Draft Guidance for Seed Testing at Forest Commission Approved Forest Tree Seed Testing Facilities

Contents

1. INTRODUCTION

On 1st January 2003, the Forest Reproductive Material (Great Britain) Regulations 2002 implementing EU Directive 1999/105 came into force. The directive replaces two previous EU directives on forest reproductive material (FRM). The new GB regulations replace the Forest Reproductive Material Regulations 1973 and 1977.

1.1 Seed Testing

1.1.1 Under the new regulations, all seed of the 46 controlled species and the genus *Populus* (see www.forestry.gov.uk/frm for the full species list) which is marketed must be tested. See the publication *Forest Reproductive Material: Regulations controlling seed, cuttings and planting stock for forestry in Great Britain* for a definition of marketing. The statutory tests are as follows (see regulation 19(2)):

a). percentage by weight of pure seed, other seed and inert matter

b). germination percentage of the pure seed or where this is impractical, the viability percentage

c). weight of 1000 pure seeds

d). number of germinable/viable seeds per kilogram

1.1.2 The regulations require only that statutory testing be carried out once on each seed lot. There is no requirement to test seed annually, therefore there is no statutory seed testing year, or to retest any smaller lots split off from the original lot.

1.1.3 The frequency and standard of any retesting will be a commercial decision for the seed supplier. However the statutory tests must be carried out after mixing seed lots (see 'Mixing lots of reproductive material' in *Forest Reproductive Material: Regulations controlling seed, cuttings and planting stock for forestry in Great Britain*).

1.1.4 There is no requirement to statutorily test seed that is not marketed.

1.2 Approved seed testing facilities

1.2.1 One of the significant differences in the new directive and regulations is the way in which seed testing is handled. Under the previous regulations, seed testing could only be handled by an Official Seed Testing Station. However, there is now no requirement to nominate an official testing station. Instead, any facility which wishes to carry out seed testing to meet the requirements of the regulations, must practise "**internationally accepted techniques**" as defined by the Commissioners, and obtain prior approval from the Forestry Commission (FC).

1.2.2 The purpose of this guidance is to provide illustrations of what the Forestry Commission considers to be internationally accepted techniques. This guidance is therefore not exhaustive and it will be for each testing facility seeking approval to show that their practices are internationally accepted.

1.2.3 The FC guidance relates mainly to species controlled under the regulations, but also include information on testing the most common native tree and shrub species which are not controlled.

1.2.4 Both the International Seed Testing Association (ISTA, www.seedtest.org) and Association of Official Seed Analysts (AOSA, www.aosaseed.com) publish seed testing procedures suitable for use in the testing of tree and shrub seeds.

1.3 Weighed replicate testing

1.3.1 The number of germinable seeds per kg is normally derived from two attributes; germination percentage and 1000 pure seed weights. However, for certain species it is either not practically possible or it is excessively time consuming to separate out the pure seed from the inert matter in a seed lot. A special forest seed test has been devised which derives this number of germinable seeds per kg directly from a weighed amount of the seed lot, without purity separation and 1000 pure seed weight determination. This test is known as the **weighed replicate test**.

1.3.2 Weighed replicate testing as described in 4.11 will be a sufficient test for *Betula* spp., *Populus* spp., *Alnus* spp., and *Salix* spp., removing the requirement to carry out a purity analysis. Either a weighed replicate or counted germination test is allowed for germination testing of *Betula* species. For *Eucalyptus* spp. the weighed replicate germination testing is the only test recognised by ISTA.

1.4 Cutting tests

1.4.1 For highly perishable, large seeded species such as oak, a cut test is sufficient to give an indication of viability, in part because germination testing is often concluded after the seed is sown.

1.5 Seed of species that can be sold stratified or dry

1.5.1 A number of species, *Fagus sylvatica*, *Fraxinus excelsior*, *Prunus avium*, *Tilia* spp. and *Acer pseudoplatanus*, can be sold stratified or dry. Purity analysis should be carried out on the dry seed to check for species purity, other forest seeds, and the number of seeds/kg. The purity percentage has no value for stratified seed because the seed is mixed in with the stratifying medium, but the number of the number of germinable/germinating seeds per kg of treated bulk is a useful characteristic and should be given as additional information.

1.5.2 Where seed of the species set out above are being sold dry and stratified, germination or viability tests must be carried out separately for the dry and stratified lots. For example, where a seed lot of *Prunus avium* is being sold dry and also stratified, a modified excised embryo test should be performed on the dry seed before treatment has begun. A germination test should then be performed on the stratified seed.

1.6 Small quantities of seed

1.6.1 Small quantities of seed as defined in Schedule 11 of the regulations (see Tables 1A-C) do not need to be tested for either a) germination percentage of the pure seed or where this is impractical, the viability percentage or b) the number of germinable/viable seeds per kilogram. This avoids the destruction of potentially scarce seed, although for many of the species, in economic terms, the listed small quantity is very small.

1.6.2 Seed testing is part of the normal economic cost of supplying seed, but exceptionally where seed lots are very valuable, or in economic terms very small, the Forestry Commission will consider requests to authorise reduced testing. In considering any request, the Forestry Commission will take into account all the circumstances, including the size and value of the lot, the costs involved in testing, and its scarcity in the market.

1.6.3 The reduced testing authorised could allow; testing using the weighed replicate test, reducing the submitted sample to the size of the working sample, or exempting a particular lot from testing.

1.6.4 Regulation 19(3) allows the seed of any species collected during that season to be first marketed without the germination or viability test having been concluded. However, the full results of testing must be available on the supplier's document covering any subsequent marketing.

1.7 Supplier's documents

1.7.1 The data for the four tests must be set out on the supplier's document which is passed from seller to buyer. The date of the seed test must also be given. Where the seller has also carried out more recent non-statutory tests, the results from these can, additionally, be displayed on the supplier's document. However, it must be clear from the document which data is the result of statutory testing, and which has been achieved using less rigorous standards. Supplier's document templates are available from the Forestry Commission.

1.8 Additional information

1.8.1 The 'Seed Manual for Forest Trees' edited by A G Gordon, (published in 1992, ISBN 0117102717, available from the FC) should be consulted for general reference and where specifically referred to in this guidance.

1.8.2 '*Forest Reproductive Material: Regulations controlling seed, cuttings and planting stock for forestry in Great Britain*' provides general guidance on the 2002 regulations, supplier's documents and the related practices and procedures. www.forestry.gov.uk/frm

1.8.3 ISTA have a number of useful publications, including the International Rules for Seed Testing, which is updated annually, and the Handbook of Tree and Shrub Seed Testing, 1st Edition, 1991. ISTA website: www.seedtest.org

1.8.4 The Association of Official Seed Analysts (AOSA) also publishes seed testing methods and procedures used in North America. AOSA website: www.aosaseed.com

1.8.5 Useful information is also contained in Forestry Commission Practice Note 12: Handling and Storing Acorns and Chestnuts and Sycamore fruits by Peter Gosling.

1.8.6 For general info on tree & shrub seeds - Gosling, P.G. (2007). Raising trees and shrubs from seed (PDF-648K). Forestry Commission Practice Guide 18. Forestry Commission, Edinburgh.

1.8.7 For principles and practices of seed sampling and testing - Gosling, P.G. (2003). Chapter on 'Seed viability testing'. pp. 445-481 In: Seed conservation: Turning science into practice. Eds. Smith, R.D., Dickie, J.B., Linington, S.H., Pritchard, H.W. & Probert, R.J. Publ. Royal Botanic Gardens, Kew. 1023pp.

1.8.8 For principles and practices of seed sampling and testing tropical and sub-tropical tree seeds, but with much in common with temperate species - Gosling, P.G. (1998) Principles of sampling and testing tropical and sub-tropical tree seed. In: Tropical and sub-tropical Tree and Shrub Seed Handbook. Eds. Poulson, K., Parratt, M.J.R. and Gosling, P.G. Publ. International Seed Testing Association, Zurich, Switzerland. 204pp.

1.8.9 The United States Department of Agriculture, Forest Service has produced a "*Woody Plant Seed Manual*" which is available at: http://www.nsl.fs.fed.us/wpsm/

2 SAMPLING

2.1 Introduction

2.1.1 To evaluate the true quality of a seed lot, a sample from it must be tested. A sufficiently large sample is needed so that all the properties of the lot are uniformly represented.

2.1.2 To obtain accurate results, which could be compared with official, control samples (regulations 28 and 29); the size of samples taken, and the methods used should be as near as possible to those used in international practice.

2.1.3 Sampling of any seed lot requires training and practise. For trees species with large seeds or seeds with a less uniform shape the more difficult it is to obtain representative samples.

2.2 Definitions

2.2.1 **Seed lot** is a specified quantity of seeds or fruits of the same species that is physically and uniquely identified. In practise it will be harvested at approximately the same time and processed and stored as a bulk and is likely to be marketed under a single Master Certificate. Within ISTA there are maximum weights for seed lots, this principle has been adopted by the Commissioners, see Table 1A-C. The quantity that defines a small seed lot is also given in the Tables.

2.2.2 **Primary sample** is a small portion taken from one point in the lot during one sampling operation.

2.2.3 **Composite sample** is a thorough mixture of all the primary samples taken from the seed lot.

2.2.4 **Submitted sample** is the sample that is actually submitted to the testing facility (see 2.3). For very valuable seed lots the size of the submitted samples and the working sample are the same.

2.2.5 **Working sample** is that part of the submitted sample used for the initial purity and germination tests. In this guidance the working sample is generally half the submitted sample except for the large seeded species.

2.2.6 **Retained sample** is that part of the submitted sample that remains untouched after the tests are completed and which is retained by the seed testing facility for future comparisons or re-testing.

2.3 Submitted sample

2.3.1 The submitted sample weights/sizes are listed in Table 1A-C and are the same for routine testing or for official control purposes. For all other purposes the

submitted sample sizes should be at least those listed in the table. The submitted sample may comprise either the whole, or a sub-sample, of the composite sample. The minimum submitted sample size is generally at least twice the working sample size, but can be much larger.

2.3.2 These weights have been based on the weight required to contain at least 2500 seeds of each species, except for the large-seeded species where a maximum of 1000 g has been applied as in the ISTA Rules.

2.3.3 Where any batches of e.g. *Quercus* spp. are delivered to the buyer prior to sampling the whole lot, a representative sample of the batch must be taken. This sample must be adjusted to reflect the relative size of the batch to the whole seed lot and then mixed with the final bulk sample, which is then reduced to the required submitted sample weight.

2.3.4 For the very large seeded *Aesculus*, *Castanea*, *Corylus* and *Quercus* spp., a submitted sample of at least 500 individual seeds or fruits is permitted, because of the large weight of the seeds.

2.4 Mixing a seed lot before sampling

2.4.1 Before a sample is taken the lot must be thoroughly mixed to ensure that it is as homogenous as practicable.

2.4.2 For diagrams of mixing apparatus see the figures. Where there is no such apparatus available, bulk seed can be satisfactorily homogenised by repeated moving and mixing a bulk of seed by shovel on a clean level floor until it appears homogeneous. For a large seed lot or seed with appendages, it may be necessary to mix and move the seeds six times or more to achieve a satisfactory result.

2.4.3 Knowledge of the physical characteristics of the seeds will give the sampler an idea whether he can expect sampling problems. Seeds that flow easily and have no appendages can be mixed quite readily, and sampling normally causes no problem.

2.4.4 For some species it is very difficult to see whether or not the sample is truly homogenous e.g. those species such as *Larix*, *Abies* and *Betula* that often contain a high percentage of empty seeds. Although the lot may mix easily, the heavier seed may settle out when the lot is handled and transported, giving rise to sampling problems.

2.4.5 Seeds with wings, e.g. *Acer* and *Fraxinus* spp., are particularly difficult to homogenise in large bulks. Large seeded species such as *Aesculus*, *Castanea*, *Corylus* and *Quercus* spp., are also difficult to mix properly because of the large volume of seed and the danger of mechanical damage when they are handled at high moisture contents immediately after harvest.

2.4.6 It is possible to carry out a test for homogeneity of a seed lot but this is not normally done because it is very time consuming. However, an indication of a lack of homogeneity can be found by examining each primary sample taken from the bulk.

2.4.7 Samplers should therefore acquire experience in homogeneity/heterogeneity for as many species as possible.

2.5 Labelling of a seed lot

2.5.1 At sampling a seed lot may be loose in bulk or in sacks or other containers, but each sack or container must always be marked with a label identifying it as part of a single lot.

2.6 Taking primary samples

2.6.1 The primary samples should be as similar in size as possible.

2.6.2 For most species, a stick- or sleeve-type sampling probe should be used for taking primary samples; but for seeds which do not flow easily, special techniques are necessary (see 2.8).

2.6.3 Primary samples may also be taken as the seeds are fed into containers, provided that the sample taken covers the complete cross section of the seed stream, and that the seeds sampled do not bounce out again.

2.6.4 When sampling any sack, container or pile it is essential that samples are drawn from all levels of the seed, e.g. top, middle and bottom of a sack; and surface, middle and deep layers of containers and piles. This is necessary to guard against the possibility of some unseen separation of seed types (such as empty seeds) or inert matter (such as fruit remains) during sack filling or creation of the pile.

2.7 Stick- or Sleeve-Type Sampling Probes

2.7.1 The stick- or sleeve-type sampling probe consists of a brass tube that fits closely in an outer casing in which it can be turned. The casing has a pointed end. Both tubes have three open slits, through which the seeds can flow into the hollow spaces of the inner tube if the slits of the two tubes are brought into alignment by turning the casing. The tubular sampling probes are made in various lengths and diameters, depending upon the different types of seeds and sizes of containers to be sampled. Dividers must be inserted in the tube so that each slot is separate preventing seeds from the upper portion of the lot flowing down the whole tube, thereby causing an over-representation of the upper layers in the container. If the dividers are missing small corks pushed into the

tube can make effective dividers. See the figures for pictures of acceptable sampling equipment.

2.8 Sampling by hand

2.8.1 For some types of seeds, especially those that do not flow easily e.g. large seeds, those with wings (*Acer, Fraxinus*), and those with hairs (*Populus, Salix* when the seed is sold with the hairs on), the sample can only be taken satisfactorily by hand.

2.8.2 The fingers must be tightly closed around the seeds when the hand is withdrawn.

2.8.3 However, it is difficult to get samples from the deeper layers in containers and sacks. Some sacks must be partially or completely emptied to get access to take samples. This applies especially to large-seeded species (i.e. *Aesculus, Castanea, Corylus, Fagus* and *Quercus* spp.) where it is impossible to remove a handful of the seeds from even half way down a sack so it will require emptying the sack, and spreading it out evenly to take a representative sample.

2.9 Sampling Intensity

2.9.1 The maximum seed lots sizes are given in Tables 1A-B.

2.9.2 For seed lots in bags or containers that are uniform in size and capable of holding 15 to 100kg of seed, the following is the minimum sampling intensity:

1-4 containers	3 primary samples from each container
5-8 containers	2 primary samples from each container
9-15 containers	1 primary samples from each container
16-30 containers	15 primary samples from the seed lot
31-59 containers	20 primary samples from the seed lot
60 or more containers	30 primary samples from the seed lot

2.9.3 For seed lots in bags or containers less than 15kg capacity, the bags or containers should be grouped together in sampling units not exceeding 100kg, and each sampling unit should be regarded as a bag or container in 2.9.1 above. Sampling of each unit should be done by means appropriate to the type of seed and type of container.

2.9.4 For seeds in large crates or containers of more than 100kg capacity or in open piles, the sampling intensity should be as follows:

Up to 500kg	At least five primary samples
501-3000kg	One primary sample for each 300 kg but not less than five
3001-5000kg	One primary sample for each 500kg but not less than ten

2.10 Post-sampling procedure in the seed store

2.10.1 All the primary samples taken should be thoroughly mixed to form the composite sample. If the composite sample is too large to use as the submitted sample it is reduced to the size of the submitted sample by repeated halving.

2.10.2 The submitted sample weights are given in Table 1A-C. The submitted samples must be labelled as coming from that seed lot. The label must be attached to the sample. Samples must always be sealed.

2.10.3 Once the submitted sample has been taken it must be packed to prevent damage during transit to the testing facility. Samples are the responsibility of the sampler and must be dispatched to the testing laboratory without delay.

2.10.4 Samples for moisture content determination must be packed in moisture proof containers from which as much air as possible is excluded. In addition samples for germination and viability testing only but which are of low storage moisture content should also be packed in moisture proof containers.

2.11 Sampling in the seed testing facility

2.11.1 On arrival at the seed testing facility, after registration of the submitted sample, it should be thoroughly mixed and randomly divided to produce the working sample. The weight of the working sample is about half that of the minimum submitted sample size set out in Table 1 except for the large seeded species where the working sample 400 seeds drawn at random from the 500 seeds of the submitted sample for *Corylus avellana* and 500 seeds for *Castanea sativa*

and *Quercus* spp. (Tables 1A and 1C). These and any inert matter must be drawn at random. For very valuable seeds the working sample is the submitted sample (see 1.6).

2.12 Methods for obtaining the working sample

2.12.1 The working sample is obtained using the following equipment and methods.

2.12.2 The **Mechanical Method** using the **Soil or Riffle Divider** is suitable for all kinds of seed except very large seeds and those that do not flow easily. The equipment has 18 slots and randomly divides a sample into two approximately equal parts. The whole submitted sample should first be mixed by passing seed through the divider and recombining the parts at least twice before reducing. The sample is reduced by running it through the divider repeatedly, and keeping or discarding each half depending on the sample size required. The process is repeated until a working sample of about the required size (but not smaller) is obtained. See figures for an example of how to obtain a 1kg submitted sample from a 1.6kg composite samples by continual halving.

2.12.3 **Manual Methods** require no complicated or expensive pieces of apparatus. However, experience is required in order to achieve satisfactory results.

2.12.4 For the **Random Cups Method** six to eight small cups or beakers are distributed at random on a tray. After being mixed thoroughly, the submitted sample is shaken out uniformly over the whole tray. The seeds that fall into the cups form the working sample. However to obtain more or less the exact weight required for each species, cups and trays of different sizes must be used.

2.12.5 For the **Continuous Halving Method** no special apparatus is needed. After mixing thoroughly, the submitted sample is spread evenly over a smooth flat surface. With a flat blade the sample is divided approximately into two halves. One half is discarded and the other re-mixed and the process repeated until the desired working sample but not less, is obtained.

2.12.6 The **Spoon Method** requires only a smooth flat surface, a flat blade and a spoon. After thorough mixing, the seed is shaken out over the surface. Small amounts of seeds are taken from not less than five randomly selected places on the tray, using the spatula to push the seeds into the spoon. The process is repeated, taking small amounts of seeds in this way until the prescribed amount of the working sample is obtained. This process can only be used satisfactorily with seeds equal to or smaller than those of *Crataegus monogyna*.

2.13 Problems in obtaining random working samples

2.13.1 Those seed types that give problems in obtaining the submitted sample in the seed store also present difficulties in obtaining the working samples in the seed

laboratory. No mechanical or physical methods described can be used for sampling *Aesculus*, *Castanea*, *Corylus* or *Quercus* spp. Mechanical methods can only be used with difficulty for large, winged seeds and seeds with hairs. Such seeds will not fall through the partitions of a riffle without some manual assistance. For these species, the only method of obtaining a randomly selected working sample is to use the continuous halving method.

2.13.2 Problems that can occur during the random selection of the 100 seed replicates from the working sample are described at 4.8.5.

2.14 Storage and retention of samples

2.14.1 Where testing cannot begin as soon as the sample reaches the seed testing facility, the sample should be kept in suitable containers in a cool, well-ventilated room or a refrigerator. For some species that do not store well it is important that the analysis is started as soon as possible. For seeds with high moisture content e.g. *Aesculus*, *Castanea*, *Quercus* spp., evaporation should be reduced as much as possible while still allowing the seeds to breathe.

2.14.2 Suppliers must keep their retained samples for nine months from the date of initial testing to allow for retesting in the event of dispute. Provided these samples have arrived at a low moisture content they can be stored without change of quality for very long periods by placing them in sealed plastic bags at 3-5°C. However, seeds with high moisture contents will lose quality rapidly unless given thermo-therapy. Stored seeds of *Aesculus*, *Castanea* or *Quercus* spp. are unlikely to give repeated test results in tolerance with the initial tests unless the repeat tests are carried out very soon after the initial test. For these species no retained sample need be kept.

2.14.3 For very expensive or scarce seed lots, the retained sample may be returned to the owner except for 25 seeds, which should be retained for reference purposes.

3 ANALYTICAL PURITY ANALYSIS

3.1 Introduction

3.1.1 Analytical purity analysis is one of the basic seed tests. In agriculture, the purchaser of seed wants to know how much non-seed matter he is paying for in a seedlot, including how much weed seed there is in it and particularly the germination percentage of pure seed.

3.1.2 With forest seed the position is rather different. Forest seed producers invariably deal with wild populations of seeds and the processing equipment has usually not been specially designed. As a result, forest seed lots may contain seeds of different tree species. Because no equipment may be available to improve the purity percentage of tree seeds, often little practical use is made when this characteristic is reported in a purity analysis, however it does provide the mechanism to extract the pure seed to test. Similarly, because most forest seed is grown under nursery seed bed conditions where many obviously different species can be easily rogued out, the other crop seeds present may not cause many practical difficulties, such as wild oats do in cereals.

3.1.3 However purity analysis of forest seeds can be very important for other reasons. The number of seeds in a kilogram capable of germinating can vary greatly from lot to lot because of the great variation in nature between different seed lots of the same species. Unless seedbed sowing densities are modified to take account of this, seedbeds may either be very sparse or far too crowded. For forest seed therefore the important characteristic is the number of germinable seeds per unit weight. Therefore, a seed weight determination is a fundamental part of purity analysis of tree seed.

3.1.4 The second additional important aspect of forest seed purity analysis is the need for authentication of the species being tested. In agriculture, no seed producer mistakes oats from barley or wheat. In forestry, closely related species of trees often grow side by side allowing mixing of species by mistake. Therefore the authentication of the species on the label is a fundamental part of the purity analysis of the seed of tree species. For some species and for some small seed lots, as explained in 1.3, a weighed replicate test is used and the standard purity separation does not have to be performed. However it is still important that the species in the seed lot is authenticated and this procedure is described at 3.2.

3.2 Pure seed

3.2.1 Seeds of the species which predominates in the sample. It includes all botanical varieties, cultivars, provenances and origins (ecotypes) of the predominant species.

3.2.2 Any whole seed of the species described, plus parts of such seed more than half, as well as any clearly identifiable immature, undersized, shrivelled, diseased or germinated seeds of that species.

3.2.3 Pieces of seed units larger than one half of their original size, provided they retain part of the seed coat.

3.2.4 In seeds of *Robinia pseudoacacia*, *Abies*, *Larix*, *Picea*, *Pinus* spp and *Pseudotsuga menziesii*, when the seed coat is entirely absent the seed unit is regarded as inert matter.

3.2.5 Drawings of the pure seeds of each of the genera included in the Regulations are shown in the figures. They have been taken from the ISTA's Handbook of Tree and Shrub Seed Testing and were drawn by Joost van der Burg with modifications by Andy Gordon on the diagrams for *Picea* and *Pinus* spp. to take account of modifications in the ISTA rules.

3.2.6 For *Picea* and *Pinus* spp., when the seeds have part of the integument which is the tissue which joins the wing to the seed adhering to them, they should be regarded as "winged seed" and left intact. They should be weighed and reported separately, but after weighing they should be re-combined with the pure seeds and the mixture used for counting out the germination replicates.

3.2.7 For some genera, *Abies*, *Larix*, *Pseudotsuga* and *Carpinus betulus*, it is difficult to completely remove the integument without damaging the seed. In these cases, the wing only if present, is detached and placed in the inert matter fraction. For other genera, *Acer*, *Betula*, *Fraxinus*, *Populus* spp, *Salix* spp. and *Ulmus* spp., in which the wing of the seed is an integral part of the seed coverings, the wing is regarded as part of the pure seed. For those genera e.g. *Picea* where the wing detaches naturally, it is not included in the pure seed fraction.

3.2.8 The seeds of many species of Alnus, *Betula*, *Larix*, *Picea*, *Pinus* and *Quercus*, are difficult to distinguish with certainty from each other. When in doubt the analyst should only report the pure seed percentage as the genus i.e. all confirmed *Quercus* spp. seeds should be treated together.

3.2.9 Damaged seeds are often difficult to evaluate. Where no damage is evident seeds are classed as pure seeds or other seeds, even if it is obvious that they are empty. Individual seeds should not be turned over to see if they are damaged on the other side. If there are cracks or other openings in the seed coat and it can be clearly seen that not more than half of a true seed is present, the unit must be classed as inert. If the seed is severely damaged, the analyst must decide if the remaining undamaged solid part of the seed is larger than one half the original size, and apply this rule accordingly. If in doubt, the seed unit is classed as pure seed.

3.3 Other seeds

3.3.1 Seeds of any species other than that of the pure seed are regarded as other seeds. The distinguishing characteristics as defined for pure seeds are also used for other seeds. The seeds of many species within the same genus of tree seeds can be remarkably similar in size and shape, and at times the analyst is faced with a very difficult task.

3.4 Inert matter

3.4.1 All other matter and structures not included in the pure seed or other seed fraction. This includes fruit parts, wings, bark, stones, insect parts, nematodes, fungal bodies, soil, grass and seeds of species other than the predominant species, in which it is readily apparent that no true seed is present. It also includes broken pieces of seed, half or less than half the original size, and seeds where the entire seed coat has disappeared.

3.5 The laboratory procedure

3.5.1 A purity analysis is performed on a smooth surface with the aid of a pointed instrument by separating the working sample into the three component parts; the pure seed, inert matter, and other seeds.

3.5.2 For the large seeds of *Aesculus*, *Castanea*, *Corylus* and *Quercus* where the submitted sample is a minimum of 500 seeds, the working sample is 400 seeds drawn at random from 500 seeds (see Table 1).

3.5.3 The working sample is closely examined to ensure that it is predominantly composed of the species as labelled. It is then accurately weighed to the correct minimal number of decimal places depending on the working sample size.

3.5.4 If the working sample is 1.000 to 9.999g this should be weighed to a minimum of 3 decimal places, 10.00 to 99.99g to a minimum of 2 decimal places, 100.0 to 999.9g to a minimum of 1 decimal place, greater than or equal to 1000g to a minimum of 0 minimum places.

3.5.5 The working sample is separated into the three component parts and each fraction weighed to the same level of accuracy. Provided the difference between the original weight and the sum of the three fractions is less than 5%, the analysis can be accepted. If the difference is greater than 5% the reason for this discrepancy needs to be investigated and the analysis may need to be repeated on the other half of the submitted sample or weights recombined and started again.

3.5.6 The purity is the weight of pure seed divided by the original sample weight expressed as a percentage and reported to one decimal place. Values for inert

matter and other seeds found in the purity test are calculated in the same way. If any value is below 0.05% this should be reported as 'Trace'. If following rounding for each component the total percentage of pure seed + inert matter + other seed is 99.9% or 100.1% then the 0.1% needs to be added or deducted from the largest value (normally the pure seed value). Reporting values that come to a total of 100.0% and 'Trace' is acceptable.

3.6 Differentiation of seed of other species

3.6.1 The appearance of seeds of a different genus in a seed lot such as *Acer* spp. seed in *Fagus sylvatica* or *Pinus* spp seed in *Picea* spp. are generally easy to spot during the physical separation of the pure seed.

3.6.2 However, in conifers where a species may have a natural distribution covering thousands of miles, the size and weight of the seed in a single species will vary greatly sometimes by as much as 50%. Therefore the size of seed of e.g. *Picea abies* from the circum-polar regions of Sweden and Finland will be very similar to the size of the seed of *Picea sitchensis* from the Queen Charlotte Islands in Canada or the north of Scotland. Within any one population of these species there is also wide between tree variation in both colour and size. However, the general shape of all *Picea* spp. seeds is very similar.

3.6.3 In the same way, seed of individual species of the same conifer genus covered by the Regulations can look very like the seed of another species in the genus. *Abies alba* seed can look very similar to seeds of *Abies cephalonica*, *Abies grandis* and *Abies pinsapo*.

3.6.4 *Larix decidua*, *Larix kaempferi*, particularly the hybrid between them *Larix* x *eurolepis* and *Larix sibirica* from all provenances, can look very similar.

3.6.5 Seed of *Pinus brutia*, *Pinus halepensis* and *Pinus nigra* grown under some conditions can also look very similar.

3.6.6 With the broadleaved species covered by the regulations, it is only the seeds of *Alnus glutinosa* and *Alnus incana*, *Betula pendula* and *Betula pubescens*, *Fraxinus* angustifolia and *Fraxinus excelsior*, *Populus* spp. and *Quercus* spp. where species authentication can be difficult.

3.6.7 However the seeds of *Acer pseudoplatanus* can be difficult to distinguish with certainty from the seeds of several other *Acer* spp. grown as ornamentals in the UK. Likewise seeds of *Prunus avium* with seeds of other *Prunus* spp. grown in the UK, and both *Tilia cordata* and *Tilia platyphyllos* with other *Tilia* spp. growing in the UK. Here again, the seed analyst cannot be expected to certify species purity from a small seed sample.

3.6.8 The only truly effective way is a close supervision of collection sites and, where species like *Alnus glutinosa* and *Alnus incana*, *Betula pendula* and *Betula*

pubescens and *Quercus cerris*, *Quercus petraea* and *Quercus robur* are growing in mixed stands, collection from the individual trees.

3.6.9 Despite the between tree variation that unquestionably exists in the seed of the above closely related species, they all show some general characteristics. When these characteristics are seen in bulk, they can help to differentiate the species, although when individual seeds are compared side by side they will still prove difficult to differentiate with 100% certainty.

3.6.10 All the other seeds found are weighed together to the correct number of decimal places as determined by the working sample size see 3.5.4 and the whole expressed as a percentage of the original sample weight to give the Other seed %. If any value is below 0.05% this should be reported as 'Trace'.

3.7 Dealing with inert matter

3.7.1 All items of inert matter are weighed together to the correct number of decimal places as determined by the working sample size see 3.5.4 and the whole expressed as a percentage of the original sample weight. If any value is below 0.05% this should be reported as 'Trace'.

3.7.2 The component parts of the inert matter, such as insect larvae, can be particularly interesting to the seed producer, and should be brought to their attention in the report.

3.8 Calculation of the 1000 pure seed weight

3.8.1 To allow the seed to be sown at an optimum sowing density, the quality of the lot must be expressed as germinable or viable seeds per kilogram. Therefore, it is necessary to calculate the number of seeds in a given weight. This is done by calculating the 1000 pure seed weight.

3.8.2 From the pure seed fraction separated out during the purity analysis, eight replicates of 100 seeds taken at random are carefully counted out, and each replicate is weighed to at least two decimal places. The ISTA method of ensuring that there has been no unwitting bias during the selection of the eight lots of 100 seed, is to calculate the variance, standard deviation and coefficient of variation for the set of eight results. Provided the coefficient does not exceed 4.0, the uniformity of the eight results is acceptable. See figures for an example calculation.

3.8.3 However a simpler method although not giving the same level of accuracy as the ISTA method is acceptable. The average weight of the eight replicates is calculated and the difference between the lightest and heaviest is worked out. Provided this difference is not greater than one tenth of the average weight, the uniformity can be regarded as acceptable. If the result is not acceptable, eight

more replicates of 100 seeds should be drawn and the results calculated on all 16 replicates. From the average weight of 100 seeds the weight of 1000 pure seeds can be calculated.

3.8.4 If available a suitable electronic seed counter could also be used.

3.8.5 The 100 seed replicates counted out at random for the 1000 pure seed weight determination are then used for germination, tetrazolium, excised embryo or X-ray testing (see relevant sections).

3.9 Calculation of the number of seeds per kg

3.9.1 In order to calculate the number of seeds per kg in the original seed lot from which the submitted sample is taken, the following formula should be applied:

No of seeds per kg = 1000/T x 1000 x P/100

Where:

T = 1000 pure seed weight in grams and P = Purity % from the purity test

3.10 Purity testing in the weighed replicate test

3.10.1 One of the fundamental attributes of the weighed replicate test is that a purity separation does not need to be performed. However, authentication of the labelled species is still essential. Therefore, it is important during the random sampling of the four or eight weighed replicates and the germination assessment, to study the individual seeds to confirm that the predominant species is the one that appears on the label. Any other seeds should be identified, and the ratio of pure to other seeds noted on the final report.

4 THE GERMINATION TEST

4.1 Introduction

4.1.1 The germination test is the most important test of seed quality because even the most pure seed lot is worthless if it will not germinate. However, seeds of many tree species do not germinate readily because they naturally exhibit significant amounts of dormancy. Few tree species, if any, will have been subjected to any selection pressures to produce rapid germination and a long spread-out period for germination may well aid a tree species' survival.

4.1.2 It will be impossible to make a deeply dormant seed lot germinate in the nursery without special treatment, but it will also be difficult to assess its ability to germinate. For such seed lots, tests of the seed's viability have been developed to identify whether the seeds are alive after harvest, or after storage at the start of treatment to overcome the dormancy. Viability tests are described in section 5.

4.1.3 Germination tests are carried out on filter paper (or some other cellulose compound), and on or in sand or organic based potting compost depending upon the size of the seeds and the length of time that the seeds take to germinate. In order for test results to be strictly comparable with those performed in other laboratories, standard conditions are used. When comparisons between test results in different countries are made international standards are used. To this end a set of international rules has been drawn up by ISTA in which the methods and conditions are carefully defined.

4.1.4 The ISTA rules are based upon the optimum conditions for making seed of a particular species germinate and for tree seed often use an alternating 20-30°C regime. These conditions are never found in practice in UK nursery bed at sowing time. Arguably, therefore, testing tree seed germination under a regime more akin to those found in a UK nursery bed in spring will yield more meaningful and useful results to the nurseryman, i.e. a constant 10°C. However, although a nursery bed in the spring in southern England is likely to be 10-12°C there will be daily fluctuations so it could be argued that using an alternating temperature of 10-15°C might be better. Alternating temperatures can be difficult to maintain without expensive equipment a constant 15°C might be a good compromise and easier to maintain than 10°C but both will delay germination and might not give the maximum germination. Even easier to maintain would be a constant 20°C which is one of the ISTA suggested temperature regimes for some of the conifer species. The temperature regimes need to be monitored so the germination temperature is maintained within ± 2°C.

4.1.5 Recent research in Poland and France has shown that for many deeply dormant broadleaved species, alternating temperature regimes of 3-20°C or 5-15°C have yielded consistently better germination percentages after dormancy breaking, than 20°C constant or 20-30°C alternating. For all these alternating regimes the warmer temperature is given for eight hours with light if required.

4.1.6 Table 2 gives the acceptable germination conditions for tree seed testing in FC approved laboratories.

4.1.7 The following methods and apparatus are illustrative of the routines and equipment that can be used for carrying out germination tests under the FRM Regulations.

4.2 Germination apparatus

4.2.1 In all germination facilities an accurate record of the germination regime must be kept. Ideally a continuous record but if this is not possible the temperatures should be checked manually at least once per day. If an alternating temperature regime is used each temperature should be checked at least once per day and checks made to ensure the temperature change over period is achieved within 3 hours (ideally within 30 to 60 minutes). Alternating temperatures can also be achieved by immediate transfer between germination facilities at different temperatures.

4.2.2 All temperatures need to be controlled within at +/-2°C of the set temperature.

4.2.3 Different types of germination apparatus are available:

a). The **open-surface incubator** in temperature controlled room involves a series of open tanks of water, individually heated to the desired temperature regime with the germination tests suspended above the water surface on glass strips with water provided by a wick. The tanks are all housed below a source of light in a room carefully maintained at set temperatures. This system is capable of providing controlled temperatures but is uneconomical of space, costly, and does not easily allow more than one temperature regime to operate at any one time.

b). The **temperature controlled incubator** which can take many forms and must be able to control light durations and may need to provide alternating temperatures regimes. A simple incubator can consist of a unit in which the temperature is controlled by a circulation fan/air conditioning unit and fluorescent tubes provides light (see figures).The individual replicates of the test are each contained in separate plastic or glass germination boxes.

c). A walk-in **germination room** where lighting and shelving are fitted in a small, centrally heated/air conditioned room, is by far the most

economical means of providing adequate conditions for carrying out germination tests. The individual replicates of the test are each contained in separate plastic or glass germination boxes that are placed on the shelves. The shelves should not be placed immediately over the source of heat/cooling and a circulation fan should be used to ensure an even temperature distribution (see figures).

4.3 Germination boxes and dishes

4.3.1 Three of the apparatuses described above require germination boxes for providing the actual germination environment. These boxes are made of clear plastic or glass and the seeds are placed on top of filter paper. The filter paper sits on top of a platform held above a small reservoir of water from which it is fed by means of a wick to the paper above. The tight fitting lid maintains the humidity around the seeds at a high level (see figures).

4.3.2 For large seeds such as *Aesculus*, *Castanea*, *Corylus* and *Quercus* spp., larger plastic boxes are used which are filled with damp sand into which the 100 seed replicates are pushed (see figures). However, because the full germination test of *Aesculus*, *Castanea* and *Quercus* seed takes approximately one month to perform, by which time many newly harvested seed lots will already be sown in the nursery, such tests are not mandatory. Instead a viability (cutting) test will be acceptable (see section 5), although if possible this should be backed up by a germination test. *Corylus* seeds will only germinate after lengthy cold pre-treatment. They would normally be tested by a viability test, but after pre-treatment they will germinate in sand.

4.4 Germination media

4.4.1 For most germination tests some type of paper or other cellulose based medium is used. For large seeds, sand is commonly used although for samples showing heavy bacterial or fungal infections, organic potting media can be substituted.

4.4.2 Any type of uniform absorbent paper can be used. It is normally used flat with a supply of water provided by a string or paper wick from a water reservoir below. Paper is used for seeds not expected to exhibit deep dormancy up to the size of *Pinus radiata*. Crimped papers are used when a high proportion of small seeds are found to be infected with mould.

4.4.3 Sterile sand moistened to a standard amount is normally used for all seeds larger in size than *Pinus radiata*, and for smaller seeds which due to their dormancy, are expected to take a long time to germinate. The seeds may either be pressed into the surface of the sand (TS) or covered by the sand (S). The container should be kept sealed to prevent water loss, but if this happens it should be re-moistened with a water spray.

4.4.4 Any test carried out in sand may also be carried out in organic growing medium. It does have good natural buffering properties which helps it overcome the effect of fungal infection on seeds. For this reason it is sometimes substituted for sterile sand for badly contaminated seed lots.

4.5 Light

4.5.1 Light is normally applied when testing the seeds of many tree and shrub species. For most species, there is little scientific evidence that light is actually required as germination appears not to suffer. However, a number of conifer species and newly collected *Betula* and *Alnus* seeds have been shown to be responsive to different wavelengths of light, but this response usually disappears when the seeds are chilled before germination.

4.5.2 In contrast, more than 16 hours of light per day have been found to be harmful to seeds of *Pinus pinaste*r. Where light is thought to be advantageous it should be given for a minimum of 8 in every 24 hours, and should coincide with the warmer temperature (in an alternating temperature regime). Total darkness appears not to affect the ability of some species to germinate, but light during the assessment of germination is usually necessary.

4.5.3 Cool white fluorescent tubes are the best source of light, since both direct sunlight and incandescent light inhibit germination to some extent because of the far-red wavelength they contain. Fluorescent lamps emit very little far red illumination, and their emissions in the red wavelengths promote germination. The light should be of uniform intensity to all the samples, and should not create temperature gradations at the seed surface.

4.6 Temperature

4.6.1 The temperature regimes are detailed in Tables 2 and 3 and the actual temperatures achieved must be recorded and within +/-2°C of the set point. See 4.2 for details.

4.6.2 In some species, the temperature change itself stimulates germination, so the change at the seed surface should occur within one hour. If no temperature change is possible over weekends, the lower temperature should be maintained.

4.6.3 A special case is *Fagus sylvatica*, for which a very low germination temperature, 3° to 5°C, is prescribed.

4.7 Germination test - procedure

4.7.1 The working sample is subjected to either a purity analysis and 1000 pure seed weight determination followed by a germination test or sampling for weighed replicate testing.

4.7.2 For those species where a standard germination test is to be performed (see Table 2) then either four or eight replicates are taken at random from the eight 100 seed replicates counted out in for the purity analysis.

4.7.3 For those species and seed lots where a weighed replicate test is to be performed (see Table 2 and below), four or eight replicates of the weight specified in Table 2 are drawn at random and weighed to two decimal places.

4.7.4 Small differences in density often manifest themselves during the counting out of germination replicates, and replicates for the 1000 pure seed weight determination.

4.7.5 If there is any doubt about the ability to secure random samples the working sample should be repeatedly divided in two, preferably in a soil divider, until as near as possible to a 100 seed sample has been obtained. Starting with the working sample each time, this procedure should be used to obtain all the required replications. Small samples of seeds to make up 100 seed replications should be obtained in the same manner. It is essential that 'selection' of seeds by moving them in such a way that light, empty seeds separate in a non-random way from heavy, filled ones, is avoided.

4.8 The standard germination test

4.8.1 Non-dormant seeds can be set to germinate immediately after the seeds have been separated during the 1000 pure seed weight determination. Four replicates are spread evenly over the surface of four germination substrates, ensuring a water supply is in place.

4.8.2 Some species have very large seeds which require special treatment. If germination boxes which can accommodate 100 large seeds are not available, replicates of 50 seeds or 25 seeds can be used. Here, four pairs of boxes with 50 seeds or four quartets of boxes with 25 seeds are grouped together at random, and labelled. Each group of 100 seeds is counted as one replicate.

4.8.3 When seeds with large wings are tested, it is perfectly acceptable to remove the wings so that the 100 seeds in a replicate can fit into one container.

4.9 Dormancy and pre-chilling

4.9.1 For moderately dormant conifer, and newly harvested lots of Alnus and *Betula* spp seeds, to be tested, it is necessary to give them a short period of cool moist

treatment (known as pre-chilling) before they are set to germinate as above. Two sets of four replicates are therefore chosen at random from the eight replicates separated for the 1000 pure seed weight determination. For pre-chilling, one set of four replicates is placed in a moist environment at 3 to 5°C for 21 or 28 days (a germination box placed in a refrigerator provides these conditions). The results from the so called 'double' tests allows a comparison between prechilled and non-prechilled and therefore provides useful information on the level of dormancy in the seed lot.

4.9.2 In order to gauge whether the treatment has been effective, the set of replicates which have received the pre-chilling should be set to germinate at exactly the same time as the four replicates which have not had the pre-chilling. When this is not possible, the same germination conditions should be provided for the two halves of the test.

4.9.3 Some species are deeply dormant and require very lengthy treatments to promote germination. For practical reasons quick tests of viability are preferred for these categories of untreated seed see section 5. However, when such seeds are sold ready stratified, they should be given a germination test which should generally be carried out in sand.

4.9.4 *Robinia pseudoacacia* exhibits quite deep dormancy which can be easily and quickly overcome. In common with nearly all members of the Leguminosae, its seeds exhibit a condition known as hardseededness. This is caused by a thin waxy layer on the outside of the seed coat (testa) and/or thickened seed coats which prevents water from being absorbed. When the seeds first ripen only a small proportion of the seeds (up to 20%) do not have hard seeds and can germinate. The remainder stay hard and will not imbibe until seed coat is weakened or ruptured by some means. This can be achieved by a process known as scarification see 4.10.

4.10 Scarification

4.10.1 The most effective although most time consuming method of overcoming hard-seededness is to weaken the seed coat by manual scarification, i.e. puncturing it with a sharp instrument. A prick or a scratch on the surface as far away as possible from the radicle, which is located near the hilum, can be effective, but is not as reliable as chipping a small piece of the seed coat from the edge of the seed opposite the radicle. This should be as small as possible so that the minimum amount of damage is done to the cotyledons, to avoid introducing infection and causing difficulty in assessing normal.

4.10.2 A second method which is less laborious but rather fiddly to perform and for which the preparation may be spread over two or three days involves the use of boiling water. A known volume of seeds is placed in a known volume of boiling water and allowed to cool down to room temperature. Left overnight, an assessment can be made of the effectiveness of the treatment by seeing how many seeds have swollen. This should be compared with the proportion which became swollen when a different sample of untreated seed was placed in cold water. If necessary, the swollen seeds can be sieved out of the lot and the remaining hard seeds again immersed in a known volume of boiling water. This can be repeated until all the seeds have swollen overnight. This treatment can take a long time to perform and not all seeds will begin their germination at the same time. Using only the first seeds to lose their hardseededness would introduce some bias into the test and should be avoided.

4.10.3 It is important to use known volumes of seed and water so that the treatment can be accurately repeated. If only a small volume of seeds are put into a large volume of boiling water, the seeds will be subjected to a warmer temperature for longer than when a large volume of seeds are placed in a small volume of water. Placing the seeds in continuously boiling water should be avoided without carefully checking how long the seeds can withstand such treatment. Water at lower temperatures can also be used but clearly will not be as effective as boiling water.

4.11 The weighed replicate test

4.11.1 The weighed replicate test is a recognised test for *Betula* spp. and the only recognised international test for some 50 species of Eucalyptus. It can also be used for testing other species such as *Alnus*, *Populus* and *Salix* spp., where the seeds are small and the product sold contains a lot of small particles which are difficult and time consuming to remove.

4.11.2 The test can also be used for routine tests of small seed lots where the labour cost of the full test would exceed the commercial value of the seed lot. A licence to perform a weighed replicate test for these small lots must be obtained from the Forestry Commission prior to the test. When weighed replicate tests are performed on small lots normally subjected to the standard germination test, the weight of the weighed replicates to be used are listed in the comments column in Table 2.

4.12 Weighed replicate testing - procedure

4.12.1 No purity separation is carried out. The germination tests are performed on four or eight replicates of a weighed amount of seed, instead of four or eight

replicates of 100 seeds. These weights are provided for each qualifying species in Table 2.

4.12.2 The submitted sample is thoroughly mixed and randomly split in two to provide the working sample. The working sample is again mixed. Enough seed is randomly separated out four or eight times, either by the process of repeated halving or by using the method (see 2.12.6), to provide approximately the desired weight of seed. This has been calculated beforehand to provide approximately 100 seeds in the weight selected.

4.12.3 The procedure thereafter follows the standard germination test. The recommended temperature regimes, pre-chill requirements and length of test for all the species covered in the regulations plus those of some common native tree and shrub species are to be found in Table 2.

4.12.4 At the end of the germination period the number of seeds which have germinated per replicate are counted and expressed per unit weight, from which the numbers germinating per kg are calculated.

4.13 Assessment of the germination test

4.13.1 Seeds should be allowed to germinate, and the seedlings to develop to the point where the appearance of essential structures allows the analyst to decide whether the seedling will develop into satisfactory plants under favourable conditions in the ground. These are called normal seedlings.

4.13.2 All such seedlings are removed and counted; at the same time any seeds that are so mouldy that they are a threat to neighbouring seeds, should also be removed and the number recorded. Some of the larger seed types when germinated on top of paper will take several weeks to develop to full seedlings.

4.13.3 A rule of thumb has been developed which states that where the emerging radicle has reached four times the length of the seed and shows no evidence of decay where it emerges from the seed coat, it can be assessed as a normal germinant.

4.13.4 The same procedure is repeated on a weekly or more frequent basis until the end of the recommended test period. If a large number of seeds have started to germinate between the last two assessments, the test should be extended by an extra week.

4.13.5 At the end of the test, all seedlings that have not developed normally are counted separately and are classified as abnormal seedlings. They may take a whole variety of forms.

4.13.6 After having removed normal and abnormal seedlings at the end of the test, the ungerminated seeds should be counted and assessed for their condition.

4.14 Normal seedlings

4.14.1 The radicle should have elongated into the primary root, the plumule should have started to develop normally, and the remainder of the seedling should appear healthy.

4.14.2 Intact seedlings with a well developed root system, shoot axis, specific number of green cotyledons and a terminal bud.

4.14.3 Seedlings with slight defects; limited damage on the primary root, hypocotyl or epicotyl and cotyledons.

4.14.4 Seedlings with secondary infection; where it is clear that a normal seedling has been made abnormal by infection from another seed or seedling.

4.14.5 Typical normal seedlings are illustrated in the figures.

4.15 Abnormal seedlings

4.15.1 Seedlings with stunted, broken, missing, constricted and spindly primary roots as well as seedlings where the primary root does not respond to gravity (negative geotropism), or has decayed through primary infection

4.15.2 Seedlings with hypocotyl or epicotyl deeply cracked or broken, split right through, missing, constricted, tightly twisted, forming a loop or spiral, spindly, glassy or decayed from primary infection

4.15.3 Seedlings with cotyledons which are swollen, curled, deformed, broken, separate, missing, discoloured, necrotic, glassy or have decayed as a result of primary infection.

4.15.4 Seedlings where the terminal bud is missing, deformed, damaged or decayed as a result of primary infection.

4.15.5 Typical abnormal seedlings are shown in the figures.

4.16 Ungerminated seeds

4.16.1 Hard seeds are seeds that have not been able to imbibe water due to their hardseededness. This condition occurs frequently in *Robinia pseudoacacia*.

4.16.2 Fresh seeds are detected by slicing the ungerminated seeds with a sharp instrument. Such seeds have imbibed water and have fresh viable looking tissues but have not germinated. Sometimes they show signs of producing

chlorophyll in the embryo. If there are more than 5% fresh seeds these should be stained in tetrazolium solution to confirm that they are viable.

4.16.3 Dead seeds are seeds which, on being sliced open with a sharp instrument, exhibit signs of decay, discolouration and may be covered in mould.

4.16.4 Empty seeds are seeds which, on being sliced open, are shown to be completely empty or contain only some residual tissue.

4.16.5 Insect damaged seeds are seeds that, on being sliced open are shown to have contained the larvae of an insect.

4.17 Comments on seedling analysis

4.17.1 It is possible to learn something of the history of a seed lot by studying the appearance of the seeds and seedlings during germination. In theory, a newly harvested lot should germinate well with few, if any seedlings showing abnormal development. As a seed lot ages, and depending to some extent upon the conditions in which it is stored, it will begin to have a higher incidence of abnormal seedling development. On the other hand, if a new seed lot is damaged during initial processing, it may well show a significant amount of abnormal germination from the outset.

4.18 Fungal growth

4.18.1 The seeds of *Abies* spp. have resin vesicles on their surface which rupture easily, and trap fungal spores and bacteria. During testing these species are therefore prone to exhibit significant amounts of mould which can affect germination. Freshly processed seeds which germinate vigorously will usually be unaffected by the mould, but older seeds germinating less vigorously are often overwhelmed by the fungal growth leading to death of the seedling (see figures).

4.18.2 All types of seeds when stored at higher than recommended moisture content will show increased levels of fungal contamination during the seed test.

4.18.3 When fungal growth is excessive, dead and decaying seeds should be removed as early as the first assessment, and noted. In extreme cases, all ungerminated seeds should be removed and washed in distilled water, before being put back onto a new germination substrate. Using crinkled paper can help to reduce the spread of the mould. The final resort is to repeat the test using sand or preferably organic growing media, instead of a paper substrate. Such seed lots will almost certainly show slow and irregular germination in the

nursery, and the analyst should bring this to the attention of the producer so that a suitable fungicidal treatment can be used in the nursery.

4.19 Calculation of results

4.19.1 The result of the germination test is calculated as the average of the four 100 seed replicates. Sub-replicates of 50 or 25 seeds are combined into 100 seed replicates at the start of the test, to avoid bias at the end of the test. The percentage, which is reported on the certificate, is taken to the nearest whole number with 0.5 rounded up to the next figure. The percentage of abnormals, fresh, dead, empty, and insect-damaged seeds is calculated in the same way.

4.19.2 When reduced numbers of seeds per replicate are used, the percentage germination should be calculated and used for reporting and testing the tolerance of the results (see below).

4.19.3 Using the average germination percentage, the number of seeds per kg, and the purity percentage, the number of germinable seeds per kg can be calculated. By adding the number of normal seedlings, abnormal seedlings and fresh ungerminated (stained) seeds, the number of viable seeds per kg can also easily be calculated.

4.20 Tolerances

4.20.1 The result of the test is only reliable for trade purposes if the difference between the highest and lowest replicate is within accepted tolerances. To check the reliability of the result, the average percentage from the two or four replicates is calculated and compared with Table 6A for full 100 seed replicate tests or Table 6B for weighed replicate tests. The results are acceptable if the difference between the highest and the lowest replicate does not exceed the tolerance indicated for that particular average germination percentage or total germination count. If the difference is greater than is listed in the table, the test should be repeated using the same submitted sample.

4.20.2 If the variation in the germination results is not within tolerance limits, it is recommended that a further set of replicates is tested. Only the in-tolerance results should be reported. If this continues to be a problem the cause should be investigated and possibly a re-sample or new working sample taken from the seed lot.

5 TESTING THE VIABILITY OF SEEDS

5.1 Introduction

5.1.1 If a seed is not dormant or if the treatment of a seed to make it germinate has been effective, most seeds will germinate fully by the end of a four week germination test. Provided the treatment time for dormant species is short, as with leguminous seeds, four weeks is not too long to wait for a result. If however the treatment lasts for more than four weeks and the full test result is not known sooner than eight weeks, the information may be redundant by the time it is available.

5.1.2 Time is also important for testing large seeded broad-leaved species such as *Aesculus*, *Castanea* and *Quercus* spp. Most nurserymen sow these species very soon after harvest rather than storing them until the spring because of their poor storability. A germination test lasting five weeks is of little use if the seed lot is already sown.

5.1.3 For these reasons, quicker tests of the seed lot's capacity to germinate are required. Apart from tests of *Aesculus*, *Castanea* and *Quercus* spp., a rule of thumb has been adopted by authorities in many countries that if any test takes longer than eight weeks to perform, then an alternative using a quicker method should be used. These tests, because they do not actually measure the germination percentage, are known as viability tests.

5.2 Methods of performing viability tests

5.2.1 Tetrazolium staining is probably the most widely used method. It is subjective and involves the use of a compound that differentially stains living and dead tissues. It requires a lot of experience to obtain reliable results.

5.2.2 The excised embryo test involves the physical growing on top of filter papers, of embryos which have been excised from the dormant seeds. Therefore it is an objective test. Living seeds remain fresh and may even grow. Dead seeds decompose when subjected to normal germination conditions.

5.2.3 The cutting test has been used widely for over a century and is the straightforward cutting of the seed with a sharp instrument to assess the condition of the embryo and endosperm.

5.2.4 A fourth method in which full and potentially viable seeds can be detected is by using X-ray methods which will not be described here.

5.3 Testing the viability by staining methods

5.3.1 2,3,5 triphenyl tetrazolium bromide or chloride is a salt which in solution is a pale yellow but which, when oxidised by the dehydrogenase enzymes present in living tissues, produces an insoluble product, formazan, which has a deep red colour.

5.3.2 The test is performed on 100 seed replicates which have been obtained as per the standard germination test. In order to obtain results that could be reproduced in another laboratory the seeds must be prepared according to a standard method, the tetrazolium solution must be made in a standard way, and the timing and method of inspection must also be standardised.

5.3.3 However, it is not necessary to be totally standardised in the methods and materials used. Instead of a buffered solution being used to make the 1% solution, distilled water or de-ionised water can be used (buffered solutions are used to correct water with pH problems). Nor is it necessary to use exactly 1% solution e.g. if a faster result is wanted, a more concentrated solution can be prepared. Non-standardised methods are commonly used, and set out in the ISTA handbook on Tetrazolium Testing.

5.3.4 The preparation of the seeds is very important. The seeds are first soaked, or for very dry old seeds, wrapped in some wet medium to prevent cell wall rupture during rapid uptake of water. Methods are described in various rule books, but do not need to be strictly followed unless comparative tests are being made between laboratories.

5.3.5 It is important to do as little damage as possible to the embryo as it is exposed and/or removed from the seed coat or other seed structure. The harder the seed coat e.g. *Pinus cembra*, or harder the endocarp in e.g. the stone of *Prunus avium*, the harder it is to excise the embryo without doing any damage.

5.3.6 In endospermic seeds, both the embryo and the associated endosperm of each seed are placed in the solution. For conifers, it is gametophytic tissue which surrounds the embryo and is placed with the embryo in the solution. For non-endospermic seed, only the embryo is placed in the solution.

5.3.7 Once the 100 embryos and/or endosperm are prepared, they are placed into the solution of the salt. This is then warmed to 30°C +/-2°C in the dark for as long as it takes for the stain to generate.

5.3.8 The staining develops gradually and should be examined over time. This allows the way that it reacts with the different tissues to be studied. One assessment after the seeds have been in the solution overnight may miss some important staining patterns. Even dead tissue can sometimes contain enzymes which react

with the tetrazolium, but the appearance of such tissue is distinctly different and may take on a very dark red appearance. Living tissue has a lustrous appearance whereas moribund tissue is not turgid and has a dull appearance. The use of a needle under the binocular microscope to prod the different tissues is therefore useful (see figures).

5.3.9 Tissues which has been recently injured (during the preparation of the seed), release enzymes from their cells and will temporarily yield a very rapid, almost scarlet, stain. For the same reason tissues whose cells have been ruptured by freezing or some other factor can sometimes take on an almost black red appearance. If the whole embryo has not been exposed, the part with unhindered access to the solution may stain more rapidly than tissue covered by e.g. the testa.

5.3.10 Accurate interpretation of staining by Tetrazolium requires a great deal of experience before the results will correlate well with germination test results. It also is very time-consuming to prepare four replicates of 100 seeds and to test them by tetrazolium. For this reason, even though a result can be obtained in under a week, the test is seldom performed in its entirety. In this guidance four replicates on reduced numbers of seeds have been proposed. The species for which the reduced Tetrazolium tests apply are listed in Table 5.

5.3.11 A very few types of seed e.g. *Quercus* spp. contain storage material that blocks the development of the red coloured formazan. These will not stain red and can only be assessed by the simple cutting test described below. The green colour of the embryos of *Acer* spp. species mask the red colour, and also make assessment of these species difficult.

5.3.12 Indigo carmine is not so frequently used but is much cheaper and easier to obtain. It stains dead tissue blue and leaves living tissue its natural colour. The methods of preparation and assessment are the same as for tetrazolium. Again, it requires considerable expertise to obtain reliable results that match germination percentages.

5.4 Testing for viability using excised embryos

5.4.1 An alternative method to test for viability is based upon the principle that when live embryos are removed from a seed and incubated, they remain fresh and vital, often grow, and may even start producing chlorophyll. Dead, diseased and damaged embryos start to decay.

5.4.2 In the excised embryo test all the embryos in a replicate are excised carefully from the seeds. They are incubated on top of paper in warm moist conditions

for up to 14 days at a temperature of 20 to 25°C, with light for at least eight hours per 24 hours. The incubation boxes are identical to those described for the standard germination test. Those embryos that are viable remain alive and look healthy, whereas dead tissue or tissue which contains dead areas will start to decompose. In some species, when the embryo is exposed to light it begins to create chlorophyll and turns green. This sometimes occurs only in the embryo touching the substrate, as an inhibitor is leached from the embryo (see figures).

5.4.3 Because the embryo is going to be incubated under warm conditions for a considerable time, it is important that it does not suffer any more than slight damage as it is excised from the seed. This can be particularly difficult when the seed coat or endocarp are very hard. Proper preparation of the seed with soaking in water before and between removal of each seed or fruit covering is important. Sharp scalpels, fine pointed tweezers and mounted needles are all used to assist the excision.

5.4.4 It is extremely time consuming to carry out a test on four replicates of 100 seeds to compare it with a normal 4 x 100 germination test. For this reason it is seldom used in practice, but as a reduced test on domestic seed lots it is useful, providing information on the influence of seed coverings on the germination of newly harvested seeds. In this guidance, four replicates on reduced numbers of seed have been proposed.

5.4.5 The modified excised embryo test is a modified routine which, although it increases the length of time needed for the test, is still quicker than the standard germination test and reduces the amount of labour required.

5.4.6 In this guidance, for the modified excised embryo tests a reduced number of seeds in replicates are also proposed. The relevant species and amounts are listed in Table 5.

5.4.7 After thorough soaking of the seeds, replicates of the specified number are placed on moist paper or in moist sand for large seeds, and put into the normal germination conditions for two weeks. At the end of this period, each seed is examined for evidence of mould and decay. Those that are obviously dead or moribund can be rejected and counted. Those that are apparently still sound are then excised carefully as in the full test. If during the excision, it is clear that some apparently sound seeds have embryos that are beginning to decay, they can also be rejected and counted. In the end only healthy embryos are placed on top of paper for incubation with a minimum eight hours of light per 24 hours. Those remaining healthy or turning green after 14 days are assessed as being viable.

5.4.8 The % viable is the number of viable seeds/total number of seeds x 100.

5.5 Testing for viability using the cutting test

5.5.1 The simplest and oldest test of all is the cutting test. The cutting test is used very widely, and in this guidance is the preferred method for testing the quality of *Aesculus*, *Castanea* and *Quercus* seeds. These seeds do not react with the Tetrazolium stain in the normal way.

5.5.2 To be completely comparable with a standard germination test, four replicates of 100 seeds should each be cut open and assessed. As a transverse cut through the nut of *Aesculus*, *Castanea* and *Quercus* spp. is an integral part of the full germination test of these species, the labour input to perform a cutting test is no greater than that for a full test, and should be fully performed. The variation between the results of the four replicates should be assessed as per the standard germination test.

5.5.3 When the results of the four replicates are out of tolerance, a germination test must be carried out on the 100 seeds left over from the submitted sample (see Table 4A). In such cases, the seller should note in the supplier's document the tolerance failure, set out both the cutting and germination test results, and emphasise that the buyer should thoroughly mix the received batch before sowing.

5.5.4 In the cutting test, the seed or fruit is cut open usually length-wise, with a sharp instrument using a sliding action. This allows empty seeds to be identified and discarded. The full seeds are examined to obtain an indication as to whether the contents are fresh and alive, or dead or dying.

5.5.5 The most important part of the seed is the embryo. If it is damaged in any way the seed is unlikely to have been able to germinate. Experience is required to interpret the condition of the embryo with accuracy. Because there is no aid (tetrazolium staining or actual growth) to assist in the assessment, the cutting test tends to be less reliable than these others.

5.5.6 The seeds should be counted out at random into the four replicates of 100 seeds, and soaked for up to 24 hours. They are then cut longitudinally and assessed according to the condition of the radicle and plumule, and the position of any necrotic areas in relation to these organs. Necroses on the outside of the endosperm or cotyledons well away from the growing points would not have influenced the ability of the seed to germinate, and should be assessed as viable.

5.5.7 The presence of necrotic areas in the radicle of *Quercus petraea*, particularly if the seed had already started to germinate, requires very careful consideration. If it has reached far up into the seed near where the two cotyledons and plumule join, then the chances are that the seed would not germinate. In contrast, seeds of other *Quercus* spp. are much more able to withstand necrotic areas on their radicles after they have begun to germinate.

5.5.8 With experience, cutting tests can provide assessments of viability, which compare quite closely with the germination percentage for non-dormant seeds and the viability of dormant seeds when tested by either the tetrazolium or excised embryo test. However, until experience is gained, significant deviations will occur because of a failure to take account of the isolated spots of damaged or moribund tissue, particularly in critical areas of the embryo. Prodding such areas with a needle can help to identify changes in the condition of tissues over quite small distances.

5.5.9 The cutting test does not lend itself to testing many of the other broadleaved species in the regulations. This is because the seeds are either too small or have an outer covering which is so hard, that even after soaking, cannot be cut through easily with a sharp instrument. The exception is seed of *Fraxinus* spp. that can be sliced through easily after a soak. For these species, a period of incubation at 20 to 25°C is useful (as in the excised embryo test) as it allows damaged tissue to decay or not, which can be picked up relatively easily in the cutting test. If in doubt, the embryo halves should be carefully removed from the endosperm for closer examination.

5.6 Calculation of results

5.6.1 The result of all viability tests is calculated as the average of the four replicates. The percentage is taken to the nearest whole number with 0.5 rounded up to the next figure. When reduced numbers of seeds per replicate are used in the tests, the percentage germination should be calculated and used for reporting and testing the tolerance of the results (see Table 6).

5.6.2 Using the average viability percentage, the number of seeds per kg and the purity percentage, the number of viable seeds per kg can be calculated.

5.7 Tolerances

5.7.1 See section 4.20 and Table 6A for information on tolerances.

6 MOISTURE TESTING

6.1 Introduction

6.1.1 Moisture testing is not a prescribed test under the FRM regulations but can be useful to allow customers to determine if seed lots need to be dried for successful storage or how much water might need to be added for bulk pre-chilling of seed lots.

6.1.2 The principle of moisture testing is to take a representative sub-sample from the seed lot weigh it, dry it in an oven and then reweigh it. The loss in weight is treated as water loss and the % calculated.

6.2 Moisture testing - procedure

6.2.1 Tree seeds often have high oil content and therefore an oven temperature of between 101 and 105°C for a drying period of 17 hours +/- 1 hour is recommended. For large seeded species such as *Quercus* spp. the seed are cut open to facilitate uniform drying in the oven.

6.2.2 Tins are used to dry the samples. The diameter of the drying tin determines the sample size for the moisture test. Using a tin with a diameter of between 5 and 8cm the sample weight should be 4.5g +/- 0.5g. With a drying tin diameter of greater than 8cm the sample weight should be 10.0g +/- 1.0g. The weights should be weighed to 3 decimal places.

6.2.3 Once the drying period is complete the tins are removed from the oven. To avoid the sample absorbing moisture from the atmosphere the tins are placed into a sealed container with moisture absorbing crystals to cool to room temperature before re-weighing.

6.3 Calculations

6.3.1 The % moisture is calculated by the following formula:

((weight of the tin and sample before drying – the weight of the tin and sample after drying) / the weight of sample before drying) *100

e.g. ((45.500 – 44.250) / 10.500) * 100 = 11.9%

The values are calculated and reported to 1 decimal place.

6.4 Tolerances

6.4.1 For more accurate determination of moisture content two replicates should be tested and the results only reported if the average is within set limits. See Table 6C for the allowed tolerance limits.

7 QA REQUIREMENTS

7.1 Introduction

7.1.1 Having a Quality Assurance Manual is becoming a normal prerequisite of testing laboratories. With the philosophy of continual improvement becoming an expectation equipment needs to be regularly calibrated and traceable to national standards. Simple steps can be taken to help ensure that tests are not subject to excessive influence from sources such as media or temperature and should be incorporated into the routine procedures at the FC approved testing laboratories.

7.2 Balance calibration

7.2.1 Balances should be calibrated using weights traceable to national standards. Balances also need to be regularly verified as accurate with in-house calibration weights using weights near to the regular sample weights used. Limits need to be set at which balances would be taken out of service, for example when a 10g calibration weight is weighed on a two-place balance the balance should weigh 10g within +/- 0.01g, or when weighed on a four-place balance +/- 0.0001g. One approach to in-house verification of balances is to check balances on the first use each day and keep a record of this. Other time intervals for verification can be set but if balances are found to be outside of calibration all affected tests since the last acceptable verification should be retested.

7.3 Temperature monitoring

7.3.1 Incubators need to have their temperature monitored regularly and the temperature measuring devices (i.e. mercury or digital thermometers) need to have a certificate of calibration traceable to a national standard and to be checked on a regular interval, for example once per year.

7.4 Media checks

7.4.1 The pH of the water at the surface of the media used for germination testing should be checked to be within a pH of 6.0 to 7.5. The media can be used if the pH is outside this range if there is evidence to show this does not affect germination. The pH check can be done with a pH meter or pH strips. New batches of media should be checked before use.

7.4.2 As well as the pH of new batches of media a phytotoxicity test should be done by germinating sensitive species such as *Betula* spp. on samples of both the new and old batches of media as a comparison. Other species can be used to achieve the aim which is to detect any sub-standard germination media before it is used in a test.

7.5 Training

7.5.1 A structured training programme is essential for uniformity in analysis and to provide confidence in results