Evaluating Quality

I. Sampling

A. Introduction

Sampling is the process of taking a small part or quantity of something for testing or analysis; it is the first step in seed testing. In sampling, it is essential to obtain: (1) a sample of proper size and (2) a sample representative of the main seedlot. The results of the laboratory tests can only show the quality and characteristics of the sample submitted for the analysis; therefore, the validity of test results for a large seedlot is determined by the success of obtaining a representative sample. Sampling seedlots for quality evaluation must be done systematically, using appropriate techniques, tools, and procedures, to ensure that the seed sample represents the entire lot.

B. Objectives

1. Quantify a seedlot according to accepted standards.
2. Determine sampling intensity according to size and characteristics of the seedlot.
3. Learn appropriate sampling instruments and techniques according to recognized standards.

C. Key Points

The following points are essential in seed sampling:

1. Laboratories can only measure the properties of the sample; the sampler must ensure that the sample truly represents the seedlot.
2. Submitted samples should contain at least 2,500 seeds (except for very large seeds of certain species).
3. Drawing the sample must be completely random.
4. Proper packaging and labeling of the sample are essential.

D. Definition of Terms

Relevant terms are defined as follows:

1. **Lot—a** specified, physically identifiable quantity of seeds.
2. **Primary sample—a** small quantity of seeds taken from one point in a seedlot
3. **Composite sample—formed** by combining and mixing all the primary samples taken from a seedlot
4. **Submitted sample — the** sample submitted to the testing laboratory
5. **Working sample—a** subsample taken from the submitted sample in the laboratory
6. **Subsample** — a portion of a sample obtained by reducing the sample by recognized methods (table 13).

Table 13. — *Weights of lots and samples for shrubs and trees (ISTA 1985)*

Species	Maximum weight of seedlot	Submitted sample	Working sample for purity analysis
	Kilograms	*Grams*	*Grams*
Acacia spp.	1,000	70	35
Ailanthus altissima	1,000	160	80
Alnus rubra	1,000	15	2
Castanea sativa	5,000	500 seeds	500 seeds
Cedrela spp.	1,000	80	40
Eucalyptus camaldulensis	1,000	15	5
E. globulus	1,000	60	20
E. tereticornis	1,000	15	5
Morus spp.	1,000	20	5
Pinus halepensis	1,000	100	50
P wallichiana	1,000	250	125
Quercus spp.	5,000	500 seeds	500 seeds
Robinia pseudoacacia	1,000	100	50

E. Sampling Intensity

A sample is obtained by selecting small portions at random from various positions in a seedlot and combining them.

1. **Calculating primary samples —** Each composite sample must be made up of at least five primary samples.
2. **Seedlot size—For** international trade in tree seeds, a maximum size of a seedlot for most species has been set at 1,000 kg ± 5 percent (table 13).

F. Sampling Procedures

There are three common sampling tools or techniques:

1. **Triers** are used for free-flowing seeds. The steps are:
   a. Close the gates before inserting the trier into the drum.
   b. Insert the trier into the drum.
   c. Open the gates.
   d. Close the gates.
   e. Remove the trier.
   f. Dump the seeds.
2. **Soil dividers** are used primarily for small lots. The steps are:
   a. Pour the seeds through the divider several times for mixing.
   b. Divide the sample into halves, quarters, etc.
3. **Extended hand method** is used for chaffy, winged, or other nonflowing seeds. The steps are:
   a. Extend the fingers, and insert the hand straight into the seeds.
   b. Close the hand, and withdraw a primary sample.

G. Preparation of the Sample

1. **Composite sample —All** primary samples are combined and mixed (table 13).
2. **Working sample —** The submitted sample is reduced to a working sample by:
   a. Mechanical divider method
   b. Random cups method
   c. Modified halving method
   d. Spoon method
   e. Manual halving method
3. **Extra seeds—** The remainder of the submitted sample should be stored to permit retesting if necessary. International Seed Testing Association (1985) recommends storing for 1 year.

H. Sources

For additional information, see Association of Official Seed Analysts 1988, Edwards 1987, International Seed Testing Association 1985.

II. Moisture Content

A. Introduction

The first measurements taken in seed testing are moisture, purity, and weight. All of these measurements are important, but moisture is the most critical one. Seed moisture levels can influence or indicate seed maturity, longevity in storage, and the amount of pretreatment needed for rapid germination.

B. Objectives

1. Learn the principles of official seed testing for moisture.
2. Apply these principles in practical exercises.

C. Key Points

The following points are essential to testing for moisture content:

1. Official testing procedures are prescribed in detail.
2. Many tests may be unofficial, and different methods may be used, but accuracy and precision are still essential.
3. Large recalcitrant seeds present special problems that official testing rules have not yet adequately addressed.

D. Definition of Terms

Relevant terms are defined as follows:

1. **Sample, submitted—** the sample of seeds submitted to a seed-testing station; it should be twice the size of the working sample.
2. **Sample, working—** a reduced seed sample taken from the submitted sample in the laboratory
3. **Seedlot** — a specified quantity of seeds of reasonably uniform quality

E. Moisture Measurements

1. **Importance**
   a. Is the most important factor in viability retention.
   b. Controls insect and disease activity (table 5).
   c. Affects the relationship of weight to number of seeds.
2. **Frequency —Moisture** is measured:
   a. After extracting and cleaning.
   b. When seeds are placed in storage.
   c. Periodically during storage.
   d. When seedlots are shipped.
3. **Procedures—Accurate** results are ensured by:
   a. Using the submitted sample.
   b. Measuring immediately on receipt.
   c. Expressing results as a percentage of fresh weight (wet weight), not dry weight.
4. **Methods — Moisture** content can be measured by four methods:
   a. Ovendrying method—Critical points are:
      (1) Heat samples for 17 ± 1 hours at 103 ±2 °C.
      (2) Use forced-draft ovens.
      (3) Place samples in glass or metal containers.
      (4) Leave space between cans in the oven.
      (5) Cool the samples in desiccators.
      (6) Keep ambient humidity less than 70 percent in the laboratory if possible.
      (7) Weigh to the nearest milligram.
      (8) Grind or cut large seeds or seeds of high moisture content.
      (9) Predry if seed moisture exceeds 17 percent in seeds that must be ground, or 30 percent in other species.
      (10) Check tolerance on results. Moisture tolerances for tree seeds are more liberal than those for agricultural seeds (table 14).

Table 14. — *Tolerance levels for differences between two determinations of moisture content of tree and shrub seeds (ISTA 1985)*

Seed size class	Seeds per kilogram	Initial moisture	Tolerance
	Number	*Percent*	
Small seeds	>5,000	<12	0.3
Small seeds	>5,000	>12	0.5
Large seeds	<5,000	<12	0.4
Large seeds	<5,000	12-25	0.8
Large seeds	<5,000	>25	2.5

b. Electric meters:
   (1) Are not allowed for official ISTA tests but are very useful.
   (2) Are based on electrical resistance or capacitance and are accurate to within 1 percent on free-flowing seeds.
   (3) Require construction of calibration charts.
   (4) Are available in various models:
      (a) Motomco — based on capacitance and very accurate
      (b) Radson (Dole or Seedburo) — a reliable model in the United States
      (c) Dickey-John or Insto — based on capacitance
      (d) Super-Beha — widely used in Europe

c. Infrared instruments are small, infrared ovens with built-in balances, which use a gravimetric method based on drying time.

d. Laboratory methods for research:
   (1) Karl Fischer method
   (2) Toluene distillation
   (3) Nuclear magnetic resonance (nondestructive)
   (4) Infrared spectroscopy

F. Summary— See table 15.

G. Sources
For additional information, see Bonner 1981b; International Seed Testing Association 1985, sections 9, 9A; Willan 1985, p. 227-230.

III. Purity and Weight

A. Introduction
After moisture content has been determined, the submitted sample is ready for purity and weight determinations. These determinations are a vital part of official seed testing and practical seed use, with legal ramifications in both domestic and international seed trade.

Table 15. — *Suggested test procedures for tree seed moisture (Bonner 1981b)*

Seed size class	Accurate measurement or ISTA official test	Rapid estimate
Small seeds, low oil content (e.g., *Platanus, Robinia)*	Oven: 103 ± 2 °C for 17 ± 1 hours. Sample: 4 to 5 g	Electric meter Sample: 80 to 200 g, depending on type
Small seeds, high oil content (e.g., *Abies, Pinus, Tsuga, Zanthoxylum)*	Oven: 103 ± 2 °C for 17 ± 1 hours. Sample: 4 to 5 g or Toluene distillation	Electric meter Sample: 80 to 200 g, depending on type
Large seeds, low oil content, moisture <20% *(e.g., Nyssa)*	(1) Grind or equivalent (2) Oven: 103 ±2 °C for 17 ± *1* hours. Sample: 4 to 5 g or enough to equal weight of five seeds	Microwave drying Sample: 4 to 5 g or enough to equal weight of five seeds
Large seeds, low oil content, moisture >20%, (e.g., *Aesculus, Quercus)*	(1) Predry to <20% at 130 °C for 5 to 10 minutes (2) Grind or equivalent (3) Oven: 103 ±2 °C for 17 ± 1 hours. Sample: enough to equal weight of five seeds	Microwave drying Sample: enough to equal weight of five seeds
Large seeds, high oil content (e.g., *Carya, Fagus, Juglans)*	(1) Grind or equivalent (2) Oven: 103 ± 2 °C for 17 ± 1 hours. Sample: enough to equal weight of five seeds or Toluene distillation	Microwave drying Sample: enough to equal weight of five seeds

B. Objectives

1. Learn the principles of official seed testing for purity and weight.
2. Apply these principles in practical exercises.

C. Key Points

The following points are essential to determine seed purity and weight:

1. The line between true seeds and trash can be ambiguous for some tree seeds, especially those that are dewinged.
2. Patience and good eyesight are needed.
3. The smaller the seeds, the more difficult the purity test will be.

D. Definition of Terms

Relevant terms are defined as follows:

1. **Purity—proportion** of clean, intact seeds of the designated species in a seedlot, usually expressed as a percentage by weight
2. **Sample, submitted—the** sample of seeds submitted to a seed-testing station; it should be twice the size of the working sample
3. **Sample, working—a** reduced seed sample taken from the submitted sample in the laboratory on which some test of seed quality is made
4. **Seedlot** — a specified quantity of seeds of reasonably uniform quality; maximum lot size is 1,000 kg (5,000 kg for *Fagus* and larger seeds)

E. Purity

1. **Procedure—** The ISTA (1985) rules are followed for purity testing. The steps are:
   a. Reduce the submitted sample (after mixing) to the working sample by:
      (1) Mechanical dividers
      (2) Random cups
      (3) Modified halving
      (4) Spoon method
      (5) Manual halving (chaffy, winged, and large seeds)
   b. Divide the working sample into fractions of
      (1) Pure seeds
      (2) Other seeds
      (3) Inert matter
   c. Weigh and express each as a percentage of the total sample weight
2. **Pure seed component —** This component contains:
   a. Intact seed units of the desired species
   b. Pieces of seed units larger than one-half the original size, even if they are broken
3. **Tree seed specifics**
   a. Seeds of Leguminosae, Cupressaceae, Pinaceae, and Taxodiaceae with seedcoats entirely removed are inert matter.
   b. In *Abies, Larix, Libocedrus, Pinus elliottii,* P *echinata, P. rigida, P taeda,* and *Pseudotsuga,* wings or wing fragments are detached and removed and placed in the inert matter fraction. Other *Pinus* spp. retain wing fragments (see "a" above).
   c. For samaras, wings are not removed (e.g., *Acer, Fraxinus, Cedrela,* and *Swietenia).*
   d. For drupes, the fleshy coverings are not removed.
   e. In *Eucalyptus,* for species with small seeds, a simplified procedure is used; only other seeds and inert matter that is obviously of nonseed origin are removed.
   f. For Leguminosae, if any portion of the testa is present, it must be classified as pure seed.
   g. If species distinctions are impossible, only the genus name is given on the certificate.

F. Seed Weight

1. **Determination—The** ISTA (1985) rules are used to properly determine seed weight. Either the whole working sample or replicates from it are used.
   a. Working sample — Weigh the entire pure seed fraction.
   b. Replicates — Count and weigh 8 replicates of 100.
2. **Reporting results — Results** are reported in one of two ways:
   a. 1,000-seed weight
   b. Seeds per gram (or per kilogram, ounce, or pound)

G. Sources

For additional information, see International Seed Testing Association 1985, sect. 3, 3A, 10; Willan 1985, p. 198-202, 221.

IV. Germination Tests

A. Introduction

Good seed testing is the cornerstone of any seed program, no matter what kind of seeds: agricultural, forestry, agroforestry, or ornamental. The quality of the seeds used must be measured and described. Seed testing may have legal ramifications because of its connection to seed sales. For this reason, the International Seed Testing Association (ISTA) coordinates international efforts to standardize seed testing. The quality of seeds must be known to make efficient and effective use of them in reforestation or afforestation programs.

B. Objectives

1. Identify the international organizations that deal in tree seed testing and how they derive their prescriptions.
2. Learn the principles of germination testing and how they are applied in the laboratory for standard conditions.
3. Practice actual germination testing in the laboratory.
4. Learn proven techniques to analyze germination data and how these data can be expressed.
5. Learn the application of germination test results to practical nursery and field conditions.
6. Learn techniques for rapid estimates of seed quality when time and/or proper facilities are absent or limited.

C. Key Points

The following points are essential for conducting germination tests:

1. Laboratory germination tests are designed to provide the optimum conditions for germination and to determine the full germination potential of the seeds under these conditions.
2. The primary conditions to be considered are temperature, light, aeration, and moisture.
3. Rapid estimates of germination are just that - estimates; they are not as accurate as germination tests.
4. If more than 60 days are required for a germination test, analysts should use a rapid estimate for official testing.
5. Germination testing in the course of research may require different methods and equipment from official testing.
6. No matter how standardized the test prescriptions are, the judgment of the analyst must prevail in the laboratory.

D. Definition of Terms

Relevant terms in germination testing will be defined according to the glossary developed by the Seed Problems Project Group of the International Union of Forestry Research Organizations (IUFRO) (Bonner 1984a). These terms are defined as follows:

1. **Abnormal seedlings-in** seed testing, seedlings that do not possess all normal structures required for growth, nor show the capacity for continued development
2. **Filled seed-a** seed with all tissues essential for germination
3. **Germination -** resumption of active growth in an embryo, which results in its emergence from the seed and development of those structures essential to plant development
4. **Germination** capacity-proportion of a seed sample that has germinated normally in a specified test period, usually expressed as a percentage (synonym: germination percentage)
5. **Germination energy-proportion** of germination that has occurred up to the time of peak germination, the time of maximum germination rate, or some preselected point, usually 7 test days. (The critical time of measurement can be chosen by several means.)
6. **Germination percentage-** (see germination capacity)
7. **Hard seeds - seeds** that remain hard and ungerminated at the end of a prescribed test period because their impermeable seedcoats have prevented absorption of water
8. **Peak germination-the** specific time when rate of germination is highest. It may be derived in many ways (see germination energy).
9. **Pretreatment - any** kind of treatment applied to seeds to overcome dormancy and hasten germination
10. **Purity-proportion** of clean, intact seeds of the designated species in a seedlot, usually expressed as a percentage by weight
11. **Sample, submitted-the** sample of seeds submitted to a seed-testing station
12. **Sample, working-a** reduced seed sample taken from the submitted sample in the laboratory, on which some test of seed quality is made
13. **Seedlot -a** specified quantity of seeds of reasonably uniform quality
14. **Seed quality-a** general term that may refer to the purity, germination capacity, or vigor of a seedlot
15. **Sound seed -** a seed that contains in viable condition all tissues necessary for germination
16. **Tolerance - a** permitted deviation (plus or minus) from a standard. In seed testing, the permitted difference between or among replicated measurements beyond which the measurements must be repeated
17. **Vigor - seed** properties that determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions.

E. Quality Evaluation

For satisfactory evaluation of germination, the following principles are fundamental:

1. Sampling must be good; tests describe the sample only.
2. Testing at standard, optimum conditions ensures that:

a. Absolute maximum potential of the lot is determined.
b. Standard conditions can be duplicated by all laboratories for test comparison.

F. Methodology

Satisfactory germination testing depends on proper methods:

1. **Pure seed component** —Only the pure seed component is used in the test (4 replications of 100 seeds each).
2. **Environmental conditions —** Temperature, light, moisture, and medium must be carefully controlled.
   a. Temperature requirements differ according to species. International Seed Testing Association prescriptions should be followed.
   b. Light requirements are also spelled out in the ISTA rules.
   c. The germination medium must be nontoxic; it can be either natural or synthetic material.
      (1) Natural materials include soil, sand, peat, and other organic materials.
      (2) Synthetic materials include blotters, paper towels, cellulose wadding (Kimpako), filter paper, agar, and cloth.
3. **Moisture—Excessive** moisture is a common problem in many tests.
4. **Equipment —** Germination equipment must be dependable.
   a. Cabinet germinators
   b. Jacobsen tables
   c. Walk-in rooms
   d. Small containers (petri dishes and plastic boxes)
5. **Test Procedures**
   a. Pretreatment
      (1) Micro-organism/pathogen treatment.
      (2) Overcoming dormancy (delayed germination)
         (a) stratification (prechill)
         (b) chemical treatment (nitrates, hydrogen peroxide, and growth regulators)
         (c) scarification for hard seeds
   b. Placement of samples
   c. Counting
      (1) Define "germinated seed"
      (2) Count frequency; weekly is the minimum
      (3) Recognize abnormal seedlings; common abnormalities are albino seedlings, stunted roots, negative geotropism, "endosperm" collars, and necrotic areas
   d. Length of test
   e. Determining condition of ungerminated seeds
6. **Tolerance and retesting**
   a. Review the concepts for official testing.
   b. Analysts should be aware of other reasons for a retest:
      (1) Too much dormancy; additional prechill needed.
      (2) Too much fungal infection; increase distance between seeds on blotter or test in sand or soil.
      (3) Normal/abnormal distinction unclear.
      (4) Evidence of human error.

G. Additional Testing Considerations
1. Thermogradient plates
2. Greenhouse or nursery bed tests
3. Testing by weight (e.g., *Eucalyptus* and *Betula)*
4. *Quercus* and other large seeds can be cut in half

H. Reporting Results
1. **Germination capacity**
2. **Rate of germination**
   a. Germination energy
   b. Mean germination time (MGT)
   c. Time for a certain proportion of germination to occur (e.g., number of days for 50 percent of the seeds to germinate)
   d. Germination Value (GV)
   e. Peak Value (PV)

I. Practical review

J. Sources

For additional information, see Bonner 1984a, 1984b; Czabator 1962; Edwards 1987; International Seed Testing Association 1985, sect. 5, 5A, 11; Willan 1985, p. 202-227.

V. Rapid Tests: Cutting, Vital Stains, Excised Embyro, and Hydrogen Peroxide

A. Introduction

The standard for judging seed quality is always a germination test under optimum conditions. Under certain circumstances, however, germination tests are not possible, and so-called "rapid tests" must be used to estimate seed quality. When performed properly, rapid tests can furnish valuable information to seed users, analysts, and managers.

B. Objectives
1. Learn the different types of rapid tests and how to perform them.
2. Recognize the limitations of each test and when it should be used.

3. Examine the interpretation of test results.

C. Key Points

The following points are essential to perform rapid tests:

1. The cutting test is the quickest and simplest and can be extremely useful with fresh seeds.
2. Tests with vital stains reveal more than just potential germination, but interpretation is subjective.
3. X-ray radiography is the most expensive, but not necessarily the best, of the rapid tests. It is very effective for some situations.
4. Leachate conductivity is a new and promising method.

D. Use of Rapid Tests

Rapid tests are used when one of the following conditions occurs:

1. **60-day rule of ISTA**—If a germination test requires more than 60 days to complete, then a rapid test should be used.
2. **Requested by user**
3. **Seed supply is limited**
4. **A quality check is needed during collection**
5. **There are other test objectives**

E. Sampling

The same sampling principles and precautions apply for rapid tests as for standard germination tests.

F. Test Methods

There are six rapid tests that have applications with tree seeds.

1. **Cutting**
   a. Technique: Seeds are cut in half lengthwise and all tissues are examined.
   b. Evaluation: Good seeds are firm, with good color.
   c. Advantages
      (1) Quickest and cheapest
      (2) Can be performed in the field
      (3) Is accurate on fresh seeds
   d. Disadvantages
      (1) Has size limitations
      (2) Produces poor results with stored seeds
      (3) Is a destructive test
2. **Vital stains**
   a. Technique: Embryo and storage tissues are exposed by cutting and then stained. Location and intensity of staining indicate viable or dead tissue.
   b. Stain options:
      (1) Tetrazolium chloride (TZ) (most widely used) stains live tissues red.
      (2) Indigo carmine stains dead tissues blue.
      (3) Selenium or tellurium salts.
   c. Evaluation (TZ only):
      (1) Sound tissue should stain carmine.
      (2) "Topographic stain" analysis is the most accurate, but it is the most difficult to standardize.
      (3) The ISTA (1985) prescribes TZ for certain dormant species.
   d. Advantages
      (1) Fast, stains can be read within 24 hours
      (2) Inexpensive
      (3) Equipment needs are simple
   e. Disadvantages
      (1) Labor-intensive
      (2) Difficult to obtain uniform penetration of the stain
      (3) Difficult to interpret the stain
      (4) Requires practice and experience
      (5) Destructive test
3. **Excised embryo**
   a. Technique: Seeds are cut open, and the embryos are incubated in dishes.
   b. Evaluation
      (1) Viable seeds are green and white, with some growth.
      (2) Nonviable seeds are dark or moldy, with no growth.
   c. Advantages
      (1) Simple equipment needs
      (2) Actual seed performance is tested
      (3) Easy to evaluate
   d. Disadvantages
      (1) Labor-intensive
      (2) Requires practice for proper excision
      (3) Slow (10 to 14 days)
      (4) Destructive test
4. **Hydrogen peroxide**
   a. Technique: Seedcoats are cut to expose the radicle, and the seeds are incubated in 1-percent hydrogen peroxide. Radicle growth is measured.
   b. Evaluation: Based on radicle growth.
   c. Advantages
      (1) Inexpensive test
      (2) Partially objective
      (3) Simple preparation
   d. Disadvantages
      (1) Not practical for very small seeds
      (2) Tested only on conifers
      (3) Destructive test
      (4) Slow (7 to 8 days)
5. **X-ray radiography**
   a. Technique: Intact seeds are exposed to soft x rays, and the images that are captured on film are examined.
   b. Evaluation: Evaluation is very subjective.

c. Advantages
(1) Fast
(2) Provides a permanent image
(3) Nondestructive
d. Disadvantages
(1) Equipment is expensive
(2) Extensive training is required
(3) Interpretation is subjective

6. **Leachate conductivity**
a. Technique: Seeds are leached in deionized water for 24 to 48 hours; electrical conductivity of the leachate is then measured.
b. Evaluation: Relationship of conductivity to germination must be established for each species.
c. Advantages
(1) Requires no expensive equipment
(2) Fast and simple
(3) Objective measurement
(4) Nondestructive
d. Disadvantages
(1) Indirect measurement of seed quality
(2) Unknown factors still cause trouble

G. Sources
For additional information, see International Seed Testing Association 1985, annex to chap. 6, app. B; Leadem 1984; Willan 1985, p. 221-226.

VI. Rapid Tests: X Rays and Leachate Conductivity

A. Introduction
Like other rapid tests, radiography offers a quick estimate of seed quality when there is no time for a complete germination test. The application of x-ray radiography in seed science is one of the few technologies that originated with tree seeds instead of agricultural seeds. It has not yet fulfilled its early promise, but there are many applications with seeds. Many rapid estimates of seed quality have major drawbacks: high cost, subjective interpretations, excessive time, etc. The leachate conductivity method offers a test that meets all requirements: low cost; fast, objective measurements; easy procedures; and nondestructive. Although relatively new, it shows great promise.

B. Objectives
1. Review x-ray theory, and see how x rays can be used in seed radiography.
2. Learn the principles of seed radiograph interpretation.
3. Examine the physiological basis for leachate testing.
4. Learn the leachate methodology.
5. Recognize the advantages and the disadvantages of both techniques.

C. Key Points
The following points are essential to an understanding of these two methods:
1. Many types of seed damage can be detected by x-ray testing.
2. Embryo development can be measured precisely, but exact correlations with germination are not possible.
3. The use of contrast agents can increase the amount of information obtained from radiographs; however, many of these agents kill the seeds.
4. Many special radiographic techniques are available, but most require equipment associated with medical x-ray technology.
5. As seeds deteriorate, cellular membranes are damaged, allowing the leaching of many substances from the seeds.
6. Many chemical groups can be detected, but electrolytic activity is the easiest to measure.
7. Good estimates of quality are possible with many species, but germination tests are still preferred as the standard measurement of seed quality.
8. The conductivity method is promising, but more research is needed.

D. X rays

1. **Theories**
a. X rays are electromagnetic energy of very short wavelengths. X rays penetrate materials that absorb or reflect light, and are themselves absorbed by the target object.
b. Radiographs are pictures of the object formed by the x rays that pass through the object and strike a photographic material.
(1) Radiograph quality is defined by contrast, density, and definition of the image.
(2) Quality is controlled by kilovoltage (kV), milliamperage (mA), exposure time, focus-film-distance (FFD), and object-film-distance (OFD).

2. **Methods**
a. Equipment: Several types of x-ray equipment are available commercially.
b. Film: Several film choices are available, including
(1) Conventional film

(2) Polaroid film
(3) Radiographic paper

c. Contrast agents: Contrast agents are used to increase density of certain seed tissue images on the radiograph.
(1) Aqueous agents are primarily solutions of heavy cation salts (e.g., barium chloride and silver nitrate).
(2) Vaporous agents: chloroform or other halogen derivatives of alkanes.

d. Safety is an important aspect of seed radiography.

3. **Special Techniques—Mainly** for research application, they include:
a. Stereoradiography
b. Tomography
c. Xeroradiography

4. **Applications in seed testing** — X rays were first used on seeds in Sweden in 1903.
a. The most effective uses are:
(1) Determining seed anatomy
(2) Determining insect damage
(3) Determining mechanical damage

b. X rays have limited usefulness in determining viability.

E. Leachate Conductivity

1. **Major points—As** seeds deteriorate, substances can be leached in proportion to the degree of deterioration. Sugars, amino acids, and electrolytes are just some of the materials that can be measured.

2. **Techniques —** Leachate conductivity can be measured in two ways:
a. Multiple-seed analyzers
(1) Advantages
(a) Fast
(b) Receives input from individual seeds
(c) Data are printed on paper tape
(d) Some models can calculate statistics
(2) Disadvantages
(a) High cost (US $6,500)
(b) Some equipment not reliable
(c) Influence on the conductivity/germination relationship unknown

b. Single probe techniques
(1) The ISTA handbook on vigor testing (Perry 1981) includes this method for peas.
(2) Advantages
(a) Fast
(b) Inexpensive equipment
(c) Completely objective
(d) Accuracy for some species within 10 percent of germination
(3) Disadvantage: Some factors have an unknown effect.

F. Sources
For additional information on x rays, see Vozzo 1978, 1988; Willan 1985, p. 224-226. For additional information on leachate conductivity, see Bonner 1991a, Perry 1981.

VII. Vigor Tests

A. Introduction
Standard germination tests do not adequately measure the ability of seeds to germinate and produce normal seedlings under field conditions because germination tests are conducted in the laboratory under optimum conditions. Such conditions are seldom encountered in the field, so germination and emergence may be much lower than in the laboratory. Therefore, a more sensitive measurement of seed quality has been sought by those concerned with the planting quality of a seedlot. This measurement of seed quality has been referred to as seed vigor. Seed vigor tests add supplemental information about the quality of seeds to information obtained through other tests.

B. Objectives
1. Learn the concept of seed vigor and realize how it can help the seed users.
2. Become familiar with the types of seed vigor tests and know which ones are most suitable for tree seeds.

C. Key Points
The following points are essential to an understanding of vigor tests:
1. Vigor is a seed quality that may or may not be indicated by a standard germination test.
2. Vigor is most important under adverse field conditions, and it can also indicate the storage potential of a seedlot.
3. Vigor tests usually involve either direct or indirect measurements.
4. For many tree seeds, rate of germination is the best expression of vigor.

D. Definition of Terms
1. **Vigor**
a. Association of Official Seed Analysts: "Those seed properties which determine the potential for rapid, uniform emergence, and development of normal seedlings under a wide range of field conditions" (AOSA 1983).
b. International Seed Testing Association: "The sum of the properties which determine the potential level of activity and performance of the seed or seedlot dur-

ing germination and seedling emergence" (Perry 1981).

c. International Union of Forestry Research Organizations: "Those seed properties which determine the potential for rapid, uniform emergence, and development of normal seedlings under a wide range of field conditions" (Bonner 1984a).

2. **Seed quality—** "A general term that may refer to the purity, germination capacity, or vigor of a seedlot" (Bonner 1984a).

E. Seed Vigor Concepts

1. **Physiological quality—** Seedlots vary tremendously in physiological quality. This is exemplified by the different rates of germination within a seedlot, the variation in the growth rates and sizes of seedlings produced, and the ability of some seeds to produce seedlings under adverse conditions while others do not. The physiological quality of seeds is commonly called seed vigor.

2. **Physiological maturity—** Seeds reach their maximum germination capacity and vigor during the maturation process at their maximum dry weight, or the "physiological maturity" stage. Once physiological maturity has been reached, deterioration begins and continues until the death of the seed. The process cannot be stopped, but the rate of deterioration can be controlled to some extent. Different seeds decline in vigor at different rates.

3. **Deterioration — Seed** vigor declines more rapidly than does the ability to germinate. The first sign of deterioration is a loss of vigor. Thus, a seed may germinate even though some of its physiological functions may have been impaired. The ability to produce seedlings under stress conditions and the growth and yield of plants may be affected as vigor declines. Vigor is thus a more encompassing measurement of seed quality than the standard germination test.

4. **Strategy—The** general strategy in determining seed vigor is to measure some aspect of the seed performance or condition that reflects the stage of deterioration or genetic deficiency. Developing a good test for this strategy is not easy. A practical seed vigor test should:
   a. Be reproducible
   b. Be easily interpreted
   c. Indicate field performance potential
   d. Take a reasonable length of time
   e. Not require expensive equipment
   f. Not require extensive training

F. Common Seed Vigor Tests

Vigor tests can be grouped into four categories:

1. **Seedling growth and evaluation**
   a. Seedling vigor classification
   b. Seedling growth rate
2. **Stress tests**
   a. Accelerated aging
   b. Cold test
   c. Cool germination test
   d. Osmotic stresses
   e. Methanol treatment
3. **Biochemical tests**
   a. Tetrazolium chloride (TZ) staining
   b. Adenosine triphosphate (ATP) activity
   c. Glutamic acid decarboxylase activity (GADA)
   d. Oxygen uptake (respiration)
   e. Leachate tests
      (1) Sugars
      (2) Amino acids
      (3) Electrolytes
4. **Germination data**
   a. Mathematical modeling of germination response
      (1) Normal distribution
      (2) Polynomial regressions for curve fitting
      (3) Logistic function
      (4) Probit transformation
      (5) Weibull function
   b. Germination rate
      (1) Early counts
      (2) Percentiles
      (3) Mean germination time (MGT)
      (4) Germination value (GV) and Peak value (PV)

G. Recommendations For Tree Seeds—The following tests have the most potential for tree seeds:

1. **Germination rate parameters**
2. **Seedling growth tests**
3. **Tetrazolium staining for large seeds**
4. **Accelerated aging**
5. **Leachate conductivity**

H. Sources

For additional information on vigor tests, see Association of Official Seed Analysts 1983; Blanche and others 1988; Bonner 1986b; Perry 1981; Willan 1985, chap. 9.