ii) damaged and discoloured seed, and iii) immature seed. Start with this simple classification and add more categories if required. A more detailed classification may be required because the damaged and discoloured category needs to subdivided. One can subdivide based on the component (embryo, megagametophyte, or both) and/or the observed morphology (i.e., yellow, translucent contents, discoloration of the megagametophyte).

A flowchart illustrating one classification key that has been used at the Tree Seed Centre is illustrated in Figure 28 with a corresponding cutting test sheet in Figure 29 (page 22). The chart initially looks at the two most easily identified problems: rotten and immature seed. The rotten seed are definitely non-viable, but immature seed may still germinate and are differentiated based on whether the megagametophyte appears healthy. The potentially viable seed are the 'good' seed, but the slightly deteriorated seed also germinate and the best estimate of germination capacity is usually obtained by the sum of the proportions of seed in these two categories. The last two categories contain seed with a deteriorated megagametophyte with or without a deteriorated embryo. The cutting test sheet shows additional categories that are further subdivisions of those illustrated in the flowchart.

This volume provides a baseline for what mature, healthy, viable seed should look like in longitudinal section for many conifer species. It also illustrates some of the observable problems found in seed that can affect germination. Some of these problems (empty seed, totally deteriorated contents) enable you to determine that a seed will not germinate, but other problems (immaturity, bruising, fungal infection) vary in degrees and it is more difficult to predict whether germination will occur. One factor common to most species is that slight discoloration (greying) of the megagametophyte does not usually preclude germination. Providing an optimal germination environment is important. Proper record-keeping of cutting tests and comparison with the germination obtained is critical. This will help you predict the fate of the marginal seed grown in your nursery.

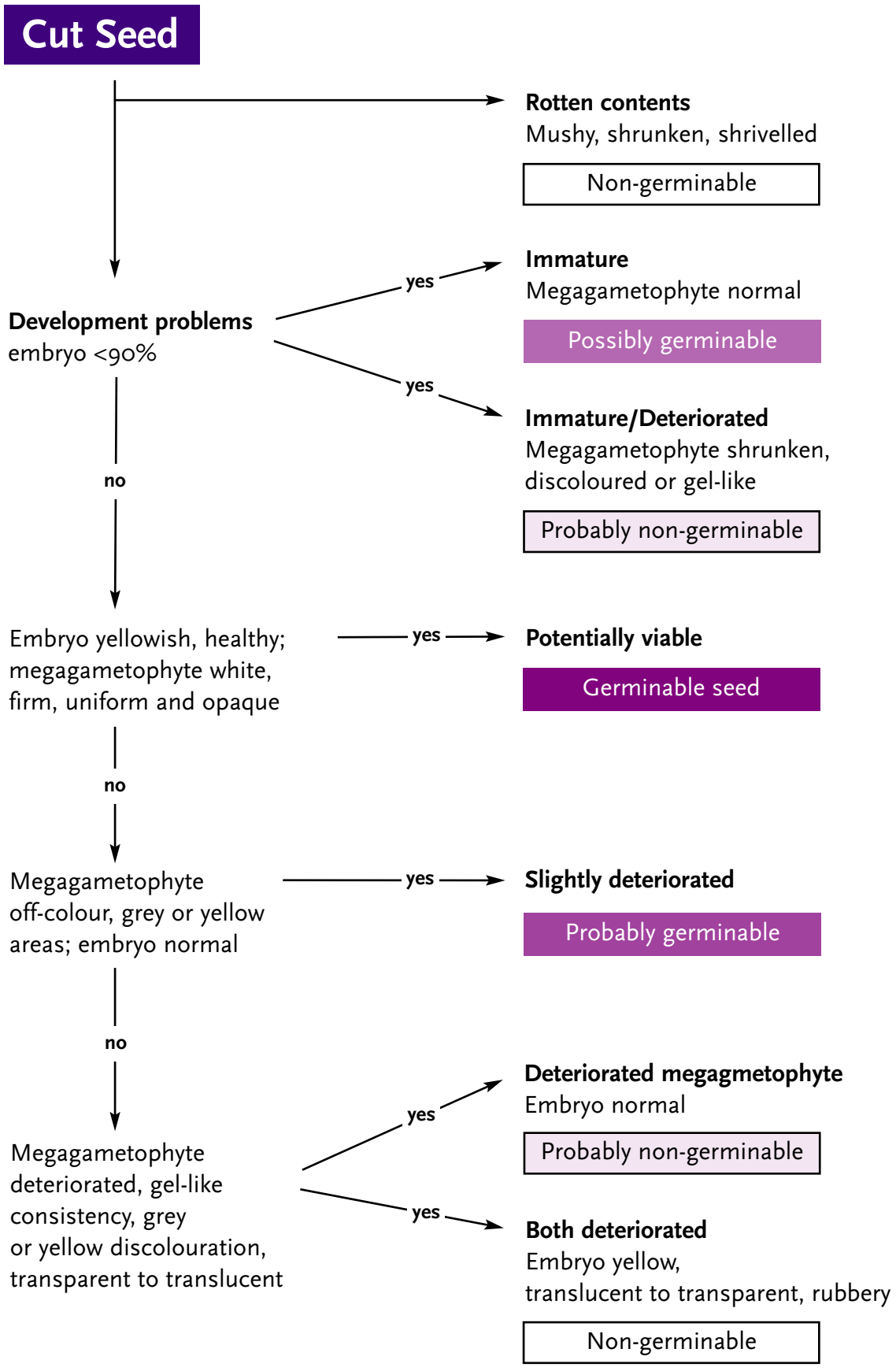

FIGURE 28

One example of a key method for classifying seed from cutting tests.

FIGURE 29

A cutting test sheet for classifying seed and recording comments.

		Viable	Questionable Viability				Immature		Non-viable			
						EMBRYO						
		Good	Good	Normal	Normal	Deteriorated translucent rubbery	Development problems	Development problems	Rotten	Other	Empty	Comments
Seedlot/ Sample	# of seeds					MEGAGAMETOPHYTE						
		Good	Good/Thin seed coat	Slight discolouration	Grey/Yellow deteriorated	Grey/Yellow deteriorated	Normal	Shrunken/ discoloured	Rotten	Other		