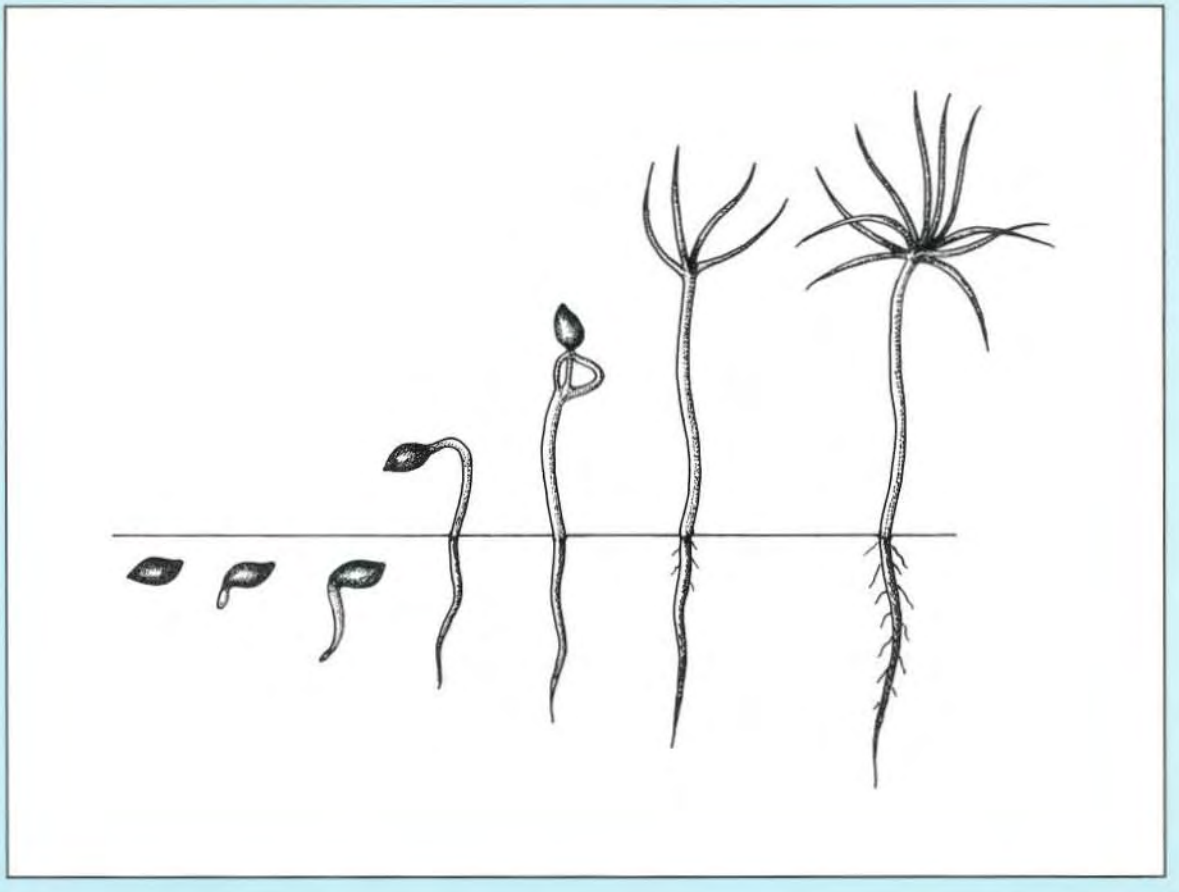
A training guide for laboratory analysis of forest tree seeds

D.G.W. Edwards and B.S.P. Wang

Pacific and Yukon Region • Information Report BC-X-356



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This report was printed with financial assistance from the Canada – British Columbia Partnership Agreement on Forest Resource Development (FRDA II).

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Natural Resources Canada

1995

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Victoria, British Columbia
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Phone (604) 363-0600

ISBN 0-662-23407-3
Cat. no. Fo46-17/356E

Printed in Canada

A microfiche edition of this publication may be purchased from:

Micromedia Ltd.
Place du Portage
165, Hôtel-de-Ville
Hull, Quebec
J8X 3X2

Canadian Cataloguing in Publication Data

Edwards, D.G.W.

A training guide for laboratory analysis of forest tree seeds

(Information report; BC-X-356)
Includes an abstract in French.
Includes bibliographical references.
ISBN 0-662-23407-3
Cat. no. Fo46-17/356E

1. Trees--Seeds--Testing--Laboratory manuals.
I. Wang, B.S.P.
II. Pacific Forestry Centre.
III. Series: Information report (Pacific Forestry Centre); BC-X-356

SD401.7E38 1995 634.9'562 C95-980126-X

Contents

Abstract

This publication describes detailed training guidelines for tree seed testing that are used at national and international levels. A historical overview of tree seed testing in Canada is also given.

The basics and requirements of testing are included along with applications to numerous species. Several exercises complete the text to emphasize theoretical and practical knowledge of testing. Since the material contains all the usual procedures of tree seed testing and follows the guidelines of the International Tree Seed Testing Association, it forms an effective document for all tree seed workers.

Résumé

Le présent document décrit des procédés détaillés des essais utilisés aux niveaux national et international de même qu'un aperçu historique des essais de semences forestières au Canada.

Les essentiels et les exigences en matière d'essais, y compris des applications à de nombreuses espèces sont données. Des exercices viennent s'ajouter pour vérifier les connaissances théoretiques et pratiques en ce qui a trait aux essais. Puisque le matériel englobe tous les procédés courants des essais des semences forestières et suit les directives de l'Association internationale d'essais des semences, le présent document peut s'avérer profitable tous ceux qui œvrent dans le domaine.

Foreword

The origin of this manual is to be found in the 1987 series of workshops on forest tree seed testing that was an unique undertaking of its kind in Canada. The workshops provided important information that has been updated so that the instruction material and the results can be shared with all those working in the field of tree seed testing.

Avant-propos

Le manuel prend ses origines d'une série d'ateliers (en 1987) sur l'essai de semences forestières qui étaient une enterprise unique au Canada. Ces ateliers ont fourni de nombreux renseignments qui on été mis à jour. Ainsi pouvons-nous partager le matériel d'instruction et les résultats avec tous ceux qui œvrent dans the domaine des essais des semences forestières.

Preface

Testing is a vital step in the efficient and economic management of seeds for plant production and direct seeding programs. It is especially critical for forest tree seeds because, although the supply from seed orchards is growing rapidly, most seeds used in forest renewal are still obtained from natural, wild populations. Differences in methods of seed collection, handling, processing and storage tend to increase, rather than diminish, the variation in viability among these wild populations. Seed testing provides a means of estimating the value and potential performance of seedlots irrespective of their provenance, collection method, and subsequent handling.

The primary aim of seed testing is to assess the quality of a seedlot so that its value for seedling production can be calculated. Seed testing is not a field test, and the seed tester makes no attempt to reproduce the conditions under which the seeds may be sown. Seed tests are carried out under standardized conditions using standard procedures, so that the results should be comparable to those produced by other seed testing facilities using the same conditions and procedures. These conditions and procedures have been developed through many years of national and international testing, and represent the best methodology known. These tests provide an estimate of expected seedling output.

While increases in reforestation in Canada have been substantial since the early 1960s, it was not until 1978 that nationwide attention was focussed on tree seed resources in this country. At a Canadian Forest Service (CFS) workshop on tree seed production and tree improvement, it was estimated that seed requirements would increase by almost 80% in the decade ending in 1987 (Morgenstern and Carlson 1979). Among the numerous recommendations formulated at this workshop, the CFS was sanctioned i) to obtain authority under the Canada Seeds Act to implement national seed certification and regulatory activities for forest tree seeds and other reproductive material, and ii) to develop national standards for seed testing.

Work on these and other recommendations began almost immediately. Regulations for safeguarding forest renewal through the control of undesirable imports were drafted, together with some basic rules for labeling and certifying seed origin and quality. As guidelines for tree seed regulations (Edwards *et al.* 1988) were being developed, the need for transferring standardized seed-testing technology to the Canadian forestry sector was recognized. It was also recognized that this could be accomplished through a series of workshops that would show forestry sector personnel various testing methods, the fundamentals of which are widely accepted internationally. These workshops would provide a model for a training guide to standard tree seed testing.

This report, which is based on the program content of the workshops, is intended as a guide to training personnel in the basics of laboratory analysis of tree seeds. An outline of how the workshops were planned and an assessment of their success is included.

Organization of the workshops

Planning for the seed testing workshops began in 1986 and the program was initiated in 1987. Developed around a core schedule that would be repeated, a series of four workshops was held at regional centres across the country. Participants were drawn from a wide variety of clients including: provincial and private seed-extraction and testing centres; private and contract nursery growers, and provincial and industrial nurseries; educational institutions; scientists who use seeds in their research; and federal and provincial foresters engaged in forest renewal.

Major funding was provided by the CFS, by the Ontario Ministry of Natural Resources, by the Canada/Nova Scotia Forest Resource Development Agreement (Federal Technical Transfer Project) and by the Canada/British Columbia Forest Resource Development Agreement (Extension, Demonstration, Research and Development Program). The fourth (final) workshop was supported entirely by the CFS; it was conducted in both official languages by means of simultaneous translation, and with sign language translation for a hearing-impaired participant.

Objectives

- To review basic seed testing technology and to improve the methodology used by seed testing agencies, seed extraction plants and commercial and private nurseries throughout Canada

- To improve seed standards, and to enhance the abilities of seed workers to interpret seed test results in relation to nursery stock production

- To reduce inefficient seed use (waste)

Benefits

- More efficient use of high-value seeds produced in seed orchards

- Improved seedling production to meet increasing demands for regeneration of current and backlog forest sites

Personnel

The core instructional materials and the program were developed by the principal instructors, D.G.W. Edwards (Pacific Forestry Centre, CFS) and B.S.P. Wang (Petawawa National Forestry Institute, CFS); C.L. Leadem (British Columbia Ministry of Forests) also made a major contribution to the program. Other contributors included R.F. Smith (Maritimes Forestry Centre, CFS), M. Adams (Great Lakes Forestry Centre, CFS), R. Hallett (Maritimes Forestry Centre, CFS), and O. Sziklai (University of British Columbia). When lecturers, assistants (those who prepared the over 1000 samples used) and the secretarial staff, are included, more than 150 individuals were involved in these unique workshops.

Program

The workshops were held at the Petawawa National Forestry Institute (PNFI), Chalk River, Ontario (September 1987), at the Nova Scotia Agricultural College, Truro, Nova Scotia (November 1987), at the Pacific Forestry Centre, Victoria, British Columbia (December 1987), and again at PNFI (March 1988). Each workshop was built around a core program that emphasized well established methodology for conducting basic seed "quality" analyses that include purity tests, seed weight determinations, moisture content and viablity tests, as well as the all-important germination tests. The role and procedures for correct seed sampling were also given special attention. Workshop participants received an instructional workbook containing summaries of the lecture materials, the hands-on exercises, a manual of quick-test methods, a Canadian tree seed testing manual, and a workshop evaluation sheet. The contents of the lecture materials - the summaries of testing methods, the exercise work-sheets and the evaluation form - form the basis of this guide. The manual of quick-test methods (Leadem 1984) and the Canadian tree seed testing manual (Edwards 1987) were published separately.

Exercises

The exercises were designed to provide as much individual hands-on experience as possible within the three-day time period. Exercises were kept relatively basic, since, for many of the participants, this was their first exposure to seed testing. A wide range of seed analyses, including purity, germination, moisture content and so-called "quick tests" for viability, including x-ray, tetrazolium and hydrogen peroxide, were carried out on specially prepared samples. A separate session was devoted to seed sampling, and included a demonstration of sampling methods, equipment, and a detailed discussion of sampling problems. Each work session ended with a question period aimed at reinforcing the lessons learned.

Additional lectures were given on seed morphology, pretreatments to overcome dormancy, seed pelletizing, and vigour. Opportunities were also provided to review current tree-seed research in Canadian laboratories.

Three workshops were held at CFS Centres with excellent conference facilities, but these proved to be less than ideal for these workshops. Lighting was poor for the detailed work that was carried out, and the work areas tended to be crowded. In Nova Scotia, the college laboratory facilities (although again crowded) were probably the best overall.

Impact

The workshops were attended by 132 participants. Although most participants were from Canada, from almost every province and territory, some visiting scientists and trainees from other countries, such as Thailand, China, and Mongolia, also attended. Together these individuals represented most of the major provincial and private seed extraction and testing centres, private contract nursery growers, and provincial and industrial nurseries throughout Canada. They also represented educational institutions, federal and provincial seed researchers, and foresters responsible for forest renewal. Holding regional workshops permitted more individuals to attend, and allowed the incorporation of topics specific to different regions.

Evaluation

The workshops were generally judged by the participants themselves to be very successful. Some participants had more previous experience than others; most had done no prior seed testing, while a few had worked in the area for several years. This led to some criticisms. Persons using this guide should be alert to the probable need to adjust pace, duration and content depending on the requirements of the participants in individual workshops in the future.

A recurring theme throughout the evaluation reports was the need for workshops dealing with seed handling from the nursery growers' point of view. Indeed, some attended the workshops expecting this to be the main topic. Questions were raised about difficulties with preparing seeds for sowing, stratification methods, how much stratification to give, what to do with stratified seeds that cannot be sown, sowing methods, types of mulching, and a myriad of other aspects of handling seeds in bulk in nursery situations. Although these questions went beyond the scope of the workshops, the lecturers answered as many as they could. This interest implied that one or more workshops on seed preparation for nursery sowing would probably benefit many individuals.

The importance of this training guide

This training guide represents a significant first step in the standardization of tree seed testing in Canada. Used in conjunction with the Canadian tree seed testing manual (Edwards 1987) and the manual of quick tests (Leadem 1984), operational seed testing will be greatly improved. Staff and personnel changes may require future workshops, which might take the form of shorter (one-day or two-day) sessions devoted to detailed specific tests – for example, the germination or x-ray tests – rather than a broad spectrum of seed quality testing methods. The information contained in this guide will crystallize the starting point for such workshops.

Acknowledgements

The series of workshops were supported in a major part by the Canadian Forest Service and by the Ontario Ministry of Natural Resources, the Canada/Nova Scotia Forest Resource Development Agreement (Federal Technical Transfer Project), and the Canada/British Columbia Forest Resource Development Agreement, (Extension, Demonstration, Research & Development Program). The assistance of personnel from the British Columbia Ministry of Forests is also acknowledged.

The authors wish to thank also C. Magnussen and S. Handke (PNFI) for the line drawings and photographs, respectively; D. Dunaway and S. Glover (PFC) for typesetting and graphic design and editing, respectively.

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General introduction to seed testing

Objectives

Of all the hazards in producing plants from seeds, the use of inferior seeds is one of the greatest. The principal objective of testing seeds is to minimize this risk by assessing the quality of the seeds before they are sown. "Seed quality" incorporates many attributes that are of interest to different people: to the seed collector, the seed processor, the seed dealer or merchant, the seedling grower, the certification authority or agency responsible for seed control. In all instances, the ultimate objective of quality testing is to determine the value of the seeds for plant production.

Responsibilities of seed testers

Those who test tree seeds should keep in mind that seeds are living organisms and their behaviour is not as predictable as that which characterizes inert or nonbiological materials. The test methods used must be based on scientific knowledge of the seeds and the accumulated experience of other seed testers, and must adhere to established standards and procedures.

Need for uniform testing methods

As tree seeds move across provincial and territorial boundaries within Canada, or are imported into or exported from this country, they may be tested in various laboratories. It is essential that these laboratories standardize tests to ensure comparable results within an acceptable range.

Sampling

Introduction

The quantity of seeds that are actually tested is usually very small compared to the size of the seedlot that it represents. No matter how accurate the test, the results can only show the "quality" (however this may be measured) of the sample.

Tree seeds often cause sampling problems because they may contain high proportions of empty seeds. Or some other condition, such as insect larvae, mechanically damaged seeds, immature seeds, may make part of the lot unusable for seedling production. The sample that is taken must represent this condition.

It cannot be over-emphasized that no matter how well the laboratory testing is performed, the results cannot be applied to the seedlot unless every effort has been made to ensure that the sample truly represents the composition of the seedlot.

Sampling objectives

a) to make sure the constituents of the sample represent the same types in the same proportions as in the seedlot, and

b) to obtain a sample of a size that is suitable for the test(s) to be carried out.

Procedure

The sample that is finally tested is obtained by taking small portions at random from different positions in the seedlot and combining them (step 1). From this, subsamples are taken until the required size for testing has been reached (step 2).

Several definitions must be recognised:

SEEDLOT, which may be small and in a single container, or large and in several containers, is a specified, physically identifiable quantity of seeds.

PRIMARY SAMPLE is a small portion of seeds taken from one point in the lot.

COMPOSITE SAMPLE is formed by combining and mixing all the primary samples taken from the lot. The composite sample is frequently larger than required so it has to be reduced in size to become the submitted sample.

SUBMITTED SAMPLE, the sample submitted to the testing laboratory.

WORKING SAMPLE is a reduced sample taken from the submitted sample in the laboratory, and on which the tests are made.

Figure 1 summarizes (as a flow chart) the principal elements of sampling.

Equipment and methods for field sampling

Primary samples can best be obtained by one of the two following methods.

a) Stick or sleeve-type trier. This is a hollow tube, with open slots along its length, that fits inside an outer sleeve, also with slots. A half turn of the tube inside the sleeve lines up the two sets of slots so that seeds can enter. Another half turn closes the openings. It is preferable to use a trier that has partitions dividing the inner tube into compartments; this prevents seeds dropping down the tube, leading to an over-representation of the upper layers in the container. Many sizes of triers are available.

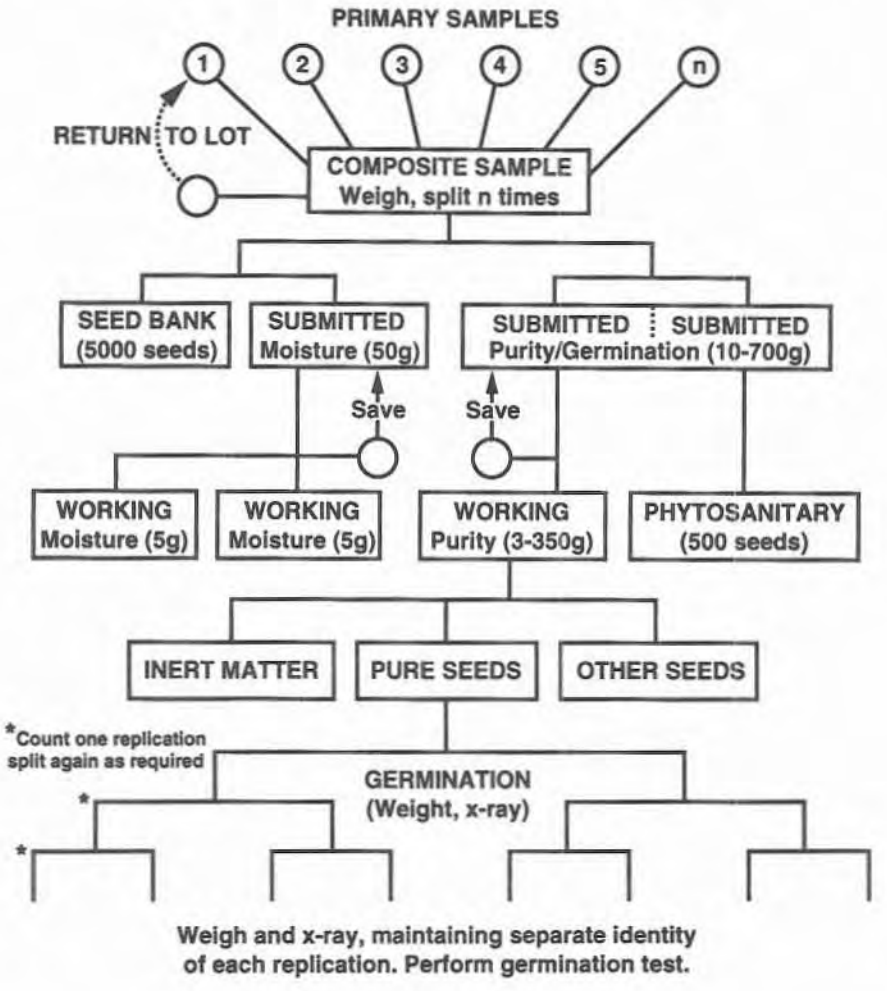

Figure 1. Flowchart of sampling

The closed trier is normally inserted diagonally into the container of seeds until it reaches the lowest layer. It is then opened by a half-turn of the inner tube and gently agitated to allow seeds to fill each compartment completely. The trier is closed by a half-turn of the inner tube, and withdrawn. (Closing the trier must be done gently so that seeds caught between slot edges are not damaged.) The seeds are emptied from the trier into a pan or onto a sheet of paper.

The seeds obtained comprise one primary sample. Depending upon the species and the size of trier used, this may provide enough seeds for the submitted sample. If not, several primary samples may have to be taken. If the seeds are in several containers, primary samples must be drawn from each one, combined in to a composite sample, then subsampled for sending to the laboratory.

b) Soil divider (riffle divider). This is a hopper into which the seeds are poured so that they are evenly distributed along its length. The seeds flow out through channels with sloping floors that deflect seeds to one side or the other since the floors of adjacent channels slope in opposite directions. The seeds are collected in two receiving pans; those in each pan comprise a representative half of the original amount of seeds placed in the hopper.

Half of the seeds are returned to the container while the other half is divided again. This is repeated until the seeds in one pan are about, but not less than, the quantity required for the submitted sample. Thus, the submitted sample is arrived at directly.

Seeds can be mixed by passing them through a soil divider three or four times, recombining the two halves from the receiving pans after each pass.

c) Manual sampling. In the absence of mechanical sampling equipment, or if the seeds do not flow freely, they may be sampled by hand. To do this, the fingers and thumb are kept straight and together, and the hand is pushed to the required depth in the container. The fingers are tightly closed around a portion of seeds and the hand is withdrawn.

Sampling intensity

Usually tree seeds are packaged in containers of one sort or another. If there are between one and five containers, each must be sampled and a total of at least five primary samples taken. (To ensure equal representation in the composite sample each container must be sampled the same number of times.) If there are between 6 and 30 containers, one in every three with a minimum of five containers must be sampled. For 31 or more containers, sample one in every five containers, with a minimum total of 10.

Weight of the submitted sample

For official testing purposes, the following minimum weights must be observed. These sample sizes can be adjusted to meet other purposes, but they provide useful guidelines for all testing needs.

i) if the sample is for a **moisture content test**, at least 50 g of seeds are required. This sample is taken separately from any for purity and germination tests.

ii) if the sample is for **purity and germination tests**, the minimum weight of seeds varies with the species; some examples are as follows:

Minimum sample weights (g)		
Species	**Submitted sample**	**Working sample**
Picea mariana	6	3
Picea glauca	10	5
Picea sitchensis	12	6
Picea rubens, Pinus banksiana/contorta	25	9
Pseudotsuga menziesii	60	30

These minimum **submitted sample** weights provide enough seeds to conduct a second test, either because the first one was abandoned (due to mold, equipment breakdown, etc.) or was unsatisfactory (out of tolerance, suspected errors in counting or seedling evaluation, etc.). The minimum **working sample** weights provide at least 2500 seeds for the purity test.

Sampling in the laboratory

The submitted sample has to be thoroughly mixed, then divided to give the proper working sample. Several methods can be used:

a) Mechanical methods. These use special apparatus to reduce the submitted sample by successive halving until a working sample of about, but not less than, the minimum amount has been obtained.

i) The soil divider can be used for this.

ii) Another apparatus, called the conical (or Boerner) divider works in a similar fashion to the soil divider, except that the channels are arranged in a circular pattern around the base of a cone.

iii) In the centrifugal (or Gamet) divider, seeds flow out of the hopper into a shallow rubber cup that is spun on a vertical shaft turned by an electric motor. Centrifugal force throws the seeds out of the cup and they fall on one side or the other of a dividing baffle. This stationary baffle diverts approximately half the seeds to each exit spout.

Using any of these dividers, the sample is split into two representative parts, one of which is returned to the container while the other is repeatedly divided until the required working sample size is reached.

b) Non-mechanical methods. These can be carried out using home-made equipment, or materials normally found in any laboratory.

i) Random cups method - small cups or beakers, usually no more than eight, are randomly placed on a tray and seeds are evenly poured over the tray. Some collect in the containers and these are combined into a larger beaker as the working sample. This process may have to be repeated if insufficient seeds collect in the containers the first time.

ii) Modified halving method - a grid of equal-sized cells is used. All cells are open at the top, but alternate cells have no bottom. The grid is placed over a tray or large sheet of paper and seeds are poured evenly over it, covering the entire grid. When the grid is lifted some of the seeds are retained, the rest being on the tray. The process can be repeated if the amount of seeds collected in the grid are more or less than required.

iii) Spoon method - seeds are poured evenly over a tray or large sheet of paper. Using a spoon and spatula, small portions of seeds are removed from at least five random positions until the required amount is obtained. This method is best used on small-seeded species.

EXERCISE 1

1. Examine and familiarize yourself with the sampling devices available.

2. Try the devices and methods to withdraw samples of seeds.

3. If you can foresee problems in sampling the materials you normally work with, discuss them with the instructors.

Questions:

1. What method (device) would you use to sample:

   a) a 200-kg lot of white spruce seeds?

   b) a 20-kg lot of black spruce seeds?

   c) a 2-kg lot of jack pine seeds?

   d) a 200-g lot from a jack pine seed orchard?

2. You have been asked to sample a large container of seeds kept in a cold storage (-15°C) facility. How would you go about this? Would any precautions be necessary, and if so what would they be?

3. If you were sampling seeds at various storage facilities on a regular basis, what kind of things would you look for?

4. If you had to send the samples you had taken to a laboratory for testing, what precautions would you take?

Purity test

Introduction

Purity tests determine the proportion of seeds in a container, and how much inert matter, such as needles or cone scales, there may be. The tests determine the composition by weight of three components:

a) Pure seeds of the crop species:

Pure seeds are the seeds of the species stated by the sender (the owner, dealer) or that are found to predominate in the test.

This includes i) intact seeds which, for most conifer species, means seeds with the wing and enclosing integument removed - except seeds of *Chamaecyparis, Cupressus* and *Thuja,* and ii) pieces of seeds resulting from breakage that are more than one-half their original size. (Broken seeds of conifer species that are less than one-half their original size, or have had their seed coats entirely removed, are regarded as inert matter - see below.)

b) Seeds of other species:

Other seeds are defined as seeds of any tree species other than that of the pure seeds. The same distinguishing characteristics as for pure seeds also apply here.

c) Inert matter:

Inert matter includes

a) broken or damaged seeds less than one-half the original size, and seeds without seedcoats.
b) soil, sand, chaff, stems, leaves, conifer needles, cone scales, wings, pieces of bark, flowers, buds, insect larvae, and all other non-seed matter.

Wings and integuments need special attention. If some seeds in the sample still bear entire wings, e.g. *Abies, Larix* or *Pseudotsuga,* or parts of integuments and wings, e.g. *Pinus, Picea, Cedrus* or *Tsuga,* they must be separated from seeds free from such impurities and weighed separately. The two weights, i.e., seeds with no wings or integuments plus those bearing such impurities, are combined to give the total component for pure seeds. The weight of those seeds bearing the impurities is to be expressed as a percentage of the total pure seeds.

In seeds of *Acer, Betula, Chamaecyparis, Cupressus* and *Thuja* the wing is regarded as part of the seed and need not be removed.

Procedure

A working sample is separated by hand into the three components. The components are individually weighed and the total weight obtained. The component weights are expressed as a percentage of the total weight, and are reported as such.

Calculations and reporting results

The percentage by weight of each of the components is calculated to one decimal place. Components of less than 0.05% are recorded as "Trace". If the result for a component is nil, report it as such. The percentage of all components must total 100.

Percentages have to be based on the combined weights of the components, not on the original weight of the working sample. This original weight is compared with the sum of the component weights to check for loss of material. Rule of thumb: if the difference between the original weight and the combined weight of the components is more than 1% of the original weight, the purity test should be repeated.

The scientific name of the species must be recorded, along with the name of each species of other seeds found. Kinds of inert matter must also be reported. When seeds of other species, or a particular kind of inert matter is found to the extent of 1% or more, the actual percentage must be reported, for example, "cone scales - 2.4%".

EXERCISE 1 **STATION NO.:**_____

1. You have been given a working sample of seeds. The weight has been recorded for you on the container.

2. Empty the sample onto your work surface and separate the material into the three components as defined above. N.B.- Undamaged seeds are regarded as pure seeds (or seeds of other species); you do not need to know if they are empty or full. Nor is it necessary to turn over individual seeds to determine the presence of holes or other damaged areas on the underside.

3. After separation weigh each component (A, B, C) to three decimal places and enter the weights in the appropriate spaces below.

4. Calculate the percentage of each component to one decimal place. Components of less than 0.05% should be recorded as "trace". If the result for any component is nil, record it as such. If you find seeds of other species, or one kind of inert matter in a percentage of 1% or more, this should be recorded also. The percentages must add up to 100.

5. The combined weights of the components (D) must be compared with the original weight (see step 1) as a check against loss of material or other error. (In an actual purity test, if the weight loss is 1% or more of the original weight of the sample, a new working sample would be withdrawn and the separation repeated.)

   Weight of the original sample: _____

		Actual %	Rounded off %
Weight of the pure seeds (A):	_____ g =	_____ %[1]	_____ %
Weight of other seeds (B):	_____ g =	_____ %[2]	_____ %
Weight of inert matter (C):	_____ g =	_____ %[3]	_____ %
Combined weight of components (D):	_____ g =		100 %

Scientific name of pure seeds: _____

other seeds: _____

type(s) of inert matter: _____

6. On completion of the exercise, return the inert matter to the container, but keep your pure seed component separate.

---

1 - Pure seeds % = A/D x 100
2 - Other seeds % = B/D x 100
3 - Inert matter % = C/D x 100

EXERCISE 2 **STATION NO.:**_____

1. Repeat the purity procedure as in exercise 1 using the second working sample.

   Weight of the original sample: _____

			Actual %	Rounded off %
Weight of the pure seeds (A):	_____ g	=	_____ %[1]	_____ %
Weight of other seeds (B):	_____ g	=	_____ %[2]	_____ %
Weight of inert matter (C):	_____ g	=	_____ %[3]	_____ %
Combined weight of components (D):	_____ g	=		100 %

Scientific name of pure seeds: _____

other seeds: _____

type(s) of inert matter: _____

4. On completion of the exercise, return your inert matter to the container, but keep your pure seed component separate.

---

[1] - Pure seeds % = A/D x 100
[2] - Other seeds % = B/D x 100
[3] - Inert matter % = C/D x 100

EXERCISE 3 **STATION NO.:**_____

1. Using a trier or other sampling device, withdraw a submitted sample for the species (for jack pine, black or white spruce, 25 g). You may have to take several primary samples and combine them into a composite sample, then subsample the composite sample to obtain your submitted sample.

2. Subdivide the submitted sample to the correct working sample size, using the soil sampler or the conical sampler. The working sample should weigh at least 3 g for black spruce, 5 g for white spruce, and 9 g for jack pine.

3. Perform a purity separation as in exercise 1.

   Weight of the original sample: _____

		Actual %	Rounded off %
Weight of the pure seeds (A):	_____ g =	_____ %[1]	_____ %
Weight of other seeds (B):	_____ g =	_____ %[2]	_____ %
Weight of inert matter (C):	_____ g =	_____ %[3]	_____ %
Combined weight of components (D):	_____ g =		100 %

Scientific name of pure seeds: _____

other seeds: _____

type(s) of inert matter: _____

4. On completion of the exercise, return all your material to the container.

[1] - Pure seeds % = A/D x 100
[2] - Other seeds % = B/D x 100
[3] - Inert matter % = C/D x 100

Answer the questions on the following page

Questions:

1. What information does a purity test provide?

2. In what way is this information useful?

3. If you were a nursery grower and you had a choice of buying two seedlots of the same species, A and B, that had the following test results:

   A - Germination 85%, Purity 92%, Moisture Content 9%,
   Price $250/kg

   B - Germination 78%, Purity 99%, Moisture Content 8%,
   Price $230/kg

   - which would you purchase? Why?

When you have answered all three questions, compare your answers with the solutions on page 64.

Weight determination - 1000 seed weight test

Introduction

This test determines the weight of 1000 seeds in the seedlot, and it is usually carried out at the same time as the purity test. The "1000-seed weight" is needed by the grower to calculate the number of seeds in a weighed container. This information is also useful to the Seed Centre in that it indicates, over a period of time, whether certain seed sources are typically large- or small-seeded.

Procedure

The test uses the pure seeds separated in the purity test. It can be carried out in one of two ways:

a) either all the seeds in the entire pure seeds component are counted. Since the weight of the pure seeds is known, then the weight of 1000 seeds can be calculated; or

b) replications are withdrawn from the pure seeds and counted.

Counting replications

The replication procedure is more commonly used since the same replications are later used in the germination tests. Eight replications of 100 seeds each are drawn randomly from the pure seeds. (For most tree species, eight replications are needed to conduct "paired" germination tests, i.e. one set of replications will be pretreated, one set will not, to test for dormancy).

Each of the eight replications is weighed to the same number of decimal places as in the purity test (3 places for the species you are working with). From these, the average weight per 100 seeds is calculated. This is multiplied by 10 to give the average 1000-seed weight.

Calculations

The variance, standard deviation, and coefficient of variation are calculated as follows:

$$\text{Variance} = \frac{n \cdot \left(\Sigma x^2\right) - \left(\Sigma x\right)^2}{n \cdot \left(n+1\right)}$$

where

x = weight of each replication in grams,
n = number of replications,
Σ = sum of...

$$\text{Standard deviation} = s = \sqrt{variance}$$

$$\text{Coefficient of variation (CV)} = \left(\frac{s}{\bar{x}}\right) \cdot 100$$

where

$\bar{x}$ = average (mean) weight of 100 seeds.

Note:

If the CV exceeds 4.0, a further eight replications should be counted and weighed, and s calculated for the 16 replications. Any replication that diverges from the mean $\bar{x}$ by more than twice the standard deviation (calculated for 16 means) can be discarded.

Coefficient of variation

Coefficient of Variation (CV) is an index of variability of data.

$$CV = \left(\frac{Standard\ deviation}{Mean} \right) \times 100$$

Example 1

x	Σx^2	$(\Sigma x)^2$
81	6561	81
79	6241	79
85	7225	85
83	6889	83
Mean $\bar{x} = 82$	$\Sigma x^2 = 26,916$	$Sum^2 = (328)^2 = 107,584$

$$\text{Variance} = \frac{n \cdot \left(\Sigma x^2 \right) - \left(\Sigma x \right)^2}{n \cdot \left(n + 1 \right)} = \frac{4 \cdot \left(26,916 \right) - \left(107,584 \right)}{4 \cdot \left(3 \right)} = \frac{107,664 - 107,584}{12}$$

$$= \frac{80}{12} = 6.666$$

$$\text{Standard Deviation } (s) = \sqrt{variance} = \sqrt{6.666} = 2.58$$

$$\text{Coefficient of Variation (CV)} = \left(\frac{Standard\ deviation}{Mean} \right) \times 100 = \left(\frac{s}{\bar{x}} \right) \cdot 100 = \left(\frac{2.58}{82} \right) \cdot 100$$

$$= \mathbf{3.15\%}$$

Example 2

x	Deviation from mean
8	0.5
7	0.5
9	1.5
6	1.5
Mean ($\bar{x}$) = **7.5**	
Standard Deviation (s) = **1.2907**	
$CV = \left(\frac{1.2970}{7.5} \right) \cdot 100$ = **17.2%**	

x	Deviation from mean
98	0.5
97	0.5
99	1.5
96	1.5
Mean ($\bar{x}$) = **97.5**	
Standard Deviation (s) = **1.2907**	
$CV = \left(\frac{1.2970}{97.5} \right) \cdot 100$ = **1.32%**	

EXERCISE 1

1. Using one of the pure seed fractions that you separated in the purity test, separate the seeds into 8 subsamples by the following method:

    a) Pour the seeds in a heap on your work table.

    b) Mix the seeds by lifting those around the edge of the heap toward the centre. A small piece of card, or a spatula, works well for this.

    c) Divide the heap into two equal parts (across the diameter) and move the two piles apart.

    d) Subdivide each pile across their smaller dimension, again moving them apart.

    e) Subdivide each of the the four small piles in the same manner.

    f) From each of the eight subsamples, count out 100 seeds. You should try to "sample" from within the entire pile, especially if it contains many more than 100 seeds. Do not select seeds; take what ever is next as you work your way through the pile.

2. Weigh each of the eight 100-seed samples to three decimal places. Record the weights in the table on the next page.

3. Make the calculations described above using the worksheet on the following page. Check to see that the CV is acceptable.

4. If there is time, repeat the exercise using the pure seeds from one of your other purity tests.

SPECIES : _____ **STATION NO.:**_____

Weight of 100-seed samples (enter in first column, "x")

Sample no. (n)	x	x^2
1		
2		
3		
4		
5		
6		
7		
8		
n=8	$\Sigma x =$	$\Sigma x^2 =$
	$(\Sigma x)^2 =$	

Mean: $= \bar{x} = \frac{\Sigma x}{n}$ = _____

Variance $= \frac{n \cdot (\Sigma x^2) - (\Sigma x)^2}{n \cdot (n+1)}$ = _____

Standard deviation (s) $= \sqrt{variance}$ = _____

Coefficient of variation (CV) $= \left(\frac{Standard\ deviation}{mean}\right) \cdot 100$ = _____

Is the CV acceptable?

The average 100-seed weight $\left(\frac{\Sigma x}{8}\right)$ = _____ g

Multiplied by 10, the average 1000-seed weight for this seed lot is _____ g

Questions:

1. Will a small-seeded seedlot have a smaller or larger 1000-seed weight than a large-seeded seedlot?

2. What factors may affect the 1000-seed weight of a seedlot?

3. How would you use a 1000-seed weight if you were a nursery grower?

Germination test

Introduction

Of all the quality measurements on seeds, none is more important than how well they will germinate. This information is the deciding factor on the value of the seedlot: for the buyer, whether to purchase the seeds; for the grower, whether to use the seeds this year or store them for future use, and at time of use, what the sowing rate should be.

Although field testing may be used, the results are normally not satisfactory since they are difficult to reproduce reliably. Thus, methods of laboratory testing have been devised to control the major environmental factors affecting germination. Combinations of the factors that provide the most regular, rapid and complete germination for a majority of the seedlots of a particular species have been developed into standardized procedures. These enable results to be reproduced within limits as near as possible to those determined by random sample variation.

Objective

The objectives of the germination test are:

i) to determine the field-sowing value of the seeds, and

ii) to compare the field-sowing values of different seedlots.

Procedure

From the pure seeds obtained in the purity test, 400 seeds are counted out at random into replications of 100 seeds. For species that are suspected, or known, to be dormant eight replications of 100 seeds are counted so two tests, one with and one without pretreatment, may be conducted concurrently.

The seeds are spread uniformly on a moist substratum, usually in a container such as a germination dish or box, and placed in a germinator set for the prescribed conditions of temperature, thermoperiod, light, photoperiod, and humidity. At set intervals and for a predetermined period of time, the number of seeds that have germinated are counted using standardized prescriptions to distinguish normal and abnormal germinants. At the completion of the test any ungerminated seeds are examined to determine if they are still fresh, and may have germinated had the test been of longer duration, or if they failed to germinate because they are empty, insect-damaged, or dead.

Some terminology is defined as follows:

GERMINATION is the emergence and development from the seed embryo of those essential structures that, for the kind of seed being tested, indicate its ability to produce a normal plant under field conditions.

GERMINATION PERCENTAGE is the proportion per 100 seeds of the sample that have produced normal germinants within a specified period.

NORMAL GERMINANTS are those seedlings that possess the essential structures that indicate their ability to produce normal plants under field conditions. (See Figs. 2 and 18. For comparisons of normal and abnormal germinants, see Figs. 2-17 and 19-21.)

ABNORMAL GERMINANTS are seedlings that are defective in one or more respects and which cannot be classified as normal seedlings. Seedlings with the following defects are classed as abnormal (see Figs. 2-17, 19-21):

i) damaged seedlings - those without cotyledons; with constrictions, splits, cracks or lesions on the essential stuctures; without a primary root (of those species for which a primary root is an essential structure).

ii) deformed seedlings - those with weak or unbalanced development of the essential structures such as spirally twisted or stunted plumules, hypocotyls or epicotyls; with swollen shoots and stunted roots; are watery or glassy; or in which development stops after initial emergence.

iii) decayed seedlings - those with any of the essential stuctures so diseased or decayed that normal development is prevented, except when there is clear evidence that the cause of the infection is not the seed itself.

FRESH SEEDS are those that have absorbed moisture and appear capable of germination, but which have not begun to germinate at the end of the test period.

DORMANT SEEDS are those that, due to a physiological condition within the seeds, to physical characteristics of the seed coat, or to the stage of development of the embryo, will not germinate under the prescribed conditions unless they are first treated to break dormancy.

DEAD SEEDS are those that are neither hard nor fresh and have not produced seedlings at the end of the test period.

EMPTY SEEDS are those that are completely empty or that contain some residual tissue showing neither endosperm nor embyo.

INSECT DAMAGED SEEDS are those that contain larvae, frass, or show other evidence of insect attack that affects the ability of the seeds to germinate.

PRECHILLING means placing the seeds on or in a moist substratum at 1-5°C for a specified period to overcome suspected dormancy. Seeds may also be prechilled by soaking in water at room temperature for 24 hours, draining the excess water, placing in a suitable glass or plastic vial or bag, and cooling at 1-5°C for a specified period.

Materials and Methods

Materials, equipment and details of testing the germination of tree seeds are described in *Methods and procedures for testing tree seeds in Canada*, Canadian Forestry Service Forestry Technical Report 36. Recommended conditions for prechilling, and alternative methods of determining seed viability are also described.

Calculation of results

The average of the four 100-seed replications, calculated to the nearest whole number, is reported as the percentage germination. The range of values for the replications must not exceed the maximum tolerated differences given in Table 3 of "Methods and procedures for testing tree seeds in Canada".

A germination test result is unsatisfactory, and the test should be repeated, when:

a) the replications exceed the tolerance limits;

b) there is evidence that the wrong conditions were used, or errors were made in seedling evaluation, inaccuracies in counting or recording results;

c) there is evidence of dormancy, phytotoxicity of the substratum, or disease.

If the test result is satisfactory, complete reporting includes the percentages of normal seedlings, abnormal seedlings, fresh ungerminated seeds, dead seeds; if any of these categories is nil, it should be reported as such. Empty seeds and insect-damaged seeds, if determined, should be reported along with the method used. The duration of the test and any pretreatment given to the seeds is also reported. If two tests, one with and one without prechill, were conducted, the results of both should be reported.

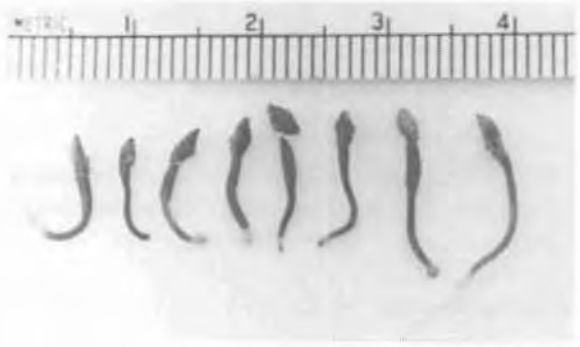

Figure 2. Germinants of western hemlock (*Tsuga heterophylla*). Seven germinants are abnormal, one is normal. The abnormal germinants have stunted or very stunted radicles. Also, all the abnormals display signs of early shedding of the seedcoat; fifth from the left has shed its seedcoat. Even if the roots were not stunted, none of these germinants would yield useable seedlings. Although they may not be true physiological dwarfs, which implies the lack of growth hormone, such germinants fail to grow because the embryos, at an early stage, lose contact with the megagametophyte. While this may amount to the same condition as dwarfing, because their true condition has not been determined, the term "miniatures" is used. (Photo: D.G. Edwards)

Figure 3. Western hemlock. Four germinants showing albinism, that is, lacking pigmentation in the cotyledons. All display stunted roots, including the fifth from the left, and all show early shedding of their seedcoats. On the right is a normal germinant. (Photo: D.G. Edwards)

Figure 4. Western hemlock. These germinants are similar to those shown in Figure 2, but their root tips (roots very stunted) are dead or dying. This is not caused by dessication, but perhaps by a fungus. On the right is a normal germinant.
(Photo: D.G. Edwards)

Figure 5. Germinants of noble fir (*Abies procera*). These germinants display the same symptoms as those described in Figure 2. (Photo: D.G. Edwards)

Figure 6. Twin embryos of Douglas-fir (*Pseudotsuga menziesii*). The second embryo is very small, its orientation is reversed (the cotyledons face the micropyle), and it is adhering to (by means of surface tension perhaps), but is not truly attached to the mid-point of the hypocotyl of the larger germinant. The larger germinant is weak, with a stunted root. (Sometimes the larger germinant will appear normal, and will be recorded as normal.)
(Photo: D.G. Edwards)

Figure 7. Douglas-fir. Triple embryos. Largest germinant is weak, with a stunted root. The second largest has a weak root also. The smallest embryo has a very stunted root. None of these germinants produced useable seedlings (after potting up).
(Photo: D.G. Edwards)

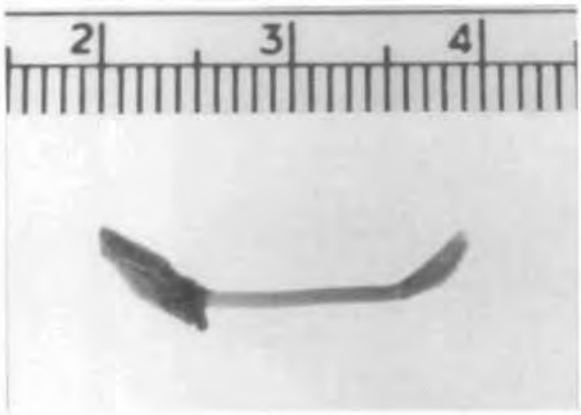

Figure 8. Germinants of grand fir (*Abies grandis*). The embryo is reversed, with the cotyledons appearing before the radicle. This embryo would not be able to develp into a useable seedling because the cotyledons are no longer in contact with the megagmetophyte, and it will remain "miniaturized" (see Fig. 2).
(Photo: D.G. Edwards)

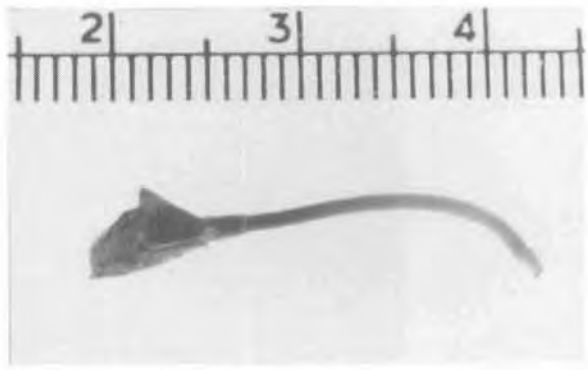

Figure 9. Grand fir. Very stunted radicle. (Photo: D.G. Edwards)

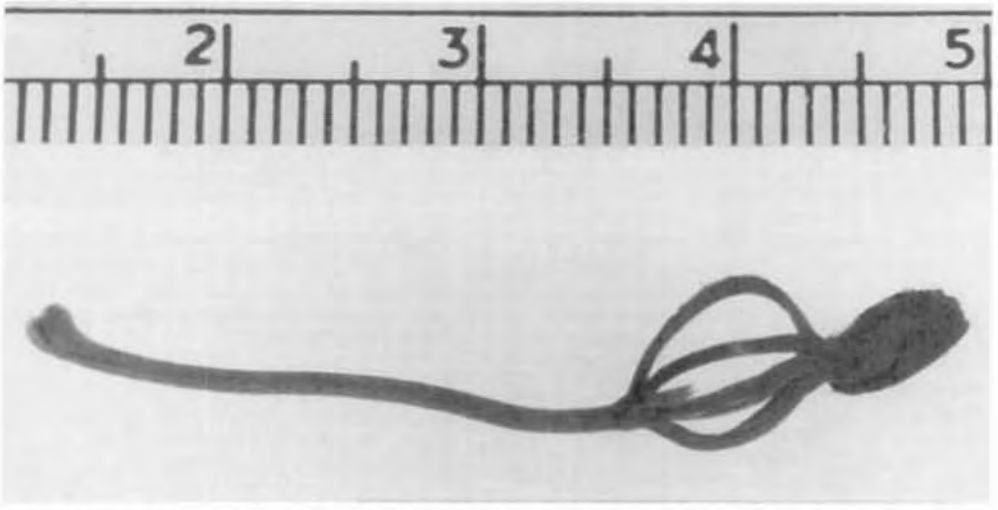

Figure 10. A western white pine (*Pinus monticola*) germinant with no radicle. (Photo: D.G. Edwards)

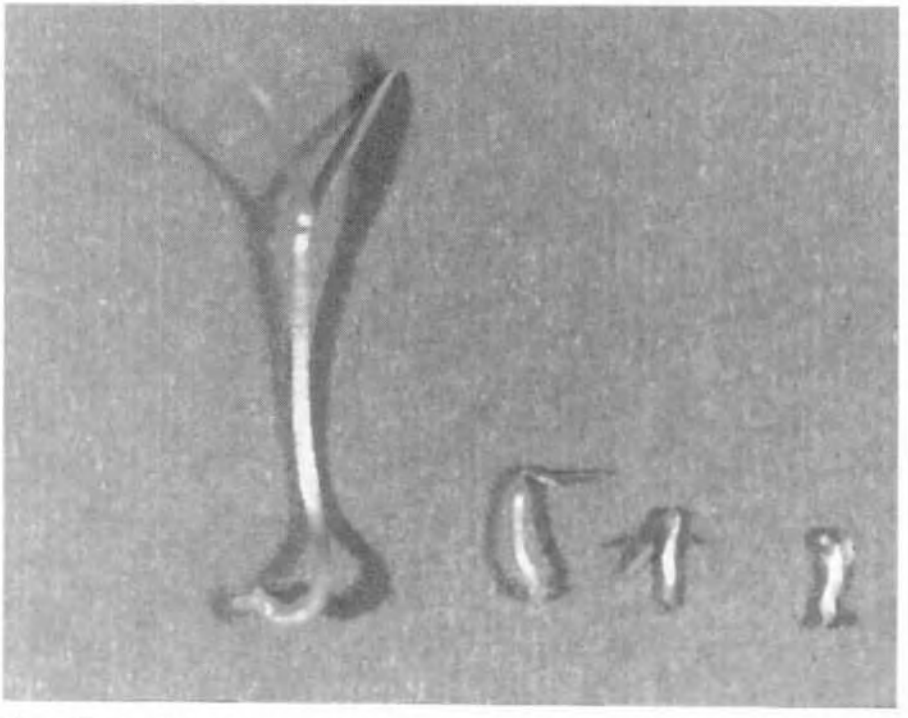

Figure 11. A yellow cedar (*Chamaecyparis nootkatensis*) germinant. "Quadruplets" (four embryos from the same seed), the largest with a stunted, diseased-looking radicle. (Photo: D.G. Edwards)

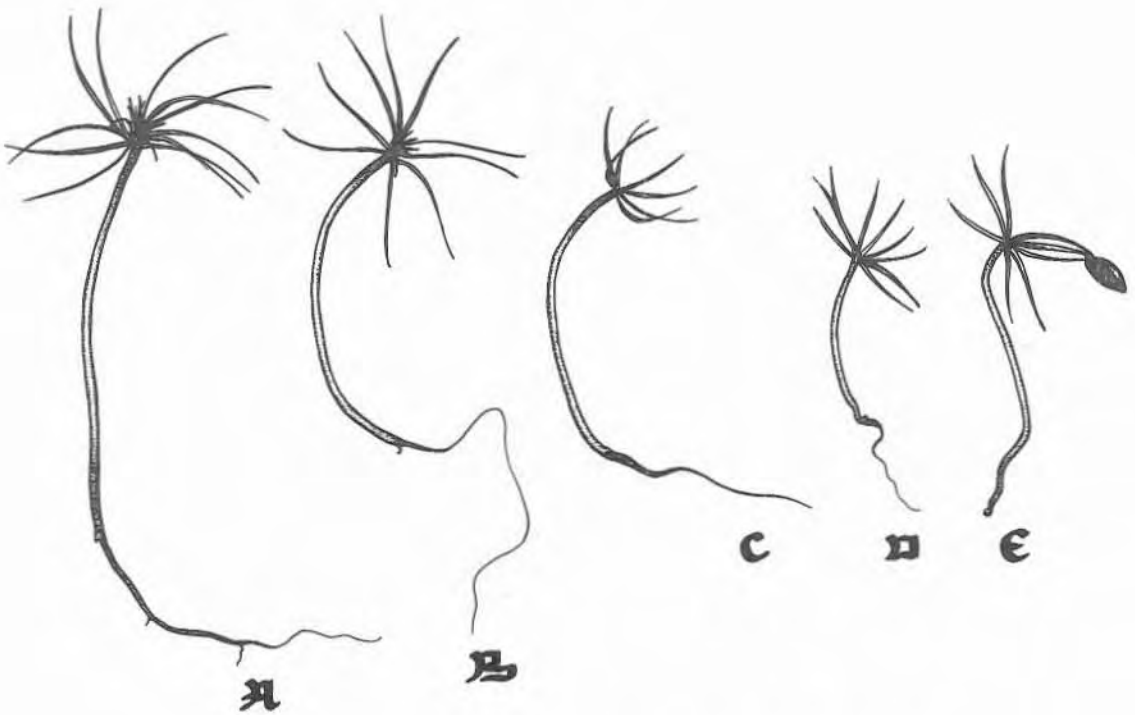

Figure 12. Germinants of eastern white pine (*Pinus strobus*). A: normal germinant, B: abnormal germinant with extended, thin root, C: roots dried out or decayed, D: thin root, E: stunted root. (Line drawing: C. Magnussen)

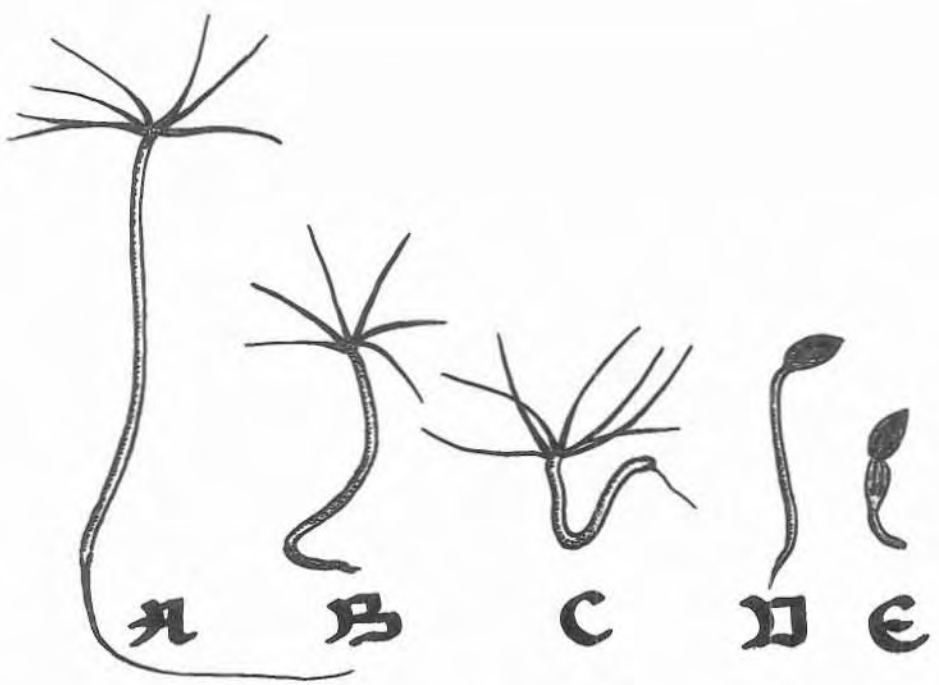

Figure 13. Germinants of red pine (*Pinus resinosa*). A: normal germinant, B: short root, C: short and thin root, short hypocotyl, D: no root, E: no hypocotyl or root. (Line drawing: C. Magnussen)

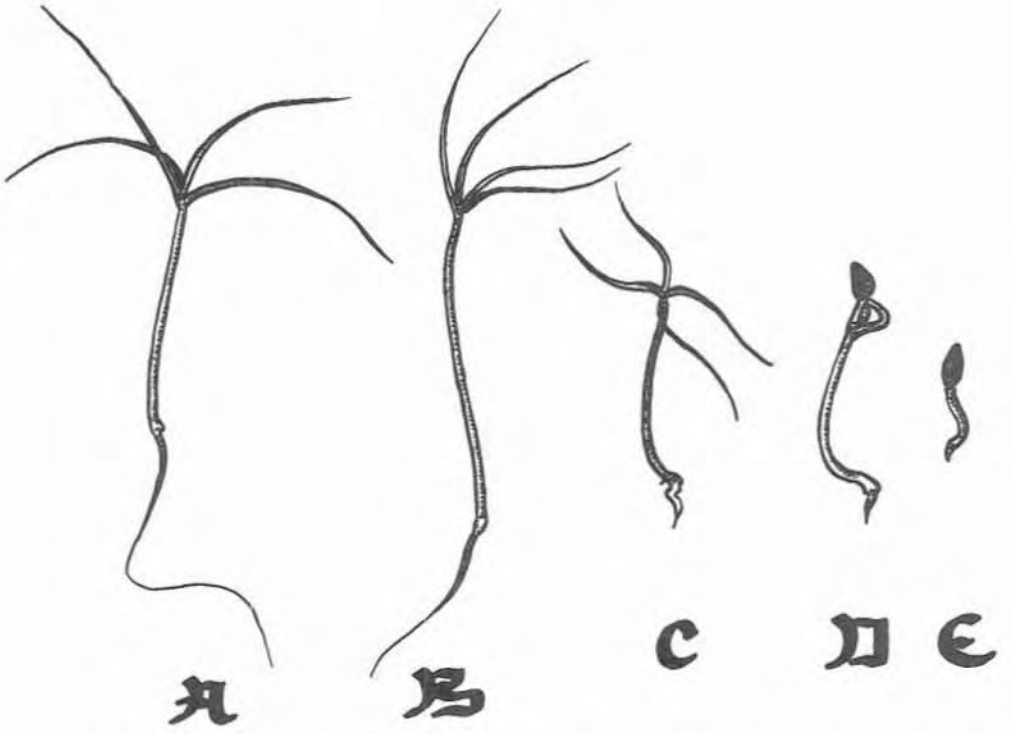

Figure 14. Germinants of jack pine (*Pinus banksiana*). A: normal germinant, B: short and thin root, C: stunted and short root, short hypocotyl, D: stunted root, E: no root and short hypocotyl. (Line drawing: C. Magnussen)

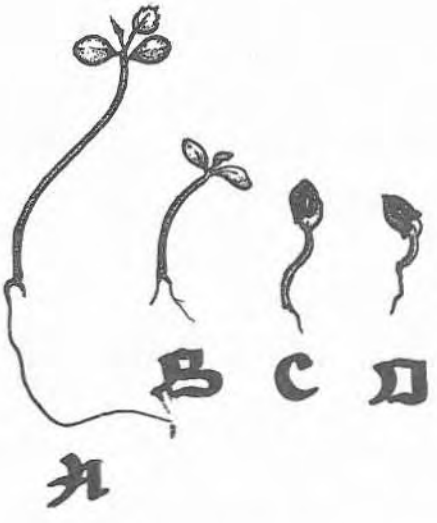

Figure 15. Germinants of yellow birch (*Betula alleghaniensis*). A: normal germinant, B: abnormal germinant with poorly-developed root and short hypocotyl, C: short hypocotyl, thin root, D: short hypocotyl, thin root. (Line drawing: C. Magnussen)

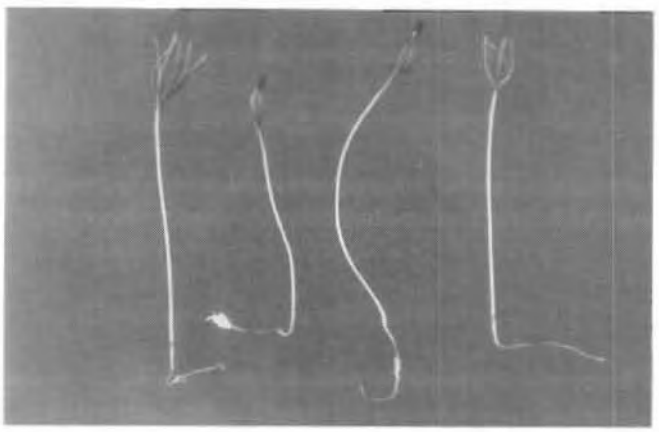

Figure 16. Germinants of eastern white pine (*Pinus strobus*). Three germinants (on the left) have extended, thin roots that did not penetrate the germination medium ("Kimpak"). Germinant on the right is normal. (Photo: B. Wang)

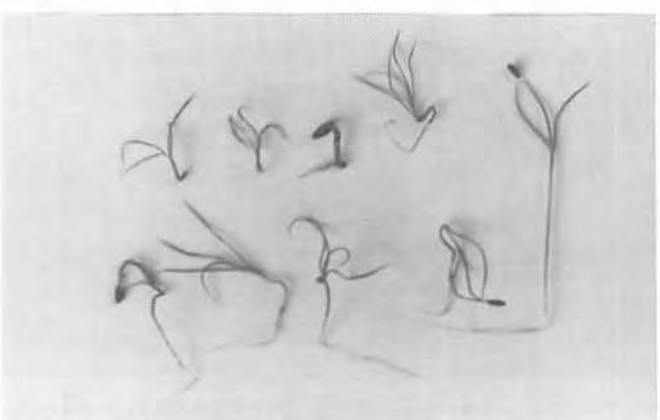

Figure 17. Germinants of jack pine (*Pinus banksiana*). Eight abnormal germinants with short and stunted roots, short and thick hypocotyls, or with neither roots nor hypocotyls. Germinant on the right is normal.
(Photo: B. Wang)

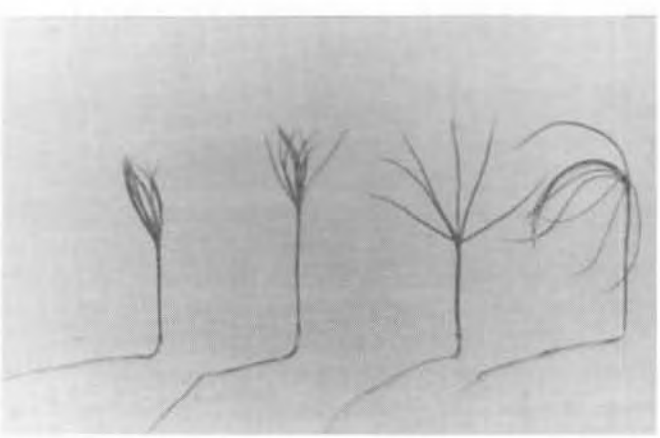

Figure 18. Normal germinants of jack pine.

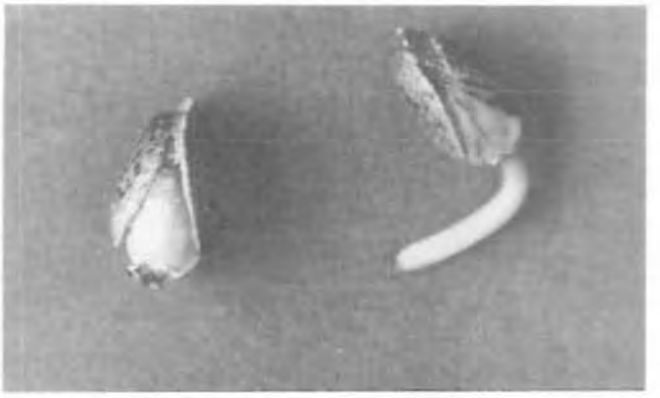

Figure 19. Abnormal germinants of eastern white pine in which (on the left) the seedcoat has split open, but there is no sign of further development, or (on the right) the radicle is very short, and development has stopped. (Photo: B. Wang)

Figure 20. Geminants of yellow birch (*Betula alleghaniensis*). The group on the right are normal germinants, while those on the left are abnormals that either have no radicles, or their radicles are very short. (Photo: B. Wang)

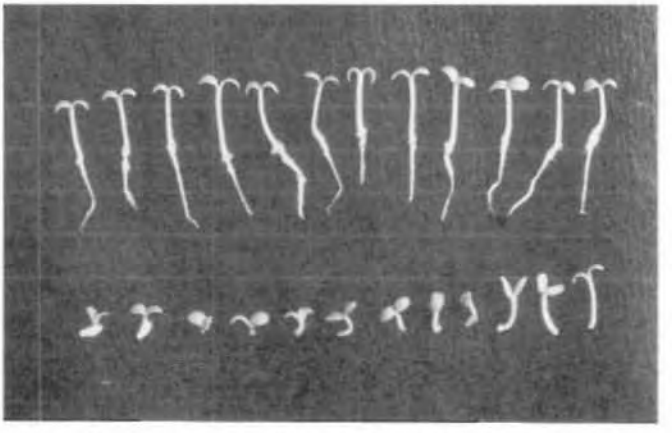

Figure 21. Germinants of plains cottonwood (*Populus deltoides* var. *occidentalis*). The germinants in the upper row are normal, those in the lower row are abnormal with no radicle or very short radicles.
(Photo: B. Wang)

EXERCISE 1 - Initiation of a germination test

1. You have been given a working sample for a germination test.

2. Using a spatula, withdraw one replication of 100 seeds.

3. Obtain a germination dish and place a layer of germination medium (Kimpak) on top of the perforated shelf. Add approximately 125 ml of water, wetting all parts of the medium; add a further 125 ml of water to the dish (underneath the shelf).

4. Sprinkle the seeds over the medium.

5. Use forceps or a small spatula to space seeds evenly. Make sure that no seeds are touching; although it is not necessary to arrange the seeds in rows, this will facilitate counting and evaluation.

6. Mark the germination box and worksheet (page 30) with the following information:
   a) Species, seedlot number;
   b) date test begins and ends;
   c) seed treatment (if any), and date treatment begins;
   d) date(s) counts are to be made.

7. Complete the worksheet for this test, putting the following information in the appropriate spaces.
   a) Species - jack pine (use scientific name)
   b) Seedlot no. - STWP Ex/001
   c) Test no. - 87-001
   d) Date of pretreatment - (N/A)
   e) Date of test - (today's date)
   f) Test period - 14 days
   g) Tested for - Workshop exercise
   h) No. of replications - 1
   i) No. of seeds/replication - 100
   j) Germination conditions: Temperature - 30°-20°C; Photoperiod - 8 h.
   k) Germination count - every 7 days

Questions:

1. What did you learn from this exercise? Did you find it useful?

2. Why does the amount of water placed in each dish have to be measured? Why does it have to be the same for each replication?

3. Why do the seeds have to be spaced apart, more or less equally?

4. Why must the samples be withdrawn randomly?

GERMINATION TEST WORKSHEET

STATION NO.: _____ Date of Test: _____

Species: _____ Test No.: _____

Seedlot No. _____ Test period: _____

Date of pretreatment: _____ Tested for: _____

No. of replications: _____ No. seeds/replication _____

Conditions: Temperature _____ ℃ Photoperiod _____

Germination count _____

Date	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average	Remarks
Total normal						
Total abnormal						

Ungerminated seeds (by cutting test):

Fresh						
Empty						
Dead						
Total	100	100	100	100		

Tolerance:_____IN:_____ OUT:_____

EXERCISE 2 - Interim germination count

1. You have been given a germination box containing a sample of seeds that were placed in the germinator 1 week ago. The first interim germination count has to be made.

2. Remove seeds that have germinated and developed into healthy seedlings with all essential structures visible. Count these seeds and then discard them.

3. Enter the number of seeds/germinants so counted in the appropriate space (top line) on the worksheet (following page). Enter today's date.

4. Examine the remaining seeds and remove any that can be positively identified as having germinated abnormally. For the interim count, this means any albino germinants, and those with the cotyledons emerging from the micropyle. Germinants showing evidence of disease are not removed during interim germination counts unless it is clear that the germinant is dead and will not develop further. Enter these counts of abnormal germinants on the worksheet.

5. Make notes of any special or unusual observations that may help explain further evaluations or test results. These might include excessive moisture in the germination medium (for example).

6. If the germination medium shows signs of excessive dryness, add water to the box. Make a note on the worksheet, including date, when this was done.

Questions:

1. Did you find it difficult to identify normally germinated seeds?

2. Did you have difficulties in distinguishing normal and abnormal germinants?

3. What is the reason for making notes of any unusual observations?

4. Why are interim counts necessary?

GERMINATION TEST WORKSHEET (interim counts)

STATION NO.: _____ Date of Test: _____

Species: _____ Test No.: _____

Seedlot No. _____ Test period: _____

Date of pretreatment: _____ Tested for: _____

No. of replications: _____ No. seeds/replication _____

Conditions: Temperature _____ ℃ Photoperiod _____

Germination count _____

Date	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Average	Remarks
Total normal						
Total abnormal						

Ungerminated seeds (by cutting test):

Fresh						
Empty						
Dead						
Total	100	100	100	100		

Tolerance:_____IN:_____ OUT:_____

EXERCISE 3 - Termination of germination test

1. You have been given a box containing a germination test that was prepared in advance for the Workshop, using either black spruce or jack pine seeds.

2. The test ends today, so count the germinated seeds as if this was the final count. (Ignore the fact that interim counts have not been made.)

3. Evaluate the seedlings as normal or abnormal according to the criteria given earlier.

4. Record your observations on the last line of the upper part of the worksheet provided for Exercise 2.

5. Perform a cutting test on any ungerminated seeds and classify them as "fresh", "dead" or "empty".

   Note - If you had carried out a TZ test, only those seeds showing adequate staining would have been counted and entered on the line "TTZ". Seeds which did not stain properly would have been regarded as "dead".

6. Some of the germinated seeds may have shed their seed coats, and these will appear to be empty seeds. (With a little practice such seedcoats can be recognised without cutting them.) The number of empty seeds is usually obtained by subtracting from 100 the total of the normal germinants (upper part of worksheet) + abnormal germinants + fresh (ungerminated) seeds + dead seeds.

7. Summarize your test results:

   i) Calculate the average normal germination.

   ii) Using the average, determine the tolerance limits from the table of tolerances found on page 34. Check the highest and the lowest values for your replications and compare the difference between them with the tolerance limits. Indicate on the worksheet whether the results are in or out of tolerance.

8. Enter your data on the test report form; use the spaces in the middle of the form under "Germination Test".

9. Complete as much of the remainder of the report as you can using the information given to you in Exercise 2.

Table of maximum tolerated ranges between replications

If the average germination percentage is	or	then the maximum tolerated range between replications is:
99	2	5
98	3	6
97	4	7
96	5	8
95	6	9
93 to 94	7 to 8	10
91 to 92	9 to 10	11
89 to 90	11 to 12	12
87 to 88	13 to 14	13
84 to 86	15 to 17	14
81 to 83	18 to 20	15
78 to 80	21 to 23	16
73 to 77	24 to 28	17
67 to 72	29 to 34	18
56 to 66	35 to 45	19
51 to 55	46 to 50	20

This table indicates the maximum range, that is, the difference between highest and lowest, in germination percentage tolerable between replications. To find the maximum tolerated range, calculate the average percentage of the replications to the nearest whole number. Locate the average in either of the first two columns and read the maximum tolerated range in the third column.

Questions:

1. Why is seedling evaluation, that is, the identification of normal and abnormal germinants, important?
2. Do you think the prescribed test period is important?
3. Why must germination counts be made frequently during the test, instead all at the conclusion?
4. Is a cutting test necessary at the end of a germination test?
5. What does the count of empty seeds depend on?

REPORT OF TEST RESULTS

STATION NO.:_____

Tested for: _____ Test No.: _____

_____ Sample Received: _____

_____ Customer Lot No.: _____

Date of Test: _____ Species: _____

Sampling and Sealing Agency: _____

Official Marks: _____

Official Seal: _____

Date of Sampling: _____ Size of Seed Sample: _____ g

Sample Represents: _____ kg of seeds in _____ containers

Year of Seed Collection: _____ Location: _____

Analysis of results:

Purity analysis			Germination test				
Pure seeds	Inert matter	Other seeds	Normal seedlings	Fresh ungerminated seeds	Abnormal seeds	Dead seeds	Empty seeds

Kind of Inert Matter: _____

Kind of Other Crop Seeds: _____

Pretreatment Date: _____ Method: _____ Temperature: _____ °C Time: _____

Date Germination Tested Started: _____ Concluded: _____

Moisture Content (wet basis): _____ %

Seed Count per kg. _____ Full Seeds by X-ray: _____ %

Processing Injury Test: _____

_____

Remarks: _____

Date: _____ Tested By: _____

Table 2. Methods for laboratory testing the germination of tree and shrub seeds (after the International Seed Testing Association 1993, Seed Sci. and Technol. 21, Suppl.)*

Notes:
The abbreviations have the following meanings:
TP: Germination test on top of paper.
S: Germination test *in* sand. (Place the seeds on a layer of moist sand, then cover them with dry sand to a depth of 10-20 mm. Assure good aeration.)
TS: Germination test *on* sand. (Seeds are pressed into the sand surface.)
TZ: Tetrazolium test.
EET: Excised embryo test
(The less desirable methods are placed in brackets in the table.)

Species	Substrate	Temperature (°C)	First count (days)	Final count (days)	Additional directions including recommendations for breaking dormancy
Abies alba *Abies balsamea* *Abies cilicica* *Abies firma* *Abies fraseri* *Abies homolepis* *Abies lasiocarpa* *Abies magnifica* *Abies numidica* *Abies sachalinensis*	TP	20-30	7	28	Prechill 21 days at 3-5°C
Abies amabilis *Abies cephalonica* *Abies concolor* *Abies grandis* *Abies nordmanniana* *Abies pinsapo* *Abies procera* *Abies veitchii*	TP	20-30	7	28	No prechill and prechill 21 days at 3-5°C. Double tests.
Acacia spp.	TP	20-30; (20)	7	21	1. Pierce seed, chip or file off fragment of testa at cotyledon end and soak 3 hours. 2. (Soak seeds1 hour in concentrated H_2SO_4. Wash seeds thoroughly in running water after acid treatment).
Acer negundo *Acer platanoides* *Acer pseudoplatanus* *Acer saccharum*	- - (S;(TP))	- - (20)	- - (7)	- - (21)	1. Use TZ. 2. (Use EET.) 3. (Prechill 2 months at 1-5°C. It is advantageous to remove pericarp before testing.) Fresh undried seeds are usually more dormant than dried and/or stored seeds.

* Additional directions can be found in the Proceedings of the International Seed Testing Association, 1993, Seed Science and Technology 21, Suppl., pages 169-177.

Table 2 continued

Species	Substrate	Temperature (°C)	First count (days)	Final count (days)	Additional directions including recommendations for breaking dormancy
Acer palmatum	- - (S; (TP))	- - (20)	- - (7)	- - (21)	1. Use TZ. 2. (Use EET.) 3. (Prechill 4 months at 1-5°C. It is advantageous to remove pericarp before testing.)
Acer rubrum *Acer saccharum*	S; (TP)	20	7	21	
Aesculus hippocastanum	TS; (S)	20-30; (20)	7	21	Soak seeds 48 hours; cut off 1/3 scar end of seed. Do not remove testa from sown portion. Fresh nuts may require prechill.
Ailanthus altissima	TP	20-30	7	21	Removal of pericarp after soaking for 24 hours may speed up germination.
Alnus cordata *Alnus glutinosa* *Alnus incana* *Alnus rubra*	TP	20-30	7	21	
Betula papyrifera *Betula pendula* *Betula pubescens*	TP	20-30	7	21	
Calocedrus decurrens	TP - -	20-30 - -	7 - -	28 - -	1. Prechill 28 days at 3-5°C. 2. (Use TZ.) 3. (Use EET.)
Caragana arborescens	TP	20-30	7	21	Pierce seeds, chip or file off fragment of testa from cotyledon end soak 3 hours.
Carpinus betulus	- (S)	- (20)	- (14)	- (42)	1. Use TZ. 2. (Incubate in moist substrate 1 month at 20°C followed by 4 months at 3-5°C.)
Castanea sativa	TS; (S)	20-30	7	21	Soak seeds 48 hours; cut off 1/3 at scar end and remove testa.
Catalpa spp.	TP	20-30	7	21	
Cedrela spp.	TP	20-30	7	28	
Cedrus atlantica *Cedrus deodora* *Cedrus libani*	TP	20; (20-30)	7	21	Prechill 21 days at 3-5°C.

Table 2 continued

Species	Substrate	Temperature (°C)	First count (days)	Final count (days)	Additional directions including recommendations for breaking dormancy
Chamaecyparis lawsoniana	TP	20; (20-30)	7	28	
Chamaecyparis nootkatensis	TP	20; (20-30)	7	28	Prechill 21 days at 3-5°C.
Chamaecyparis obtusa	TP	20-30	7	21	
Chamaecyparis pisifera	TP	20-30	7	21	
Chamaecyparis thyoides	- TP	- (20)	- (7)	- (28)	1. Use TZ. 2. (Prechill 90 days at 3-5°C.)
Cornus mas *Cornus sanguinea*	-	-	-	-	Use TZ.
Corylus avellana	- (S)	- (20; (20-30))	- (14)	- (35)	1. Use TZ 2. (Remove pericarp and prechill 2 months at 3-5°C).
Cotoneaster spp.	-	-	-	-	Use TZ.
Crataegus monogyna	- (S)	- (20-30)	- (7)	- (28)	1. Use TZ. 2. (Incubate in moist substrate for 3 months at 25°C followed by 9 months prechill at 3-5°C.)
Cryptomeria japonica	TP	20-30	7	28	
Cupressus arizonica	TP	20-30	7	28	No prechill and prechill 21 days at 3-5°C. Double test.
Cupressus macrocarpa	TP	20-30	14	35	
Cupressus sempervirens	TP	20	7	28	
Cytisus scoparius	TP	20-30	7	28	Pierce seeds or chip or file off fragment of testa at cotyledon end, soak 3 hours.
Elaeagnus angustifolia	-	-	-	-	Use TZ.

Table 2 continued

Species	Substrate	Temperature (°C)	First count (days)	Final count (days)	Additional directions including recommendations for breaking dormancy
Eucalyptus spp.					All *Eucalyptus* spp. should be tested by the weighed replicates method.
Euonymus europaea	- (TP)	- (20-30)	- (7)	- (28)	1. Use TZ. 2. (Prechill 45 days at 3-5°C).
Fagus sylvatica	TP	3-5	-	-	1. Duration of the test depends on dormancy and in an extreme case could require about 24 weeks. 2. (Use TZ).
Fraxinus spp.	- - (TP)	- - (20-30)	- - (14)	- - (56)	1. Use TZ. 2. (Use EET.) 3. (Pretreat 2 months at 20°C followed by 7 months at 3-5°C.)
Gleditsia triacanthos	TP	20	7	21	1. Pierce seeds or chip or file off fragment of testa at cotyledon end and soak 6 hours. 2. (Soak whole seeds in concentrated H_2SO_4 for as long as necessary to pit surface of testa. Wash thoroughly in water.)
Juniperus communis	- (TP; (S))	- (20)	- (14)	- (28)	1. Use TZ. 2. (Prechill 90 days at 3-5°C.)
Juniperus scopulorum	- (TP; (S))	- (15)	- (14)	- (42)	1. Use TZ. 2. (Pretreat 60 days at 20°C followed by 40 days at 3-5°C.)
Juniperus virginiana	- (TP; (S))	- (15)	- (14)	- (28)	1. Use TZ. 2. (Pretreat 60 days at 20°C followed by 45 days at 3-5°C.)
Laburnum alpinum *Laburnum anagyroides*	TP	20-30	7	21	1. Pierce seeds or chip or file off fragment of testa at cotyledon end and soak for 3 hours. 2. (Soak whole seeds1 hour in concentrated H_2SO_4 wash thoroughly in water.)
Larix decidua *Larix x eurolepis* *Larix gmelinii* *Larix laricina* *Larix sibirica* *Larix sukaczewii*	TP	20-30	7	21	
Larix kaempferi *Larix occidentalis*	TP	20-30	7	21	No prechill and prechill 21 days at 3-5°C. Double test.

Table 2 continued

Species	Substrate	Temperature (°C)	First count (days)	Final count (days)	Additional directions including recommendations for breaking dormancy
Ligustrum vulgare	-	-	-	-	Use TZ.
Liquidambar styraciflua	TP	20-30	7	21	Sensitive to drying in test.
Liriodendron tulipifera	- (TP)	- (20-30)	- (7)	- (28)	1. Use TZ. 2. (Prechill 60 days at 3-5°C.)
Malus spp.	-	-	-	-	1. Use TZ. 2. (Use EET.)
Morus spp.	TP	20-30	14	28	
Nothofagus obliqua	TP	20-30	7	28	No prechill and prechill 28 days at 3-5°C. Double tests.
Nothofagus procera	TP	20-30	7	28	
Picea abies *Picea engelmannii* *Picea koyamai* *Picea mariana* *Picea omorika* *Picea orientalis* *Picea polita* *Picea pungens* *Picea rubens*	TP	20-30	7	21	
Picea glauca *Picea glehnii* *Picea jezoensis* *Picea sitchensis*	TP	20-30	7	21	No prechill and prechill 21 days at 3-5°C. Double tests.
Pinus albicaulis	TP	20-30	7	28	Prechill 28 days at 3-5°C.
Pinus aristata	TP	20-30	7	14	
Pinus banksiana	TP	20-30	7	14	
Pinus canariensis	TP	20	7	28	
Pinus caribaea	TP	20-30	7	21	

Table 2 continued

Species	Substrate	Temperature (°C)	First count (days)	Final count (days)	Additional directions including recommendations for breaking dormancy
Pinus cembra	- - (S)	- - (20-30)	- - (7)	- - (28)	1. Use TZ. 2. (Use EET.) 3. (Prechill 6-9 months at 3-5°C.)
Pinus cembroides	S	20	7	28	Prechill 21 days at 3-5°C.
Pinus clausa	TP; (TS)	20	7	21	Sensitive to excess moisture.
Pinus contorta	TP	20-30	7	21	No prechill and prechill 21 days at 3-5°C. Double tests.
Pinus coulteri	- - (S)	- - (20-30)	- - (7)	- - (28)	1. Use TZ. 2. (Use EET.) 3. (Prechill 60-90 days at 3-5°C.)
Pinus densiflora	TP	20-30	7	21	Prechill 14 days.
Pinus echinata	TP	20-30	7	28	
Pinus edulis	TP	20-30	7	28	Light for 16 hours or more.
Pinus elliottii	TP	22; 20-30	7	28	
Pinus flexilis	TP	20-30	7	21	Prechill 21 days at 3-5°C.
Pinus glabra	TP	20-30	7	21	Prechill 21 days at 3-5°C.
Pinus halepensis	TP	20	7	28	
Pinus heldreichii	- - (TP)	- - (20-30)	- - (7)	- - (28)	1. Use TZ. 2. (Use EET.) 3. (Prechill 42 days at 3-5°C.)
Pinus jeffreyi	TP; (S) - -	20-30 - -	7 - -	28 - -	1. Prechill 28 days at 3-5°C. 2. (Use TZ.) 3. (Use EET.)
Pinus kesiya (khasya)	TP	20-30	7	21	
Pinus koraiensis	- - (S)	- - (20-30)	- - (7)	- - (28)	1. Use TZ. 2. (Use EET.) 3. (Pretreat 2 months at 25°C followed by 3 months at 3-5°C.)
Pinus lambertiana	- - (TP; (S))	- - (20-30)	- - (7)	- - (28)	1. Use TZ. 2. (Use EET.) 3. (Prechill 60-90 days at 3-5°C.)

Table 2 continued

Species	Substrate	Temperature (°C)	First count (days)	Final count (days)	Additional directions including recommendations for breaking dormancy
Pinus merkusii	TP	20-30	7	21	
Pinus monticola	- - (TP)	- - (20-30)	- - (7)	- - (28)	1. Use TZ. 2. (Use EET.) 3. (Prechill 60-90 days at 3-5°C.)
Pinus mugo	TP	20-30	7	21	
Pinus muricata	TP	20-30	7	21	
Pinus nigra	TP	20-30	7	21; (14)	
Pinus oocarpa	TP	20-30	7	21	
Pinus palustris	S; (TP)	20	7	21	
Pinus parviflora	- - (S)	- - (20-30)	- - (7)	- - (28)	1. Use TZ. 2. (Use EET.) 3. (Prechill 6-9 months at 3-5°C.)
Pinus patula	TP	20; (20-30)	7	21	
Pinus peuce	- - (S)	- - (20-30)	- - (7)	- - (28)	1. Use TZ. 2. (Use EET.) 3. (Prechill 6 months at 3-5°C.)
Pinus pinaster	TP	20	7	35	1. No prechill and prechill 28 days at 3-5°C. Light for no more than 16 hours per day. Double tests. 2. (Use TZ.)
Pinus pinea	TP	20	7	28	Soak one day prior to test.
Pinus ponderosa	TP	20-30	7	21	No prechill and prechill 21 days at 3-5°C. Double tests.
Pinus pumila	- (S)	- (20-30)	- (7)	- (21)	1. Use TZ. 2. (Prechill 4 months at 3-5°C.)
Pinus radiata	TP	20	7	28	
Pinus resinosa	TP	20-30; (25)	7	14	
Pinus rigida	TP	20-30	7	14	
Pinus strobus	TP -	22; 20-30 -	7 -	28 -	1. Prechill 28 days at 3-5°C. 2. (Use TZ.)

Table 2 continued

Species	Substrate	Temperature (°C)	First count (days)	Final count (days)	Additional directions including recommendations for breaking dormancy
Pinus sylvestris	TP	20-30; (20)	7	21; (14)	Eastern, Mediterranean provenances may require prechill 21 days at 3-5°C.
Pinus tabuliformis	TP	20-30	7	14	
Pinus taeda	TP	22; 20-30	7	28	
Pinus taiwanensis	TP	20-30	7	21	
Pinus thunbergii	TP	20-30	7	21	
Pinus virginiana	TP	20-30	7	21	
Pinus wallichiana (P. excelsa)	TP	20-30	7	28	
Platanus spp.	TP	20-30	7	21	
Populus spp.	TP	20-30	3	10	
Prunus avium *Prunus padus* *Prunus serotina*	- - (S)	- - (20-30; (20))	- - (7)	- - (28)	1. Use TZ. 2. (Use EET.) 3. (Prechill 3-4 months at 3-5°C.)
Pseudotsuga menziensii	TP	20-30	7	21	No prechill and prechill 21 days at 3-5°C. Double tests.
Pyrus spp.	- - (S)	- - (20-30)	- - (7)	- - (28)	1. Use TZ. 2. (Use EET.) 3. (Prechill 3-4 months at 3-5°C.)
Quercus spp.	TS; (S)	20	7	28	Soak seeds for up to 48 hours and cut off 1/3 at scar end of seed and remove testa.
Robinia pseudoacacia	TP	20-30	7	14	1. Pierce seeds or chip or file off fragment of testa at cotyledon end and soak 3 hours. 2. (Soak whole seeds in concentrated H_2SO_4 for as long as necessary to pit surface of testa. Wash thoroughly in water.)
Rosa spp. (sauf R. multiflora)	- (S)	- (20)	- (35)	- (70)	1. Use TZ. 2. (Prechill in moist substrate for 12 months.)
Rosa multiflora	- (T)	- (10-30)	- (7)	- (28)	1. Use TZ. 2. (Prechill 28 days at 3-5°C.)

Table 2 continued

Species	Substrate	Temperature (°C)	First count (days)	Final count (days)	Additional directions including recommendations for breaking dormancy
Salix spp.	TP	20-30	7	14	
Sequoia sempervirens	TP	20-30	7	21	
Sequoiadendron giganteum	TP	20-30	7	28	
Sorbus spp.	- (S)	- (20-30)	- (7)	- (28)	1. Use TZ. 2. (Prechill 4 months at 3-5°C.)
Spartium junceum	TP	20	7	14	Pierce seeds or chip or file off fragment of testa at cotyledon end and soak 3 hours.
Syringa reflexa	TP	20	7	21	No prechill and prechill 27 days at 3-5°C. Double tests.
Syringa villosa	TP	20-30	7	21	
Syringa vulgaris	TP	20	7	21	
Taxodium distichum	S -	20-30(20) -	7 -	28 -	1. Prechill 30 days at 3-5°C. 2. (Use TZ.)
Taxus spp.	- (S)	- (20-30)	- (7)	- (28)	1. Use TZ. 2. (Prechill 9 months at 3-5°C.)
Tectona grandis	S	30	14	28	Soak in water and allow to dry for 3 days - repeat this 6 times.
Thuja occidentalis	TP	20-30	7	21	
Thuja orientalis	TP	20	7	21	
Thuja plicata	TP	20-30	7	21	
Tilia cordata *Tilia platyphyllos*	- - (S)	- - (20-30)	- - (7)	- - (28)	1. Use TZ. 2. (Use EET.) 3. (Prechill 6-9 months at 3-5° C.)
Tsuga canadensis	TP	15	7	28	Prechill 28 days at 3-5°C.
Tsuga heterophylla	TP	20	7	35	No prechill and prechill 21 days at 3-5°C. Double tests.

Table 2 continued

Species	Substrate	Temperature (°C)	First count (days)	Final count (days)	Additional directions including recommendations for breaking dormancy
Ulmus americana *Ulmus parviflora* *Ulmus pumila*	TP	20-30; (20)	7	14	Pericarp may be removed.
Zelkova serrata	TP	10-30	7	28	No prechill and prechill 14 days at 3-5°C. Double tests.

Moisture content test

Introduction

The object of this test is to determine the moisture content of a seed sample. Seed moisture content is defined as the quantity of water lost when the sample is dried under specified conditions.

Moisture content is expressed as a percentage of the weight of the original sample, that is, it is expressed on a fresh weight basis. This is different from other expressions of moisture content for scientific purposes which are usually made on a dry weight basis. For seeds, it is more useful to the buyer to know the moisture content on a fresh weight basis; if he is purchasing a 100 kg lot of seeds at a moisture content of 20%, then he knows that 20 kg of the net weight is water. For a moisture content of 20% expressed on a dry weight basis, the weight of water in 100 kg of seeds would be approximately 16.5 kg.

Seeds contain other materials besides water so the methods used for measuring moisture content must not interfere with or change these other constituents. When heating seeds in a drying oven, for example, volatile compounds within the seeds may also evaporate causing a weight loss.

Sample shipment

To ensure that the sample sent for moisture-content testing represents the lot from which it was taken, it must be placed in an air-tight, moisture-proof container immediately on removal from the lot. As much air as possible must be removed from the container; a sealable plastic bag works well for this purpose. The sample must remain in the container until it is tested in the laboratory.

Beginning the test

A moisture content test must begin as soon as possible after the sample reaches the laboratory. In performing the test, the analyst has to make sure that the sample is exposed to the laboratory atmosphere as little as possible, especially when air-humidity is high.

Oven-drying method

The most commonly used method is to dry the seeds in an oven for a set period of time. The best type of oven to use is a forced-draught type, which provides a more uniform temperature when many samples are being dried. The seeds are dried in containers on which covers can be placed at the end of the drying period. The containers (and seeds) are then cooled in a dessicator until they reach room temperature.

Desiccator

The use of a desiccator is important. Dried seeds, on removal from a hot oven, will absorb moisture from the air very rapidly. This can be seen by putting an uncovered sample straight from the oven onto the balance and watching the gain in weight. On removal from the oven, therefore, a cover must be placed on each container which is then rapidly transferred to a dessicator to cool. Even when cool, weighing should be done quickly because the dry seeds still have a strong affinity for moisture.

Procedure

The empty container and its cover are weighed; covers and containers should have matching numbers in case they become separated at some stage. Weights are recorded to three decimal places.

In very damp weather, it is recommended that the containers and covers should be dried, and cooled in a dessicator, before they are used.

The submitted sample must be thoroughly mixed, then at least two working samples of seeds of 4-5 g each are independently withdrawn and placed in the containers. The containers, and covers, are reweighed and placed (with cover beneath the container) in an oven preheated to 103°C. Withdrawing the samples and placing them in the oven must be done very quickly to reduce to a minimum the exposure of the samples to ambient air. The remainder of the submitted sample must be returned to the container in which it was received, and resealed.

The seeds are maintained at 103 ± 2°C for 17 ± 1 hours; the drying period begins when the oven (which will have cooled down while the samples were being placed in it) has returned to the required temperature. At the end of the drying period, covers are put on their respective containers, then placed in a dessicator to cool for at least 30-45 minutes. When cool, the container with its cover and contents are again weighed.

Calculations

Seed moisture content as a percentage by weight is calculated from the following formula:

$$\text{Moisture content } \% = (M_2 - M_3) \cdot \frac{100}{(M_2 - M_1)}$$

where M_1 = weight of the empty container and cover,
M_2 = weight of container, cover and seeds before drying, and
M_3 = weight of container, cover and seeds after drying.

Thus, the difference $M_2 - M_1$ is the weight of the seeds before drying, that is, the "fresh" weight.

The average of the two (or more) determinations is calculated. The difference between the determinations should not exceed a tolerance limit which varies with seed size and moisture content (before drying). The tolerance limits are as follows:

Seed class	Seed size (no./kg)	Initial m.c. %	Tolerance %
Small	>5000	<12	0.3
Small	>5000	>12	0.5
Large	<5000	<12	0.4
Large	<5000	12-25	0.8
Large	<5000	>25	2.5

If the difference between the highest and lowest determined values exceeds the appropriate tolerance limit (0.3% or 0.5% for the small seeds you are using) the entire test should be repeated.

If the test result is within tolerance, the moisture content is reported to the nearest 0.1%.

EXERCISE 1 **STATION NO.:**_____

1. You have been given a sample of a seedlot for a moisture content test.

2. Make sure that a balance is available. Working in pairs, as quickly as possible divide the material into two equal portions, place them in the two containers provided. Put the covers on and weigh them. (To save time, the empty containers have already been weighed and you will be given these weights.) Record your data in the appropriate space below.

3. Place your samples in the oven after removing the covers and placing them underneath the containers.

4. The oven will be maintained overnight at 103 ± 2°C.

5. When you return, remove the containers from the oven, placing the covers on them as you do so, and put your samples into a dessicator. Use tongs or an insulated glove to handle the hot samples.

6. Not less than 30-60 minutes later, remove your samples from the dessicator and reweigh them. Record your data below.

7. Calculate the moisture content for each sample, and obtain the average. Compare the spread of the values with the tolerance limits given above, and record if the result is within tolerance.

(You will not be asked to repeat the test if the tolerance is exceeded.)

Species:_____

		Weight of container plus seeds			
Container number	Empty weight (M_1)	Before drying (M_2)	After drying (M_3)	M_2-M_1	M_2-M_3

Sample 1:

$$\text{Moisture content \%} = (M_2 - M_3) \cdot \frac{100}{(M_2 - M_1)} \quad = \_\_\_\_\_$$

Sample 2:

$$\text{Moisture content \%} = (M_2 - M_3) \cdot \frac{100}{(M_2 - M_1)} \quad = \_\_\_\_\_$$

Average moisture content % = _____

This is/is not within tolerance.

EXERCISE 2 **STATION NO.:**_____

1. Repeat the exercise using a different seedlot. The two sets of samples (for exercise 1 and exercise 2) can be prepared one after the other and placed in the oven at the same time.

2. Record your data below and calculate the moisture content.

Species:_____

		Weight of container plus seeds			
Container number	Empty weight (M_1)	Before drying (M_2)	After drying (M_3)	M_2-M_1	M_2-M_3

Sample 1:

Moisture content % $= (M_2 - M_3) \cdot \dfrac{100}{(M_2 - M_1)}$ = _____

Sample 2:

Moisture content % $= (M_2 - M_3) \cdot \dfrac{100}{(M_2 - M_1)}$ = _____

Average moisture content % = _____

This is/is not within tolerance.

Questions:

1. Within each seedlot, was there much variation in moisture content between the two replications? Was this variation acceptable (see tolerance table in text, page 48)?

2. How did the two seedlots differ in moisture content?

3. Would you purchase (for use in the nursery) both of these seedlots now that you know their moisture contents? Give your reasons.

4. In what way does sampling for a moisture content test differ from that for other tests?

Quick tests for seed viability

Introduction

The ability of a seedlot to produce plants is best determined by means of a growth test, the germination test. When this is not possible because of time constraints or other reasons, a quick estimate of seed viability must be made.

Three types of so-called "quick tests" are commonly used on tree seeds: the x-ray test, hydrogen peroxide test and tetrazolium test. No one test is the best in all situations, and each has its own advantages and disadvantages.

Objective

The objective of a quick test is to provide a rapid estimate of the viability of the seedlot, particularly where the seeds are dormant and a long germination test must be avoided. Rapid estimates can be valuable in circumstances where facilities do not permit a standard germination test to be conducted.

Procedure

a) The x-ray test utilizes a radiograph made in an x-ray unit that produces "soft" radiation, that is, at low voltages. Radiographic images resemble photographic negatives: those parts of the seeds that absorb more x-rays, preventing the radiation from reaching the film, show as lighter areas on the film, while those stuctures absorbing less radiation appear darker. Thus the x-rays penetrate the seed tissues according to their thickness or density. Embryos, if present, can be distinguished from the endosperm (or megagametophytic tissue) and the seed coat. The relative development of the major structures of the seeds can be evaluated permitting an estimate of the percentage of seeds that are likely to germinate.

Seeds are placed on top of a sheet of radiographic film and exposed to a predetermined amount of x-ray radiation. The film is processed and the images of the seeds are evaluated for possible germinability according to criteria for embryo and endosperm development, insect presence or physical damage. To ensure accuracy in interpreting radiographic images, x-ray tests should be compared periodically with standard germination results on the same seedlot.

b) In the hydrogen peroxide test seeds are immersed in a weak solution of hydrogen peroxide, and the embryos of germinable seeds elongate beyond their original length within a few days. It is believed that the peroxide, a powerful oxidizing agent, enhances elongation by stimulating respiration. The percentage of seeds showing a predetermined amount of elongation in a given time period provides an estimate of the seeds that are likely to germinate.

The micropylar end of the seeds is clipped off and the seeds are soaked in a dilute solution of hydrogen peroxide for 1 week, allowing embryo elongation. To ensure the accuracy of hydrogen peroxide tests, results should be compared periodically with standard germination results on the same seedlot.

c) The tetrazolium test relies on the ability of respiring tissues, which contain metabolic enzymes, to reduce a colourless solution of tetrazolium chloride (or bromide) to a red, stable formazan compound. The formazan forms as a red stain within living tissues, while non-respiring dead or damaged areas, which lack enzyme activity, remain uncoloured. The intensity of the stain and its location within the embryo and endosperm are used to evaluate the proportion of the seeds that are likely to germinate.

Seeds are placed in a solution of triphenyl tetrazolium chloride (or bromide) for several hours. A red stain forms in tissues that are actively respiring; the location of the stained area, as well as the intensity of staining, is critical in determining whether individual seeds are viable. To ensure the accuracy of tetrazolium tests, results should be compared periodically with standard germination results on the same seedlot.

All three tests require only a few days for completion, far less time than required for a germination test. For this reason they are commonly referred to as "quick tests". However, when the number of quick tests completed in one

month is compared with the number of germination results obtained in the same period, it becomes apparent that quick tests are more time consuming and costly.

Detailed descriptions of test methods are contained in "Quick tests for tree seed viability", British Columbia Ministry of Forests Land Management Report No. 18.

X-RAY TEST

EXERCISE 1 - Interpretation of an x-ray image

1. You have been given a number of seeds and a strip of x-ray film with which to observe the morphological structure of seeds.

2. Dissect a filled seed by removing the seedcoat (testa), then splitting the endosperm (megagametophyte) so that the embryo can be removed. Try to perform this without damaging the embryo.

3. Draw a two-dimensional sketch in the space provided below of your dissected seed to show its anatomy. Make your sketch quite large. Identify the seedcoat, endosperm and embryo.

4. Slice one or more seeds in half longitudinally using a razor blade. Stop when you have one filled seed cut so that all seed tissues are clearly visible and have not been disturbed.

5. Draw a sketch below of what you see. Make your sketch quite large. Identify the seed coat, endosperm and embryo.

6. Examine the x-ray film strip using a magnifier.

7. Concentrating on one of the images of a filled seed, draw a sketch of what you see, and identify the main stuctures. Identify any empty seeds on your radiograph.

8. Compare your three sketches of filled seeds.

EXERCISE 2 - Determination of embryo development, empty seeds and insect-damaged seed

STATION NO.:_____

1. You have been given a piece of x-ray film showing the images of 25 seeds.

2. Determine which seeds are filled (F), empty (E) and insect-damaged (I). Mark an F, E or I in the top row of the corresponding squares lower on this page.

3. Study the table and illustrations in your "Quick test manual", Table 4 on page 31, and Figures 14-22 on pages 33-35. Then classify the seed images on the film. Mark the classification in the bottom row of squares.

4. Note the time when you begin and when you complete this exercise.

   Start: _____ End: _____ Elapsed time: _____

Seed number

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25

5. Summarize the classification data in the bottom row of squares using the following table.

Embryo/Endosperm class	Number of seeds
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
Total	25

6. Estimate viability of the seeds by evaluating the percentage in the "germinable" classes.

Viable seeds (classes 1, 3)	= _____	= _____	%
Marginally viable seeds (classes 2, 4, 5, 6)	= _____	= _____	%
Non-viable seeds (classes 7, 8, 9, 10)	= _____	= _____	%
Total	= 25	= 100.0%	

Viability percentage = Total for classes 1-6 = _____ %

EXERCISE 3 - Determination of fresh, viable seeds at the end of the germination test

STATION NO.:_____

1. Examine the x-ray film strip which shows the images of ungerminated seeds at the end of a germination test. These seeds were dried 1-2 hours before being x-rayed.

2. Fresh, viable seeds show a high and homogenous absorption of the x-rays over the whole image on the radiograph. Dead seeds allow some radiation to reach the film, causing varying degrees of film blackening; that is, you can see some of the details of the internal structures.

3. Using these criteria, indicate fresh seeds (F) and dead seeds (D) in the corresponding squares below. Use the upper row of squares for one set of seeds, and the bottom row for the other set of seeds.

4. Note the time when you begin and when you complete this exercise.

   Start: _____ End: _____ Elapsed time: _____

Seed number

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25

4. Summarize your data.

   First set: Species _____

   Number of fresh seeds _____ = _____%

   Number of dead seeds _____ = _____%

   Total = 25 = 100.0%

   Second set: Species _____

   Number of fresh seeds _____ = _____%

   Number of dead seeds _____ = _____%

   Total = 25 = 100.0%

GERMINATION TEST

EXERCISE 4 - Germination evaluation of a seed sample

STATION NO.:_____

1. Using the information you learned from the germination test exercises, count the number of germinants (normal and abnormal) in the sample given to you.
2. Summarize the number of normal and abnormal germinants; ungerminated seeds need not be examined.
3. Calculate the germination percentage of the sample.
4. Note the time when you begin and when you complete this exercise.

   Start: _____ End: _____ Elapsed time: _____

Number of normal germinants	_____	= _____	%
Number of abnormal germinants	_____	= _____	%
Number ungerminated seeds	_____	= _____	%
Total	= 25	=	100.0%

HYDROGEN PEROXIDE TEST

EXERCISE 5 - Hydrogen peroxide evaluation of a seed sample

STATION NO.:_____

The hydrogen peroxide (H_2O_2) test is performed by clipping the seed coat at the radicle end, and incubating the seeds in 1% H_2O_2 for 1 week. Protruding embryos longer than 1 mm are then counted. A detailed description of the H_2O_2 test is given in the "Quick test manual" pages 3-5.

1. You have been given a sample of seeds that has been incubated in hydrogen peroxide. The first stage of the test (clipping at the radicle end) was carried out for you 1 week ago.

2. Early in the workshop you will be asked to count the number of "evident" seeds and to renew the H_2O_2 solution. (See the "Quick test manual" page 5.) You may find that some embryos have separated from the seed coats. Remove free embryos and empty seeds from the solution. Embryos longer than the seed coat are classified as "evident". Embryos that have not elongated are discarded, and are not included in the tally on which germination percentage is based. (They should be entered in the table below, however.) Thus, the number seeds in the final tally may be less than at the start. Record your data in the appropriate spaces below.

3. Note the time when you begin and when you complete this first part of the exercise.

4. At the completion of the test, count the number of "evident" and "slight" seeds. (See Table 1, page 5 of the manual.) Record your data in the appropriate spaces below.

5. Note the time when you begin and when you complete this second part of the exercise.

	----------Time (minutes)----------			----------Number of embryos----------		
Count	Start	End	Elapsed	Evident	Slight	Discarded
First						
Second						
Total						

6. Summarize your data for the two counts.

Total elapsed time = _____

Germination percentage based on "evident" embryos = _____ %

Germination percentage based on "evident" + "slight" embryos = _____ %

Germination percentage based on "evident" + ("slight"/2) = _____ %

TETRAZOLIUM TEST

EXERCISE 6 - Tetrazolium evaluation of a seed sample

STATION NO.:_____

The tetrazolium (TZ) test is based on defining live and dead areas of the embryo and endosperm by differential, topographic staining. A detailed description of the tetrazolium test is given in the "Quick test manual", pages 13-20. Figures 6-11, pages 18-19, provide examples of different staining patterns.

1. Early in the workshop you will be asked to place this sample of seeds in water, then cut them and place them in a tetrazolium solution.

2. Note the time you begin and when you complete this first part of the exercise in the upper table on this page.

3. At the completion of the test, remove the seeds from the TZ solution, and rinse them at least twice in water.

4. Using the criteria in Table 2 (page 16) of the "Quick test manual", classify the seeds as germinable or non-germinable, again entering your data in the appropriate spaces in the lower table on this page.

5. Note the time when you begin and when you complete this second part of the exercise.

Test stage	Time		
	Start	End	Elapsed
Soak in water			
Cutting/into TZ			
Rinse/classify			

Staining class	Number of seeds
1	
2	
3	
4	
5	
Total	25

6. Summarize your data.

Number of germinable seeds _____ = _____ %

Number of possibly germinable seeds _____ = _____ %

Number of non-germinable seeds _____ = _____ %

Total = 25 = 100%

7. Based on your results, estimate the germination percentage of the seeds.

Germination percentage = _____ %

EXERCISE 7 - X-ray evaluation of a seed sample.

STATION NO.:_____

1. Using the experience gained in the previous exercises, evaluate the seeds shown in the accompanying radiographs.

2. Record the filled (F), empty (E) and insect-damaged (I) seeds in the top row of squares lower down this page.

3. Determine the embryo/endosperm classes and record in the bottom row of squares. Refer to exercise 2.

4. Note the time when you begin and when you complete this exercise.

   Start: _____ End: _____ Elapsed time: _____

Seed number

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25

5. Summarize the classification data in the bottom row of squares using the following table.

Embryo/Endosperm class	Number of seeds
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
Total	25

(Continued on next page)

STATION NO.:_____

5. Estimate the germination percentage of the seeds by evaluating the number of seeds in the "germinable" classes.

Number of germinable seeds (classes 1, 3)	_____ =	_____ %
Number of possibly germinable seeds (classes 2, 4, 5, 6)	_____ =	_____ %
Number of non-germinable seeds (classes 7, 8, 9, 10)	_____ =	_____ %
Total	= 25 =	100.0%

Germination percentage = _____ %

Questions:

1. Under what circumstances would you choose to perform a quick test for tree seed viability?
2. Under what circumstances would you choose to perform i) an x-ray test, or ii) a hydrogen peroxide test, or iii) a TZ test?
3. What are the advantages and disadvantages of each type of quick test?
4. Do quick tests give good estimates of viability?
5. How does the variation in quick tests compare with the variation obtained in standard germination tests?
6. Should quick tests be calibrated against germination tests? Why?
7. How confident are you about quick test results?

Interpretation and application of seed test results

The seed analyst provides the nursery grower with all the information possible about a seedlot, but the analyst cannot prescribe what action the grower must take. It is up to the grower to interpret and apply the information given by the analyst.

The grower must realize that laboratory germination is almost always higher than germination in the nursery, although modern advances in nursery technology have narrowed the gap, or at least made the gap consistent and more predictable. This gap naturally results from the use of favourable, usually ideal, environmental conditions in the laboratory germination test, as opposed to the more stressful field and nursery conditions. The difference between laboratory and nursery germination typically is less for rapidly-germinating seeds of high quality, but the gap may increase as seed quality decreases. Occasionally, nursery germination may exceed laboratory results, indicating that the laboratory test probably requires improvement.

Not only are nursery environments more stressful, but they usually vary from one nursery operation to another, so the relationship with laboratory germination may change, from 95%, 75% or as low as 50% - or lower - of what was obtained in the laboratory. Each grower must be aware of this difference under his/her specific growing conditions and, through experience, he/she must develop a nursery-germination correction factor.

For each seedlot, the grower must determine the proportion of viable seeds sown that is likely to produce plantable seedlings at the time of lifting. This factor, commonly called the *tree percent*, is based not only on expected nursery germination, but on seedling survival and culling rates.

Many seed producers combine purity and germination results to obtain the *PLS*, or pure live seeds value, which is obtained by multiplying purity percentage by germination percentage, then dividing by 100. The resulting value is the percentage of the seedlot by weight that will germinate. *PLS* is useful in seed sales for determining the cost per pound (or kilogram) of good seeds in the lot, as well as for calculating sowing rates.

For the grower, the most important application of seed testing results is the calculation of sowing rate, which is the amount of seeds needed to sow a specified area of seedbed. Once the expected *tree percent*, the desired seedling density in the seedbed, and the laboratory test results are known, many growers calculate the sowing rate using the following formula:

$$W = \frac{A \times d}{n \times p \times g \times t}$$

where

- W = Weight (in pounds, grams, or kilograms) of seeds necessary
- A = Area of seedbed (in square feet or square metres)
- d = Desired final seedling density (per square foot or square metre)
- n = Number of seeds per pound, per gram, or per kilogram, determined at the time of sowing
- p = Purity percentage divided by 100
- g = Germination percentage divided by 100
- t = Expected tree percent divided by 100

As already mentioned, $(p \times g) \div 100$ is the *PLS* value, which can be substituted for p and g in the formula.

Example

Suppose:

(i) the area of seedbed (A) to be sown is 37 m^2 (approximately 400 sq. feet

(ii) there are 17,500 seeds per kg (8000 seeds per lb.) (n)*

(iii) the seedlot purity (p) is 96%

(iv) the germination capacity (g) is 88%

(v) the final seedling density (d) is to be 215 per m^2 (approximately 20 per sq.foot)

(vi) the expected *tree percent* (t) is 70

Then $$W = \frac{A \times d}{n \times p \times g \times t} = \frac{37 \times 215}{175000 \times (0.96) \times (0.88) \times (0.70)} = \frac{7955}{10350}$$

= 0.77 kg of seeds (approximately 1.7.lbs)

** The number of seeds/lb. must be based on the seed moisture content at the time of sowing. This number (seeds/lb.) will be much lower for stratified seeds, or imbibed seeds, than for dry seeds. Thus the grower must determine seeds/lb. when the seeds are ready for sowing, and not use the 1000-seed weight result. Without this on-the-spot determination, the grower likely will sow far fewer seeds than required.*

Seed-test results may have other applications. Low quality seeds, which may be also low in vigour, that is they will germinate only under a narrow range of environmental conditions, also may have poor storage potential, and are probably best sown (or sold) immediately before they deteriorate further. Similar action may be advisable if laboratory-test results indicate a high level of seed processing-damage. Low germination may be due to higher than normal dormancy, so longer stratification may be needed. Conversely, high quality seedlots usually have a good storage life, are of high vigour, meaning that they germinate well over a broad range of environmental conditions, so they can be maintained for future seedling stock production.

Workshop evaluation

1. What is your previous experience with seed testing?
   None Some Considerable

2. Was the information presented in this workshop useful?

3. What aspects did you find most useful?

4. Rate the following as either: (1) poor, (2) acceptable, (3) very good.

   a. organization of topics, etc.
   b. meeting facilities
   c. audio-visual aids
   d. laboratory equipment
   e. laboratory exercises
   f. handouts
   g. instructors and other speakers:

5. Rate the following as either (1) useful, (2) not useful, or (3) informative but not essential.

   a. sampling
   b. purity
   c. germination
   d. seed weight
   e. moisture content
   f. TZ
   g. X-ray
   h. hydrogen peroxide

6. Was the group appropriate for the type of workshop?

7. Was the pace too fast, too slow, or just right?

8. What additional information would you like to see included?

9. Would you be interested in a refresher course in the future? If so, at what interval?

10. How could the workshop be improved?

Answer to question 3, purity exercises

Assume that the seeds in both lots are identical in size, that is, the 1000-seed weight is the same for both.

Seedlot A: Germination 85%
Purity 92%
Moisture Content 9%
Price $250/kg

Suppose there were 10 000 seeds per kg:

The lot is 92% pure, so in each kg there would be only 9200 seeds.
Since 85% can be expected to germinate, each kg could be expected to yield 7820 seedlings:

i.e. 10 000 x .92 x .85 = 7820

At $250/kg, the seed cost/seedling = $250/7820 = $0.03196 (3.2 cents).

Thus, to raise 500 000 seedlings the seed cost would be $15,980

Seedlot B: Germination 78%
Purity 99%
Moisture Content 8%
Price $230/kg

The seeds are exactly the same size as in seedlot A. However, there is a difference of 1% in moisture content. This means that in each kg there would be 100 more seeds, i.e. 10 100 seeds per kg.

The lot is 99% pure, so in each kg there would be 9999 seeds. Since 78% can be expected to germinate, each kg would be expected to yield 7799 seedlings:

i.e. 10 100 x .99 x .78 = 7799

At $230/kg, the seed cost/seedling = $230/7799 = $0.02949 (2.9 cents)

Thus, to raise 500 000 seedlings the seed cost would be $14,745

Based on this information, it would appear that Seedlot B might be the better buy since there would be a savings of more than $1200 in seed costs. This difference is due mostly to the higher purity percentage of the lot, as well as the slightly lower moisture content.