Forestry Commission Seed Testing Guidance Notes

Introduction

This guidance note is designed to standardise tree seed testing only. It will cover testing facilities, testing techniques, seed quality evaluation and adherence to standard procedures.

To be included will be basic facility requirements such as building and testing equipment, standard testing techniques and procedure on recording and traceability.

All the information in this guidance note has been sourced from various publications and brought together as an easy to read document.

This guidance note is produced to accompany an annual audit and inspection which will be carried out on all registered tree seed testing facilities.

Background

The Legal requirement:

The Forest Reproductive Material (Great Britain) Regulations 2002 1 provide a system of control for seed, cuttings, and planting stock that is used for forestry purposes in Great Britain. These regulations implement European Council Directive 1999/105/EC 2 controlling the marketing of forest reproductive material. The directive applies to 46 controlled species and the genus *Populus* (see Appendix 4), where reproductive material is marketed for forestry purposes. It is intended to further include guidelines for the testing of all tree seed testing facilities and procedures that are required under the conditions to be accepted as a registered seed testing facility.

The statutory tests are as follows

1. Percentage by weight of pure seed, other seed and inert matter – this is not required if the whole seed lot is marketed as stratified seed.
2. Germination percentage of the pure seed or where this is impractical, the viability percentage
3. Weight of 1000 pure seeds
4. Number of germinable/viable seeds per kilogram

Only seed testing facilities approved by the Forestry Commission can carry out statutory seed testing in Great Britain. This is a requirement of Regulation 20 of The Forest Reproductive Material (Great Britain) Regulations 2002, which states that: "the information required...shall be obtained from assessments using techniques which – (a) are, so far as is practical in all the circumstances, internationally accepted techniques; and (b) in the case of seeds to be tested in Great Britain, have been confirmed by the Commissioners as internationally accepted techniques prior to the assessments taking place”.

The Forestry Commission considers this set of guidance notes and criteria to be part of internationally accepted seed testing techniques.
1. Seed Testing Facilities

- The building

The building in which the testing will take place in, needs to be either, a separate structure or one that can be separated by compartments in order that external contamination from damaging agents or other interference is kept to a minimum. It is essential that the building can be adequately secured to avoid unauthorised access. It is essential that the building is kept clean and tidy and is kept as a dry and bio secure environment.

Health and Safety Building Regulations apply. Having a Quality Assurance Manual is a normal prerequisite for testing laboratories.

2. Seed Testing Equipment

- Germination apparatus

Different types of germination equipment and techniques are available.

The open-surface incubator in a temperature controlled room involves a series of open tanks of water, individually heated to the desired temperature. The germination tests are 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 a set temperature.

It is essential that results are noted, monitored and recorded for reference for future information and inspection.

The temperature controlled incubator must be able to control light durations and may need to provide alternating temperature 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 provide light. The individual replicates of the test are each contained in separate germination boxes.

It is essential that results are noted, monitored and recorded for reference for future information and inspection.

Walk-in germination room where lighting and shelves are fitted in a small, centrally heated/air conditioned room. The individual replicates of the test are each contained in separate 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.

It is essential that results are noted, monitored and recorded for reference for future information and inspection.

Light is normally applied when testing the seeds of many tree and shrub species. Cool white fluorescent tubes are the best source of light, although not essential for germination.

NOTE:- 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. However, because the full germination test of such seed takes approximately one month to perform, by which time many newly harvested seed lots will already be sown in the nursery, these tests are not mandatory. Instead a viability (cutting) test is acceptable, although, if possible this should be backed up by a germination test.

**Stratification apparatus**
A suitable container placed in a standard household refrigerator will provide adequate stratification conditions for most seed lots, depending on temperature criteria. It is essential that results are noted, monitored and recorded for reference for future information and inspection.

**Moisture testing** is not a requirement under the FRM regulations, but can be useful to allow testers to ensure that seed lots are kept at the optimum moisture content.

**Weighing apparatus**
Balances will be calibrated annually using weights traceable to national standards. Results to be recorded and available for inspection.

**Temperature apparatus**
Incubators need to have temperature monitored regularly and the temperature measuring devices (i.e. mercury or digital thermometers) need to have an annual certificate of calibration traceable to a national standard. Results to be recorded and available for inspection.

**Clean water apparatus**
It is essential to ensure that water quality is maintained. A filtration system such as is used on Root Electrolyte Testing is and option. Results of water quality to be recorded and available for inspection. It has been noted that tap water may offer a better option given that it contains chlorine which may be beneficial in keeping moulds and fungi at bay.

### 3. Seed Sampling

The **primary** sample is that which is taken from the seed lot during the operation.

The **composite** sample is that which is taken from the total of the samples taken from the seed lot.

The **submitted** sample is that which is offered for testing.

The **working** sample is usually half the submitted sample.

The **retained** sample is the other half of the working sample which is kept for future re-testing if required.
**Sampling intensity guidance**

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

<table>
<thead>
<tr>
<th>Weight Range</th>
<th>Sampling Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Up to 500kg</td>
<td>At least five primary samples</td>
</tr>
<tr>
<td>501-3000kg</td>
<td>One primary sample for each 300 kg but not less than five samples</td>
</tr>
<tr>
<td>3001-5000kg</td>
<td>One primary sample for each 500kg but not less than ten samples</td>
</tr>
</tbody>
</table>

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:

<table>
<thead>
<tr>
<th>Number of Containers</th>
<th>Sampling Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-4</td>
<td>3 primary samples from each container</td>
</tr>
<tr>
<td>5-8</td>
<td>2 primary samples from each container</td>
</tr>
<tr>
<td>9-15</td>
<td>1 primary sample from each container</td>
</tr>
<tr>
<td>16-30</td>
<td>15 primary samples from the seed lot</td>
</tr>
<tr>
<td>31-59</td>
<td>20 primary samples from the seed lot</td>
</tr>
<tr>
<td>60 or more containers</td>
<td>30 primary samples from the seed lot</td>
</tr>
</tbody>
</table>

**4. Seed Scarification**

This technique is sometimes used when a particularly hard seed coat is resistant to usual germination processes. Various methods are available.

**5. Seed Stratification**

This is the process by which dormancy is broken and is usually achieved with periods of heat or cold, or both.

**6. Seed Testing Techniques**

**NOTE:** It is essential to state on the final Seed Suppliers Document (SSD) the method of seed testing that has been used.

**Germination test.**

This is probably the most important and relevant test and is essential to carry out under controlled conditions. It is noted that the ISTA (*International Seed Testing Authority*) recommendations for carrying this out are based on optimum conditions and quote germinating conditions based on 20°-30°C. However, certain seeds may need different temperatures to give the best results. This test is carried out on cellulose based products such as tissue paper, sand or potting compost depending on
seed size and length of expected germination period.

The germination test is performed on four or eight replicates of a weighed amount of seed.

The submitted sample is thoroughly mixed and randomly split in two to provide the working sample and the retained sample.

The working sample is again mixed and randomly divided four or eight times, to provide the desired weight of seed. This has been calculated beforehand to provide approximately 100 seeds in the weight selected.

The retained sample is to be kept for 9 months in case of dispute or other reasons for re-testing.

Viability Test

Tetrazolium staining is the most widely used method in Research laboratories. It involves the use of tetrazolium bromide that stains live and dead tissue. Not many seed testing stations either have the training or equipment to carry out this test.

Exised Embryo Test. (Seed Cut Test)

This is normally only carried out on large seeded, perishable species such as Quercus spp and is sufficient to give an indication of viability. When seed is being dried for storage prior to marketing, an exised embryo test can be carried out prior to stratification. Following this stratification, a normal germination test can then be carried out on the seed.

Weighed Replicate Test.

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, excessively time consuming to separate out the pure seed from the inert matter in a seed lot. A seed test has been devised which derives the 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. For Eucalyptus spp. The weighed replicate germination test is the only method recognised by ISTA.

7. Germination assessment and calculation

Assessment

After germination, all normal healthy seedlings are counted. Any seeds that are diseased, dead, unhealthy or abnormal need to be recorded. At the end of the test, the un-germinated seeds should be counted and assessed for their condition.

Calculation

The germination test is calculated as the average of the four 100 seed replicates. The percentage of germinable seeds is reported on the certificate and is taken to the nearest whole number. The percentage of abnormals, fresh, dead, empty, and insect-damaged seeds is calculated in the same way.
Using the average germination result, the number of seeds per kg, and the purity percentage then the number of germinable seeds per kg can be calculated. By adding the number of normal seedlings, abnormal seedlings and fresh un-germinated (stained) seeds, the number of viable seeds per kg can also be calculated.

<table>
<thead>
<tr>
<th>Calculation for the number of seeds per kg</th>
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<tbody>
<tr>
<td>No of seeds per kg = (1000/T) x 1000 x P/100</td>
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<tr>
<td>T = 1000 pure seed weight in grams. P = Purity % from the purity test</td>
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Accurate Calculation of Seed Quality
From the three tests performed the following results are obtained:

The percentage purity = P.
the weight of 1000 pure seeds = W in grams
the germination or viability percentage = G (or V).

The number of germinable or viable seeds in a kilogram of the bulk is calculated thus:

W (example for Carpinus betulus Purity = 96%)
1000 psw = 48.22g
Viability = 37%

\[
\frac{1000 \times 1000 \times 37 \times 96}{48.22 \times 100 \times 100} = 7366
\]

This is normally expressed to the nearest ten ie 7370 viable seeds/kg.

Quick calculation of seed quality
The following quick calculation of seed quality can be made at all stages of seed processing and treatment, provided the individual terms in the equation are all available. The worked example relates to a fairly complete estimate of seed quality at the end of processing.

four replicates pure seeds = a (number)
germination (viability) = G(V) (percent)
remaining pure seeds = b (number)
weight of original sample = x (grams)

The number of germinable (viable) seeds per kg of bulk
No of pure seeds in x = V X (No of pure seeds in x = a+b) X 1000

For example: Taking a 25g sample of Carpinus betulus four replicates of 50 seeds

a = 200

Number of viables 30 + 33 + 28 + 29 = 120

\[ V = \frac{120}{200} = 60\% \]

remaining seeds = 380 seeds

b = 380

weight of original sample = 25 g

x = 25g

number of viable Carpinus betulus seeds per kg of bulk

\[ = \frac{60 \times (200 + 380) \times 1000}{25} = 13,920 \]

8. Data Recording and Traceability.

It is essential that data recording and traceability is set out in a logical way and is easily retrievable for inspection, either electronically or within a hard copy filing system.

It is essential that electronic data is regularly backed up at the end of each working day and that this back up copy is kept in a secure place for use or reference at a later date if required.

Acknowledgements

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