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: (1) to obtain a sample of proper size, and (2) to obtain 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 about 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 below:

1. **Lot—a** specified, physically identifiable quantity of seeds. A lot, or seedlot, may be small and in a single container, or large and in several containers.
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. The composite sample is usually much larger than required for seed testing and is reduced to a smaller sample for testing.
4. **Submitted sample — the** sample submitted to the testing laboratory. It must be at least the minimum specified size and may be comprised of either all or part of the composite sample.
5. **Working sample—a** subsample taken from the submitted sample in the laboratory on which one of the quality tests is made.
6. **Subsample** — a portion of a sample obtained by reducing the sample by recognized methods (tables 19, 20) [no equivalent for table 19 is included in Student Outline, table 20 is table 13 in Student Outline I.

E. Sampling Intensity

A sample is obtained by taking small portions at random from various positions in a seedlot and combining them. From this sample, other subsamples are obtained by one or more steps. At each step, thorough mixing is followed either by progressively subdividing or by selecting at random still smaller portions and then recombining.

Table 19. — *Guidelines for minimum working sample weights for tree seeds when both purity and germination are to be tested (adapted from Bonner 1974c) [no equivalent table in Student Outline]*

Seeds per pound	Minimum working sample size	Seeds per gram	Minimum working sample size
Number	*Ounce*	*Number*	*Grams*
<2,000*	16"	less than 5*	500'
2,000-2,500	14-18	5-7	300-400
2,500-3,000	11-16	7-10	200-300
3,000-3,500	10-13	10-15	140-240
3,500-4,000	8.5-11.5	15-20	100-170
4,000-4,500	7.5-10.0	20-25	85-125
4,500-5,000	6.5-9.0	25-30	70-100
5,000-5,500	6-8	30-35	60-90
5,500-6,000	5.5-7.5	35-40	54-75
6,000-7,000	5-7	40-50	42-65
7,000-8,000	4-6	50-60	36-54
8,000-9,000	3.75-5.50	60-70	30-46
9,000-10,000	3.25-4.75	70-80	27-40
10,000-15,000	2.25-4.25	80-90	24-35
15,000-20,000	1.75-3.00	90-100	22-32
20,000-25,000	1.50-2.25	100-125	17-28
25,000-30,000	1.25-2.00	125-150	15-23
30,000-40,000	0.8-1.5	150-175	13-20
40,000-50,000	0.75-1.25	175-200	11-17
50,000-65,000	0.5-1.0	200-250	9-15
65,000-80,000	0.40-0.75	250-300	8-12
80,000-100,000	0.3-0.6	300-350	6.5-10
100,000-150,000	0.25-0.50	350-400	5.5-8.5
150,000-200,000	0.2-0.4	400-500	4.5-7.5
200,000-300,000	0.1-2.5	500-750	3-6
>300,000	0.1	>750	3

*Purity analyses are rarely required for seeds of this size.
'Sample should contain at least 500 seeds.

Table 20. — *Weights of lots and samples for shrubs and trees (ISTA 1985) [table 13 in Student Outline/*

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
Ilnus 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

1. **Calculating primary samples —** Each composite sample must be made up of at least five primary samples. When more than one sample is taken from a drum, they are taken from well-separated points. For lots of one to six drums, each drum is sampled, and at least five primary samples are taken. For lots of more than six drums, five drums plus at least 10 percent of the number of drums in the lot are sampled. For example:

   Number of drums in the lot
   1 2 3 4 5 6 7 10 23 50
   Number of drums to sample
   1 2 3 4 5 6 6 6 7 10
   Total number of primary samples
   5 5 5 5 5 6 6 6 7 10

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 20) [table 13 in Student Outline]. This maximum is recommended for all domestic transactions also. If the amount of seeds exceeds this maximum, it should be divided into lots not larger than the maximum, and each portion should be given a separate lot identity.

F. Sampling Procedures

There are three common sampling tools or techniques: triers, soil dividers, and the extended hand method.

1. **Triers—Free-flowing** seeds can be most easily sampled using tools called "triers." A trier consists of a tube that fits inside an outer sleeve with a pointed end. The tube and the sleeve have slots in their walls, and when the slots in the tube line up with slots in the sleeve, seeds can flow into the cavity of the tube. A half turn of the tube closes the openings. Triers are available in various lengths and diameters and can be inserted into the seed containers either vertically or horizontally. If used vertically, the trier must have partitions dividing the tube into a number of compartments to obtain a representative sample. 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 and let the seeds fill the trier.
   d. Close the gates.
   e. Remove the trier.
   f. Dump the seeds out of the trier.
2. **Soil dividers—These** devices are made to mix and divide soil samples, but they work well on free-flowing seeds. Soil dividers are a good tool for small lots only. The steps are:
   a. Pour the seeds through the divider several times for mixing.
   b. Divide the sample into halves, quarters, etc., until the desired size is reached.
3. **Extended hand method—Requires** no special equipment. It is recommended for chaffy, winged, or other nonflowing seeds, but it will work on any 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 —** The composite sample is prepared by combining all primary samples and mixing. If it is small enough, it can be sent as the submitted sample. If it is too large, as is common, it can be reduced with a soil divider. See tables 19 [no equivalent in Student Outline] and 20 [table 13 in Student Outline] for determining sample weights.
2. **Working sample —** The submitted sample is reduced to a working sample by thoroughly mixing the submitted sample and then repeatedly halving the sample by one of the following methods:
   a. Mechanical divider method— This method is suitable for free-flowing seeds. An apparatus divides a sample into two approximately equal parts. The sample is reduced by repeated halving

until a working sample equal to or slightly more than the prescribed size is obtained. Some types of mechanical dividers are the conical (or Boerner), the centrifugal (or Garnet), and the soil divider type.

b. Random cups method—Small cups or containers, usually not more than eight, are randomly placed on a tray and the seeds of the submitted sample are poured evenly over the tray. Most seeds fall on the tray, but those that collect in the cups are combined in a larger container as the working sample. The process is repeated until the necessary quantity of seeds is collected.

c. Modified halving method—In this 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 on a tray, and the submitted sample poured evenly over it. The grid is then lifted with the seeds collected in the cells being used as the working sample. The process is repeated until the desired sample size is achieved. A 50- by 50-mm cell size is useful for conifer seeds.

d. Spoon method—After mixing, the submitted sample is poured evenly over a tray. Small portions of seeds are removed with a spoon and spatula from at least five random positions until the required amount is obtained. This method is used only for species with small seeds.

e. Manual halving — Chaffy, winged, or large seeds are spread on trays and divided in halves with the hand, fingers extended together.

3. **Disposition of the 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 seed moisture content:

I. Procedures are prescribed in detail for official testing.

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 (ISTA 1985) 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 on which some test of seed quality is made.

3. **Seedlot** — a specified quantity of seeds of reasonably uniform quality; the maximum lot size is 1,000 kg or 5,000 kg for *Fagus* and larger seeds.

E. Moisture Measurements

1. **Importance**
   a. Moisture content is the most important factor in viability retention.
   b. Insect and disease activity occurs at certain moisture levels (table 10) [table 5 in Student Outline].
   c. Moisture content influences the relationship of weight to number of seeds.
2. **Frequency**
   a. Moisture is measured after extracting and cleaning. The seeds may have been too moist during cleaning.
   b. Moisture is measured when seeds are stored. The correct moisture content must be reached, or more drying will be necessary.
   c. Periodic checks during storage reveal whether the container seals are good.
   d. Moisture is measured when seedlots are shipped. Seeds shipped moist will lose quality rapidly.
3. **Procedures — Moisture** content is measured by:

a. Using the submitted sample.
b. Measuring immediately on receipt. However, if the sample bag is not moisture proof, the measurement will be meaningless.
c. Expressing results as a percentage of fresh weight (wet weight), not dry weight. This is the international convention for seed moisture. If dry weight percentages are needed, nomograms are available for conversion.

4. Methods — Moisture content is measured by four methods:
a. Ovendrying. Critical points are:
(1) Heat samples for 17 ± 1 hours at 103 ± 2 °C, a good overnight schedule. This is the official ISTA method.
(2) A forced-draft oven, not a gravity convection oven, is used.
(3) Glass or metal containers with rounded sides and base and close-fitting tops are used.
(4) Space equal to one container's diameter is allowed between containers on the oven shelf. The tops are removed when the containers are placed in the oven.
(5) Desiccators are used to cool the samples for 30 to 45 minutes. The container covers are replaced before they are transferred to the desiccators.
(6) Ambient humidity should be less than 70 percent in the laboratory. If not, the samples should be cooled for an additional 30 minutes in the desiccator.
(7) All weights should be to the nearest milligram. The ideal balance is a top-loading, electronic instrument.
(8) The ISTA requires grinding for *Fagus, Quercus,* and all other large, recalcitrant seeds. At least 50 percent of the ground material must pass 4.00-mm mesh sieves. However, these seeds must be predried before grinding because of their moisture content (except perhaps *Fagus).*
(9) Predrying is required if moisture exceeds 17 percent in seeds that must be ground and 30 percent in other species. Predrying should be at 130 °C for 5 to 10 minutes or overnight in a "warm place" (ISTA 1985).
(10) Tolerance is more liberal for tree seeds than for agricultural seeds because of tree seeds' large size, higher moisture, and more natural variation (table 21) [table 14 in Student Outline]. (See ISTA 1985, table 9D.)

b. Electric meters
(1) Electric meters are not allowed for ISTA official tests but are useful for quick checks during processing and storage.
(2) They are based on electrical resistance or capacitance and are accurate to within ± 1 percent on most free-flowing seeds.
(3) All meters are made for grains; thus, calibration charts must be constructed for tree seeds. [see exercise 7]
(4) Manufacturers of electric meters are:
(a) Motomco —based on capacitance and very accurate.
(b) Radson (Dole or Seedburo)an established, reliable meter in the United States, based on resistance.
(c) Dickey-John or Instobased on capacitance. These meters are portable and battery operated only.
(d) Super-Beha — widely used in Europe.

c. Infrared balances — These small, infrared ovens have built-in balances. They use a gravimetric method based on drying time that has given good results on tree seeds in many places in the world.

d. Laboratory methods for research:
(1) The Karl Fischer method uses direct measurement and is the most widely used reference method.

Table 21. — *Tolerance levels for differences between two determinations of moisture content of tree and shrub seeds (ISTA 1985) [table 14 in Student Outline)*

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

(2) Toluene distillation was once allowed in ISTA for oily seeds but has been discontinued for safety reasons.
(3) Nuclear magnetic resonance (NMR) requires sophisticated equipment and must be referenced to the Karl Fischer method; NMR can measure moisture nondestructively in an intact seed.
(4) Infrared spectroscopy is a destructive test that must also be referenced to Karl Fischer measurements.

F. Summary
See table 22 [table 15 in Student Outline].

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.

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.

Table 22. —*Suggested test procedures for tree seed moisture (Bonner 1981b) [table 15 in Student Outline]*

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

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—** the 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. For size of sample, see table 20 (ISTA 1985, table 2.A.II), or the values based on seed size (table 19) [no equivalent table in Student Outline].
4. **Seedlot** — a specified quantity of seeds of reasonably uniform quality. The 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 in purity testing. The steps are:
   a. Reduce the submitted sample (after mixing) to the working sample with:
      (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, R echinata, R rigida, P taeda,* and *Pseudotsuga,* wings or wing fragments are detached and removed and placed in the inert matter fraction. Other pines retain wing fragments. (See "a" above). Normal dewinging and cleaning should remove the wings in these four *Pinus* species. Many more species should probably be on this list. Nursery workers want clean seeds.
   c. For samaras, the wings are not removed (e.g., *Acer, Fraxinus, Cedrela,* and *Swietenia).*
   d. For drupes, the fleshy coverings are not removed.
   e. In *Eucalyptus* species with small seeds, the following simplified procedure is used: pull out only other seeds [from 1. b(2) above] and inert matter that is obviously of nonseed origin. Pure seeds will contain unfertilized and aborted ovules. Germination of most species is tested on weighed replicates.
   f. For Leguminosae, if any portion of a testa is present, it must be classified as pure seed. Broken seeds must also be larger than half the normal seed size. For *Dalbergia* and other legumes that may not be completely extracted from the pods, there are no instructions.
   g. If species distinctions are impossible, then only the genus name is given on the certificate. This can happen with many conifers.

F. Seed Weight

1. **Determination—The** ISTA (1985) rules are followed to determine seed weight. Either the whole working sample or replicates from it are used.
   a. Working sample — The working sample is the entire pure seed fraction of a purity analysis carried out in accordance with ISTA (1985, chapter 3). The working sample is put through a counting machine. Then the sample is weighed in grams to the same number of decimal points as in the purity analysis (ISTA 1985, rule 3.5.1).
   b. Replicates — From the working sample, 8 replicates of 100 seeds each are counted at random, by hand or with a mechanical counter. Each replicate is weighed in grams to the same number of decimal places as in the purity analysis (ISTA 1985, rule 3.5.1). The variance, standard deviation, and coefficient of variation are calculated as follows:
      (1) Variance = $\frac{n(\Sigma x^2) - (\Sigma x)^2}{n(n-1)}$

      Where n = number of replicates, Σ = the sum of, and x = weight of each replicate in grams.

(2) Standard deviation (a) = ✓variance
Coefficient of variation (CV)
= (cr
100 where x = mean weight of 100 seeds.

(3) If the coefficient of variation does not exceed 6.0 for chaffy grass seeds or 4.0 for other seeds, the result of the determination can be calculated. If the coefficient of variation exceeds whichever of these limits is appropriate, 8 more replicates are counted, and the standard deviation is calculated for the 16 replicates. Any replicate is discarded that diverges from the mean by more than twice the standard deviation. Two examples are:

Weight determination example 1

Lot_1 where:

2.50	8
3.12 /x	22.64
3.00	2.83
2.78 1.X²	64.52
2.97 o-	0.2530
2.42 CV	(0.2530) 100 = 8.9%
	2.83
3.02	
2.83	

Weight determination example 2

Lot 2 where:

2.80 n = 8
2.78 /x = 23.28
3.00 I = 2.91
2.94 ,;c² = 67.80
2.97 o- = 0.0866
3.01 CV = (0.0866 100 = 3.0%
2.91
2.88
2.90

2. **Reporting results-Results** are reported in one of two ways, either by a 1,000-seed weight or by seed per gram (or per kilogram, ounce, or pound).

a. 1,000-seed weight
If counting is by machine, the weight of 1,000 seeds is calculated from the weight of the whole working sample. If counting is by replicate, from the 8 or more weights of 100-seed replicates, the average weight of 1,000 seeds is calculated (i.e., 10 x mean weight). The result is expressed to the number of decimal places used in the determination (ISTA 1985, rule 10.4).

b. Seeds per gram or per kilogram, ounce, or pound.

Number per gram = 1,000 / weight of 1,000 seeds in grams
Number per pound = 453,600 / weight of 1,000 seeds in grams
Conversion is simple:
Number per gram = number per pound x (0.002205)
Number per pound = number per gram x (453.6)
Number per ounce = number per gram x (28.35)

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 how to apply germination test results to practical nursery and field conditions.
6. Learn how to make 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. Almost every test will produce some condition that is not covered by the rules (ISTA 1985).

D. Definition of Terms

Relevant terms are derived from the glossary developed by the Seed Problems Project Group of the International Union of Forestry Research Organizations (IUFRO) (Bonner 1984a) and are defined as follows:

1. **Abnormal seedlings—in** seed testing, seedlings that do not possess all normal structures required for growth or 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 (synonym: viable seed)
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. The seed manager must ensure that the sample represents the lot.
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

1. **Pure seed component—Only** the pure seed component is used (4 replications of 100 seeds each). If 400 seeds are not available, the number of seeds per replication is reduced, not the number of replications.
2. **Environmental conditions —** Temperature, light, moisture, and medium must be carefully controlled.
   a. Temperature: Constant vs. alternating temperature is a much debated point. Some variation is allowed; thermogradient plate (TGP) results show broad latitude in suitable temperatures.
   b. Light: ISTA (1985) requires 750 to

1,250 lx (lux) (75 to 125 fc Ifoot-candles]).

c. Medium — The germination medium must be nontoxic; it can be either natural or synthetic.

(1) Natural materials are not used in any type of germinator with water circulation pumps. Materials include:

(a) Soil
(b) Sand
(c) Peat and other organic materials

(2) Synthetic materials include:

(a) Nontoxic paper products such as blotters, paper towels, cellulose wadding (Kimpak®), and filter paper. (Some paper products have mold problems.)
(b) Agar
(c) Cloth

3. Moisture—Excessive moisture is a common problem in many tests. Avoid having a film of water around the seeds. Use the "finger-press" test to judge moisture in blotters. Make a depression with the tip of a finger; if it fills with moisture, the blotter is too wet.

4. Equipment — Reliable testing operations must have dependable equipment.

a. Cabinet germinators — Cabinet germinators have good temperature control and high capacity. Seeds can be placed on moist blotters on open trays or in small containers (see "d" below).

b. Jacobsen tables — The advantage of a Jacobsen table is simplicity and moisture control; the disadvantage is poor temperature control unless used in climate-controlled rooms.

c. Walk-in rooms — Temperature control is a problem; alternating temperatures are almost impossible to establish.

d. Containers — Germination containers include petri dishes (watch out for poor aeration) and plastic boxes.

5. **Test Procedures —** There are many important considerations in proper germination testing.

a. Pretreatment

(1) Micro-organism/pathogen treatment. A 10-percent sodium hypochlorite solution is a good, simple treatment for external infection; hydrogen peroxide (H_2O_2) is another good surface sterilant (30 percent for 20 minutes).

(2) Overcoming dormancy (delayed germination)

(a) stratification (prechill): cold, moist pretreatments are typical for temperate species; warm, moist treatments are also used. In some species, paired tests are prescribed (one with and one without pretreatment).
(b) chemical treatment: nitrates, H_2O_2, and growth regulators are proven agents. Testing organizations disagree about the use of chemicals in standard tests.
(c) scarification is common for hard seeds, both by mechanical and chemical means.

b. Placement of samples —Analysts need to be careful in how samples are placed.

(1) Always leave spaces between seeds on the medium.
(2) Rotate trays from bottom to top of the cabinet in all germinators to equalize light and temperature.

c. Counting— Certain guidelines are needed.

(1) Be aware of definitions of a "germinated seed."
(2) Conduct counts at least weekly. If good germination rate data are needed, three counts per week are needed. Count daily if germination is rapid (e.g., *Populus,* where 12-hour counts are taken).
(3) Recognize abnormal seedlings.

(a) Typical or common abnormalities include albino seedlings, stunted roots, negative geotropism (can be an artifact), "endosperm" collars, and necrotic areas.
(b) Abnormalities also need to be recorded during the test. Some, such as those seen in *Quercus* and *Fraxinus,* may be test artifacts.

d. Length of the tests depends on rate of germination. Most people use multiples of weeks; 2, 3, and 4 weeks are the most common. In official testing, analysts are sometimes allowed to extend the test period to allow complete germination. This must be reported on the certificate, and seed users should be alert for it in dormant species.

e. At test's end, cut ungerminated seeds to determine empty, dormant (hard), or

rotten seeds. These counts help in quality evaluation.

6. **Tolerance and retesting—Analysts** should:

a. Review the concepts for official testing. For tolerance application, see table 23 [no equivalent table in Student Outline] and the examples below:

ISTA procedure

Test 1	Test 2	Test 3 (retest)
80	78	81
83	80	90
88	95	84
90	98	87
= 85	)2 = 88	x3 = 86
R, = 10	R_2 = 20	x of 2 and 3 = 87

AOSA procedure

Test 1	Test 2	Test 3 (retest)
80	78	70
83	83	83
88	40	15
90	91	90
= 85	5E2 = 73	= 64
R, = 10	R2 = 51 drop 40,	R3 = 75 drop 15,
	)72 = 84 R = 13	i3 = 81 R = 20; retest lot

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 are good research tools for screening many temperatures.
2. Greenhouse or nursery bed tests can be useful.
3. *Eucalyptus* and *Betula* can be tested by weight. See **ISTA** (1985).
4. *Quercus* and other large seeds can be cut in half to save space and speed germination.

Table 23. —*Maximum tolerated ranges between four replicates (adapted from ISTA 1985) [no equivalent in Student Outline]*

Average percent germination*	Maximum range		Average percent germination		Maximum range
1	2	3	1	2	3
99	2	5	87-88	13-14	13
98	3	6	84-86	15-17	14
97	4	7	81-83	18-20	15
96	5	8	78-80	21-23	16
95	6	9	73-77	24-28	17
93-94	7-8	10	67-72	29-34	18
91-92	9-10	11	56-66	35-45	19
89-90	10-11	12	51-55	46-50	20

*Calculate the average germination percentage to the nearest whole number. Locate the average in column 1 or 2 and read the maximum tolerated range opposite in column 3.

H. Reporting Results (figs. 33 to 36) [no equivalent figures in Student Outline]

1. **Germination capacity—should** be expressed as a percentage of the total seeds in the replication. If there are many empty seeds, the posttest count will show this in the test result.
2. **Rate of germination—Germination** studies of many species suggest that rate of germination is a good index of vigor.
   a. Germination energy (the early count).
   b. Mean germination time (MGT). Appears to be very good when comparing treatments within lots.
   c. Time for a certain proportion of germination to occur (e.g., number of days for 50 percent or 75 percent of the seeds to germinate).
   d. Germination Value (GV).
   e. Peak Value (PV). In *Pinus,* PV has been the best measure of rate of germination (table 24) [no equivalent table in Student Outline].

I. Practical review

For suggested test conditions of tropical species, see table 25 [no equivalent table in Student Outline]. For practice on test results interpretation, see table 26 no equivalent table in Student Outline].

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.

1108 Germination Test Sheet

Study **3.2 (I)** **Species Pinus taeda**

Seeds\Rep. **50\2** Test Period 12-1-88 to 12-29-88

Temp. Regime **20 to 30 °C** Treatment 7-yr test

		Sample ID	-18 °C - 10% B	-18 °C - 10% C	
DATE	TEST DAY	A	B	A	B
12-12-88	11	3	4	1	3
12-14-88	13	3	5	5	9
12-16-88	15	2	4	1	8
12-19-88	18	12	14	9	10
12-26-88	25	19	13	22	10
12-28-88	27	0	1	1	0
12-29-88	28	0	0	0	0
NNS		39	41	39	40
Good, Ungerm. Seeds (ND)		0	0	0	0
Abnormal Germ. (NA)		0	0	0	0
Total No. of Seeds (NT)		50	50	50	50
Empty Seeds (NE)		6	5	5	4
Rotten Seeds (NNV)		5	4	6	6

Figure 33. —A *germination test sheet showing good germination results with a sample of* Pinus taeda *(no equivalent figure in Student Outline].*

Study 3.2 Sample ID 18102.1

ICUT = Day trial terminated	28
DFG = Day of first germination	11
NNS = Number of normal germinations	39
NA = Number of abnormal germinations	0
ND = Number not germinated by cut off but not empty and judged capable of germination	0
NNV = Number not germinated by cut off but not empty and judged not capable of germination	5
NT = Total number of seeds in trial	44
NE = Number found empty at cut off	0
NG = Good (NT - NE)	44
PUG = (NNS + ND)100	88.63636
PNG = (NNS/NT)100	88.63636
PNGA = ARCSIN(SQR(PNG/100))	70.29977
PAG = (NA/NT)100	0
PD = Earliest day of max ((NNS(I)/NT)/I)	25
PV = ((NNS(PD)/NT)/PD)100	3.545455
MDG = ((NNS/NT)/ICUT)100	3.165584
GV = (PV) (MDG)	11.223440

RECONSTRUCTION OF DAY-NUMBER PAIRS

Day (I)	Increment INNS (I)	Cumulative No. NNS (I)
11	3	3
12	2	5
13	1	6
14	1	7
15	1	8
16	4	12
17	4	16
18	4	20
19	3	23
20	3	26
21	3	29
22	2	31
23	3	34
24	2	36
25	3	39

Mean	18.435900
Mode	16.000000
Variance	17.094470
Std. Var.	4.134546

Figure 34. — *Computer analysis of data from figure 33, replicate A of the —18 °C, 10-percent sample. See figure 33 for some term definitions. Also, PUG = ultimate germination (includes dormant seeds); PNG = percentage normal germination; PNGA = arc sine transformation of PNG; PAG = percentage abnormal germination. For PV, MDG, and GV, see Section IV under Evaluation Quality [no equivalent figure in Student Outline].*

1108 Germination Test Sheet

Study **2.2** **Species** Pinus taeda

Seeds\Rep. **100\2** Test Period **12-4-87** to **1-1-88**

Temp. Regime **20 to 30 °C**

		Sample ID			
DATE	TEST DAY	01-A	01-B	02-A	02-B
12-14-87	10	1	0	2	4
12-16-87	12	9	5	10	20
12-18-87	14	13	10	14	15
12-21-87	17	27	19	22	16
12-23-87	19	2	12	5	6
12-26-87	22	9	10	8	3
12-28-87	24	4	6	2	1
12-30-87	26	3	5	5	5
01-01-88	28	2	5	5	5
NNS		70	72	73	75
	PNG DAE*	90	95	85	93
	ii+	92		89	
Good, Ungerm. Seeds (ND)		20	23	11	16
Abnormal Germ. (NA)		0	0	0	0
Total No. of Seeds (NT)		100	100	100	100
Empty Seeds (NE)		0	0	1	2
Rotten Seeds **(NNV)**		**10**	5	15	7

*PNG DAE = germination percentage when dead seeds, empty seeds, and abnormal germinants are included.

+Mean of replication A and B.

Figure 35. —A *germination test sheet showing moderate germination results with a sample of* Pinus taeda *[no equivalent figure in Student Outline].*

Study 2.2 Sample ID 1.1

ICUT = Day trial terminated	28
DFG = Day of first germination	10
NNS = Number of normal germinations	70
NA = Number of abnormal germinations	0
ND = Number not germinated by cut off but not empty and judged capable of germination	20
NNV = Number not germinated by cut off but not empty and judged not capable of germination	10
NT = Total number of seeds in trial	100
NE = Number found empty at cut off	0
NG = Good (NT - NE)	100
PUG = (NNS + ND)100	90
PNG = (NNS/NT)100	70
PNGA = ARCSIN(SQR(PNG/100))	56.78914
PAG = (NA/NT)100	0
PD = Earliest day of max ((NNS(I)/NT)/I)	17
PV = ((NNS(PD)/NT)/PD)100	2.941177
MDG = ((NNS/NT)/ICUT)100	2.5
GV = (PV) (MDG)	7.352941

RECONSTRUCTION OF DAY-NUMBER PAIRS

Day (I)	Increment INNS (I)	Cumulative No. NNS (I)
10	1	1
11	5	6
12	4	10
13	7	17
14	6	23
15	9	32
16	9	41
17	9	50
18	1	51
19	1	52
20	3	55
21	3	58
22	3	61
23	2	63
24	2	65
25	2	67
26	1	68
27	1	69
28	1	70

Mean	16.728570
Mode	15.000000
Variance	18.693390
Std. Var.	4.323585

Figure 36. — *Computer analysis of data from figure 35. See figure 34 for definition of terms [no equivalent figure in Student Outline].*

Table *24.-Germination of* Pinus taeda: *comparing data based on total seeds with those of filled seeds only [no equivalent table in Student Outline]*

Lot number	Germination capacity		PV*		GVt	
	Total	Filled	Total	Filled	Total	Filled
1	46	67	2.06	3.00	3.45	7.27
2	55	74	2.19	2.94	4.47	8.00
3	38	64	1.54	2.58	2.12	5.86
4	45	74	2.43	4.04	3.91	10.77
5	58	78	2.70	3.63	6.24	10.87
6	38	82	1.67	4.04	2.74	12.80
7	32	78	1.48	3.63	1.73	10.09
8	40	86	1.65	3.52	2.39	10.64
9	24	73	0.98	3.03	0.90	8.82
10	24	65	1.05	2.81	0.99	6.98
11	19	46	0.90	2.18	0.69	3.64
12	56	77	2.55	3.50	5.10	9.63
13	22	65	1.05	3.35	1.18	10.42
14	20	91	1.06	5.05	1.01	21.86
15	45	79	2.36	4.14	4.07	12.68
16	20	77	1.19	9.22	1.02	13.68
17	24	84	1.48	5.39	1.69	22.11
18	48	94	3.51	6.83	8.15	30.60

Teak value.
tGermination value.

Table 25. —*Suggested test prescriptions for selected species [no equivalent table in Student Outline]*

Species	Suggested pretreatment*	Mediumt	Temperature	Duration	Comments
			°C	*Days*	
Acacia nilotica	Sc: M, A, HW; CW 24 hr	S, B	20/30t	21	
Aesculus indica	CW 48 hr, cut off 1/3 scar end	S	20/30	21	Some sources may need prechilling
Ailanthus altissima	CW 24 hr, remove pericarp	P, B	20/30	21	
Albizia procera	SC: M, HW	B	20/30	21	
Azadirachta indica	CW 24 to 48 hr	B, S	25	21	
Bombax ceiba	none	B	25	21	
Casuarina equisetifolia	CW 24 hr	P, B	20/30	14	
Cedrus deodara	Pc: 21 days at 3 to 5 °C	P, B	20	21	
Dalbergia sissoo	CW 24 hr	B	30; 20/30	21	Remove seeds from pods
Eucalyptus camaldulens s	none	P	30	14	§Test by weight (0.1 g per replicate)
Juglans regia	Pc: 30 to 120 days	S	20/30	40	
Leucaena leucocephala	Sc: HW (80 °C, 2 min), M	B	30	14	
Melia azedarach	CW 24 to 48 hr	S	20/30	28	
Morus alba	none; CW 24 hr	P, B	20/30	28	
Pinus roxburghii	none	B	20/30	28	
P. wallichiana	none	B	20/30	28	
Populus euphratica	none	P, B	20/30	10	
Prosopis cineraria	Sc: M; CW 24 hr	B	30	21	
Prunus padus	Pc: 3 to 4 mo at 3 to 5 °C	S	20/30	28	§Tetrazolium or excised embryo preferred
Robinia pseudoacacia	Sc: M, A, HW		20/30	14	

*Pretreatment codes: A = acid scarification; CW = cold water soak; HW = hot water treatment; M = mechanical scarification; Pc = prechill; Sc = scarify.
tMedium codes: B = germination blotters; P = filter paper; S = sand.
Use 16 hr at 20 °C in dark and 8 hr at 30 °C in light.
§Prescription from ISTA rules (1985).

Table 26. —*How to interpret test results from the testing laboratory [no equivalent table in Student Outline]*

Normal	Dormant	Empty	Rotten/ dead	Abnormal	PIT*	Evaluation
		Percent - - - - - -			*Percent/day*	
95	3	1	*0*	1	6.0	Good lot; sow.
70	12	15	0	3	4.0	Too many empties; reclean.
80	0	0	13	7	2.0	Old seed? damaged?
25	2	5	60	8	0.2	Bad lot; don't sow.
85	2	3	7	3	7.0	Which of these two would be
85	10	1	4	0	2.0	best for early sowing in cold soil?
50	35	5	5	5	1.5	Repeat with prechill.
60	12	2	3	23	2.5	Too many abnormals; processing damage? genetics?
70	1	1	21	7	4.0	Too many dead; abnormal total suggests damage; recondition to remove dead seeds if possible.

Teak value.

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 can tell the analyst 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, a rapid test should be used.
2. **User request—Sometimes** the test customer chooses not to wait for germination test results and wants immediate evaluation.
3. **Limited seed supply—If** the lot is too valuable to sacrifice 400 or even 200 seeds, a nondestructive rapid test may be used. This practice is common for research or breeding lots.
4. **During collection—A** rapid test can be used to check the quality of the collected seeds and adjust plans if necessary (i.e., to increase collections if seed quality appears to be low).
5. **Other test objectives—When** other seedlot parameters are more important than germination (e.g., extent of mechanical damage and viability), rapid tests may be useful.

E. Sampling

The same sampling principles and precautions apply as in standard germination tests; proper sampling is essential.

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 no apparent decayed or insect-damaged spots, and have good color (usually white to greenish white or ivory colored).
   c. Advantages
      (1) The fastest and cheapest test
      (2) Can be performed in the field to check collections as they are made
      (3) Surprisingly accurate for fresh seeds
   d. Disadvantages
      (1) Difficult for small seeds
      (2) Poor results with stored seeds of some species
      (3) Is a destructive test
2. **Vital stains**
   a. Technique: Embryo and storage tissues are exposed by cutting, then immersed in staining solutions for up to 24 hours. The location and intensity of staining indicate viable or dead tissue.
   b. Stain options:
      (1) Tetrazolium chloride (**TZ)** (2, 3, 5-triphenyltetrazolium chloride) is the most widely used stain. Active dehydrogenase enzymes form a red insoluble dye (formazan) from the colorless TZ solution; live tissue stains red. Primary development was by Lakon in Germany. The TZ method is widely used in Western Europe. Dr. Robert Moore promoted TZ strongly in the United States, including applications for tree seeds.
      (2) Indigo carmine stains dead tissues blue. This method is common in Eastern Europe and was developed in Russia by Professor Nelyubon.
      (3) Selenium or tellurium salt tests were developed in Japan by Dr. Hasegawa. This method was the first vital stain method with seeds, but it is not used now because of metal toxicity from the salts.
   c. Evaluation (TZ only):
      Sound tissue should stain carmine. Weak living tissue allows more rapid penetration of the salt and stains a darker red. Tissue that has been injured by freezing may develop a blurred, bluish-red stain. Because dead tissues are not respiring and cannot produce formazan, they do not stain.
      (1) "Topographic stain" analysis is the most accurate, but it is the most difficult to standardize (figs. 37 through 43) [no equivalent figures in Student Outline].
      (2) The ISTA (1985) prescribes TZ for certain dormant species and supplies evaluation guides.
   d. Advantages
      (1) Fast — stains can be read within 24 hours
      (2) Inexpensive
      (3) Equipment needs are simple
   e. Disadvantages
      (1) Labor-intensive, time-consuming preparation
      (2) Difficult to obtain uniform penetration of the stain, especially in seeds with hard seedcoats
      (3) Difficult to interpret the stain intensity and distribution
      (4) Requires practice and experience
      (5) Destructive test
3. **Excised embryo**
   a. Technique: Seeds are cut open, and the embryos are carefully removed for incubation in dishes. The excised embryo test begins as the TZ test does, but the embryo is completely removed. The embryos are incubated in light at 18 to 20 °C for 10 to 14 days.
   b. Evaluation
      (1) Viable seeds are green or white, with some growth.
      (2) Nonviable seeds are dark or moldy, with no growth.
      (3) The ISTA (1985) prescribes this test for some dormant species.
   c. Advantages
      (1) Simple equipment needs
      (2) Actual seed performance is tested
      (3) Easy to evaluate
   d. Disadvantages
      (1) Labor-intensive, time-consuming preparation
      (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 incubated in 1-percent hydrogen peroxide (H_2O_2) in the dark with alternating temperatures of 20 and 30 °C. Radicle growth is measured after 3 to 4 days, and the seeds are placed in fresh hydrogen peroxide. Radicle growth is measured again at 7 or 8 days. Devel-

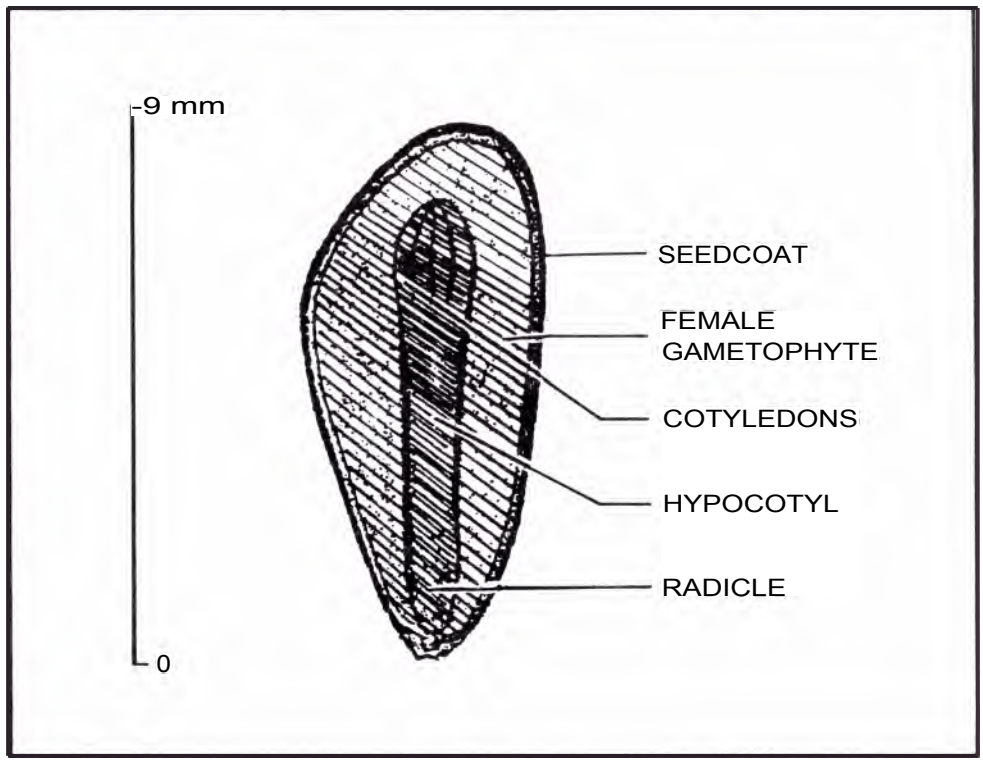

Figure 37. — Pinus: *Good stain (lined area); good seed (adapted from Krugman and Jenkinson 1974) [no equivalent figure in Student Outline].*

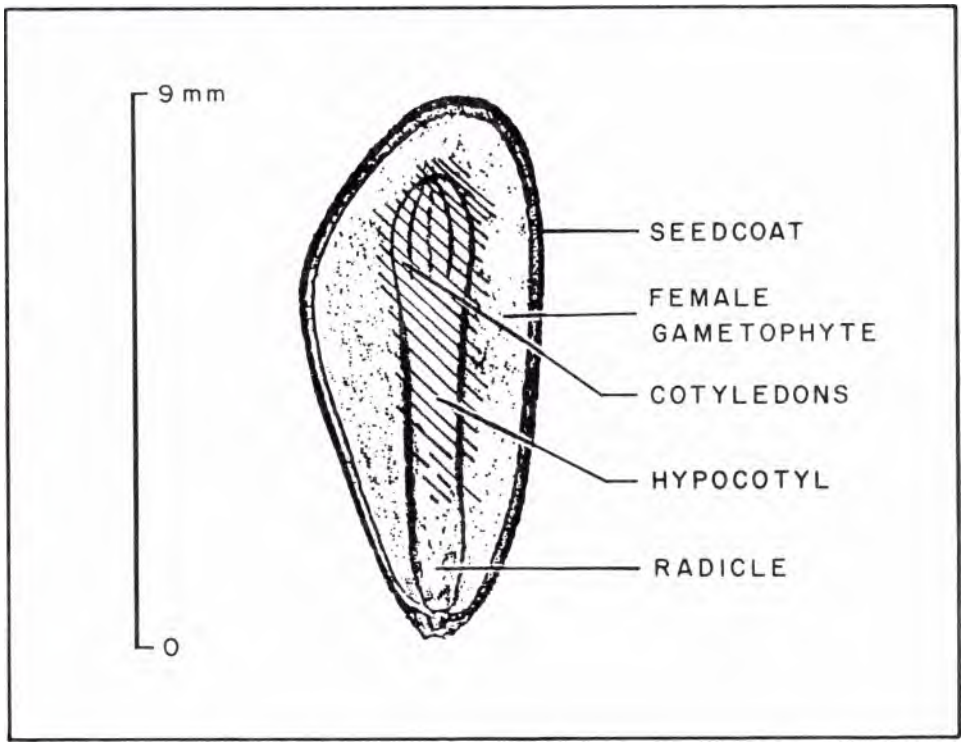

Figure 38. — Pinus: *Cotyledons weakly stained (lined area); almost no radicle stain; probably nongerminable or perhaps poor TZ penetration (adapted from Krugman and Jenkinson 1974) [no equivalent figure in Student Outline].*

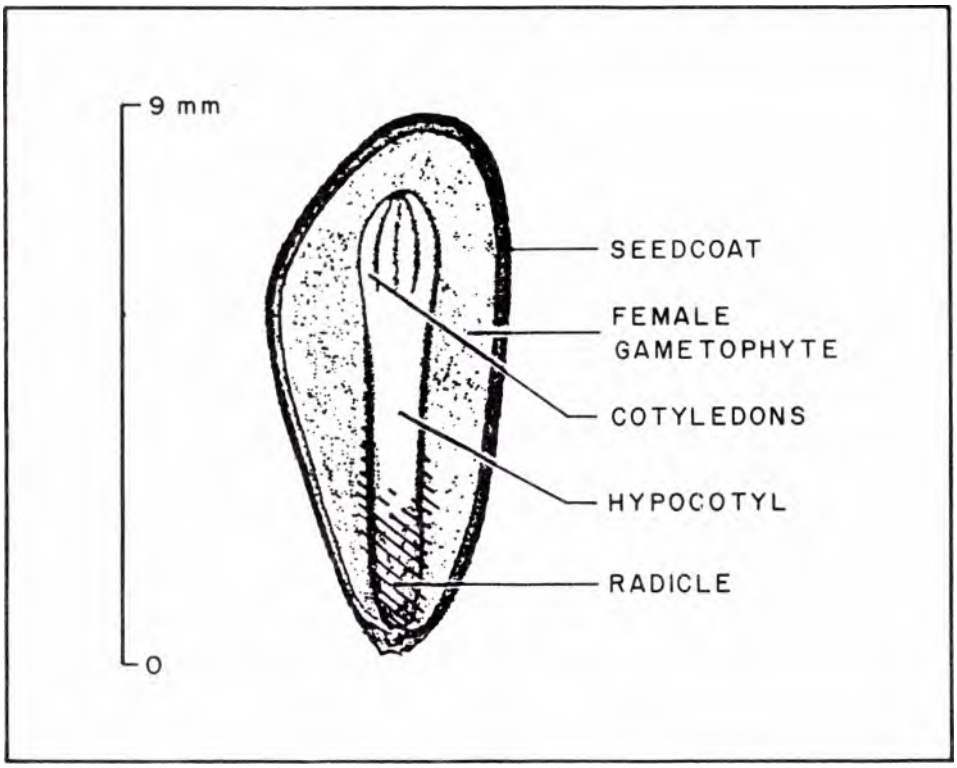

Figure 39. — Pinus: *Radicle stain only (lined area); nongerminable (adapted from Krugman and Jenkinson 1974) [no equivalent figure in Student Outline].*

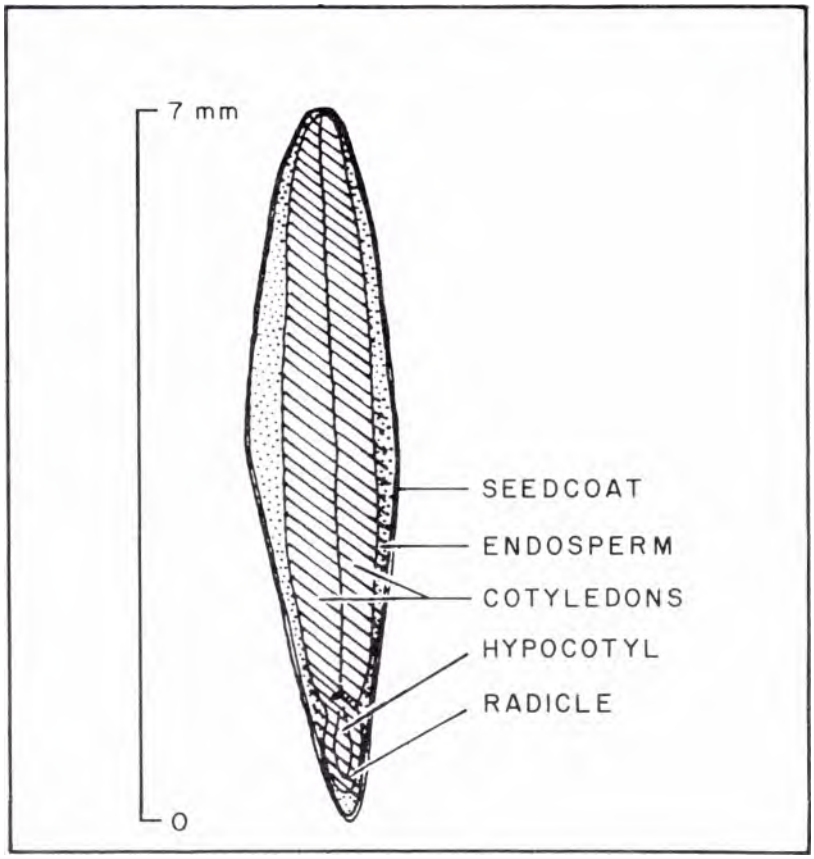

Figure 40. — Leucaena: *Complete stain (lined area); good seed (adapted from Whitesell 1974) [no equivalent figure in Student Outline].*

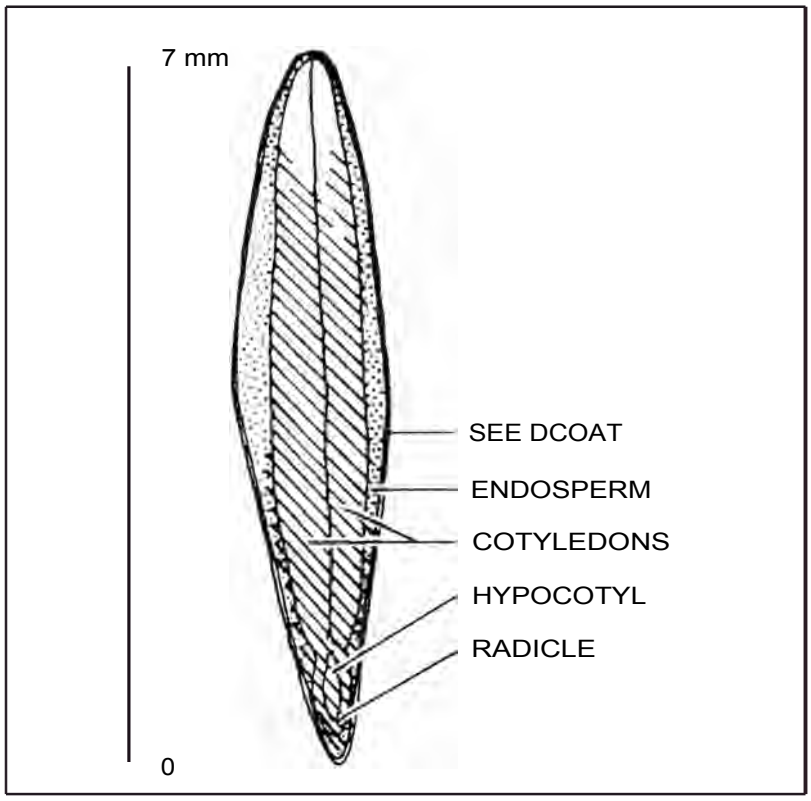

Figure 41. — Leucaena: *Unstained cotyledon tips (blank area); should germinate (adapted from Whitesell 1974) [no equivalent figure in Student Outline].*

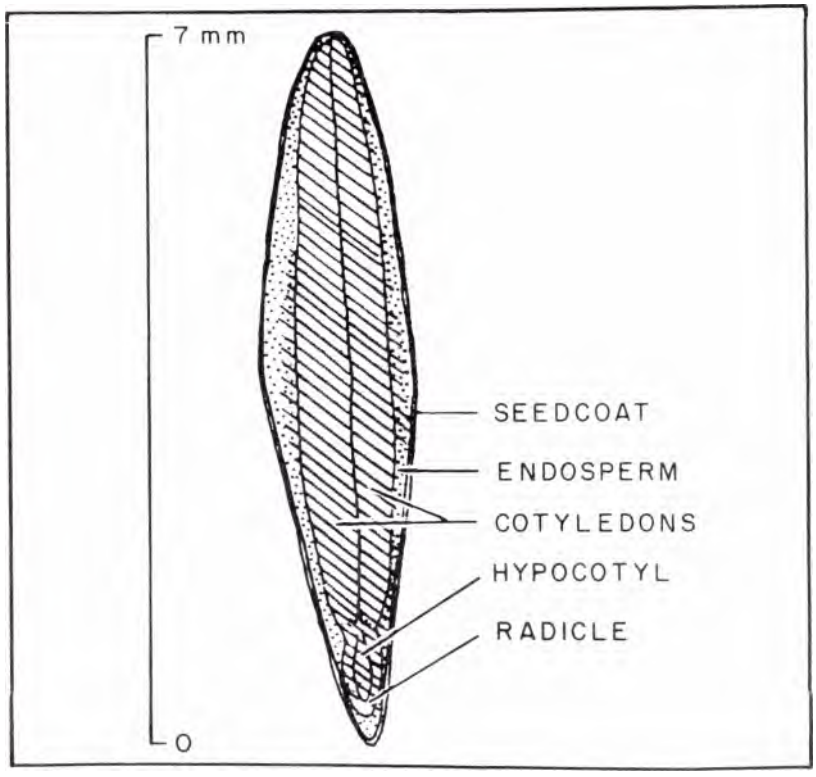

Figure 42. — Leucaena: *Less than one-third of the radicle tip is unstained (blank area); should germinate (adapted from Whitesell 1974) [no equivalent figure in Student Outline].*

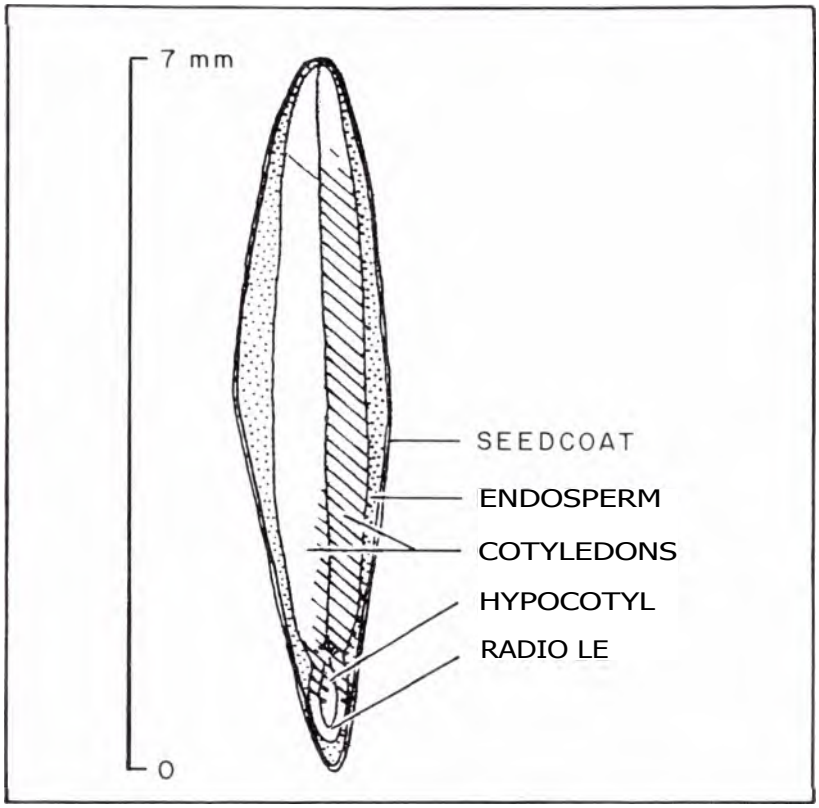

Figure 43. — Leucaena: *One cotyledon unstained and more than one-half of the radicle unstained (blank areas); nongerminable (adapted from Whitesell 1974) [no equivalent figure in Student Outline].*

oped on barley, the test is used on many North American conifers.

b. Evaluation: Based on radicle growth — 5-mm growth and up is good; 0- to 5-mm growth is uncertain; 0 growth is not viable.

c. Advantages
   (1) Inexpensive and requires no elaborate equipment
   (2) Objective (partially)
   (3) Simple preparation

d. Disadvantages
   (1) Not practical for very small seeds
   (2) Tested only on conifers so far among tree seeds
   (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 captured on film are examined. (More details are presented in the next section.)

b. Evaluation: Subjective. Enhancements are possible with contrast agents.

c. Advantages
   (1) Fast
   (2) Provides a permanent image
   (3) Nondestructive test unless contrast agent used

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, then electrical conductivity of the leachate is measured. (More details are given in the next section.)

b. Evaluation: Relationship of conductivity to germination must be established for each species.

c. Advantages
   (1) No expensive equipment required
   (2) Fast and simple
   (3) Objective
   (4) Nondestructive test

d. Disadvantages
   (1) Indirect measurement that requires testing establish the relationship of conductivity to g mination.
   (2) Still under development; some unknown facto cause trouble.

G. Sources

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

VI. Rapid Tests: X Rays and Leachate Conductivity

A. Introduction

Like other rapid tests, x-ray 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 understanding x rays and leachate conductivity.

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. The amount of absorption depends on thickness, density, and composition of the object and the wavelength of the x ray. (The shorter the wavelength the greater the energy, which means more penetration) (fig. 44) [no equivalent figure in Student Outline].

b. Radiographs are pictures of the object formed by the x rays that pass through the object and strike a photographic material (film) or fluorescent screen.

(1) Radiograph quality is defined by:

(a) Contrast — degree of difference in optical density of two adjacent fields.

(b) Density—amount of blackening of the radiograph.

(c) **Definition—** sharpness of image detail.

(2) Quality is controlled by:

(a) Kilovolt age (kV) — voltage potential between cathode and anode; increase kV to get shorter wavelength to lower contrast.

(b) Milliamperage (mA)— current applied to the cathode; increasing mA increases quantity of electrons available for x rays; too much mA results in overexposure.

(c) Exposure time (seconds) — the period that the object is exposed to x rays; increases density of the image (better to increase mA); the product of time and mA (mA-seconds) is constant for equal radiographic effect.

(d) Focus-film-distance (FFD) — distance between focal spot and film surface; increasing **FFD** decreases intensity of radiation and density.

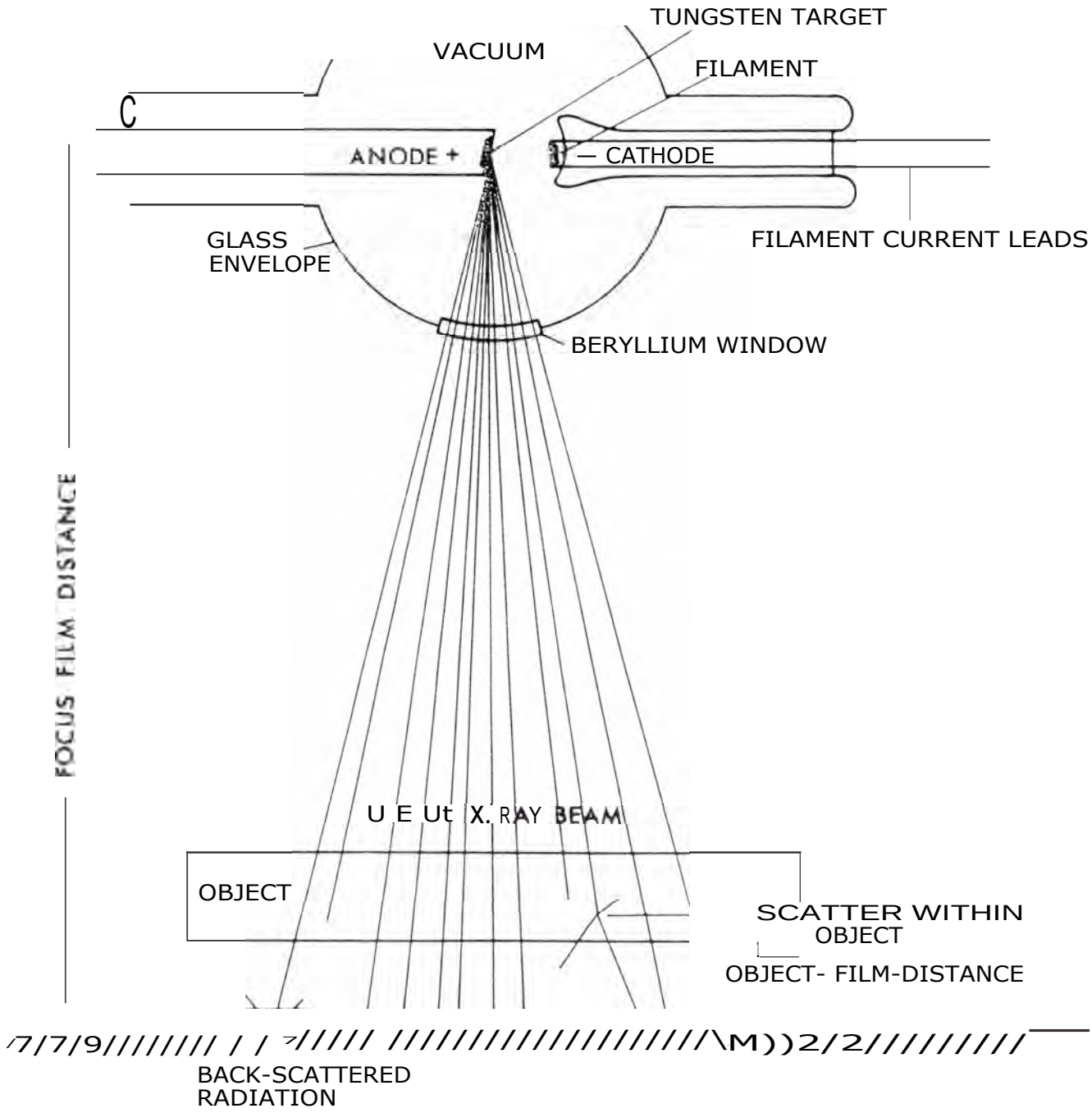

Figure 44. —*Diagramatic view of an x-ray apparatus (Simak 1980) [no equivalent figure in Student Outline].*

(e) Object-film-distance (OFD) — distance between object and film surface; in seed work, objects are usually placed directly on the film; short OFD gives better quality to the radiograph.

2. Methods

a. Equipment — Several types of x-ray equipment are available commercially, from both Europe and the United States. X-ray generation of 0 to 60 kV at 3 to 10 mA is sufficient.

b. Film — Several film choices are available:

(1) Conventional film is similar to photographic film. It requires wet development and different film speeds are available.

(2) Polaroid film provides rapid process-

ing, but is more expensive and provides less detail than conventional film. Polaroid images fade with time.

(3) Radiographic paper can be developed faster than film, but special processing is required. It shows less detail than conventional film, and images fade with time.

c. Contrast agents — Contrast agents are used to increase density of certain seed tissue images on the radiograph by treating the seeds before exposure. Water is the simplest agent; most others kill the seeds. The targeted tissues absorb the contrasting agents.

(1) Aqueous agents—primarily solutions of heavy cation salts; e.g., barium chloride ($BaC1_2$) and silver nitrate ($AgNO_3$); seeds are soaked for 1 hour after full imbibition, and salts impregnate dead or damaged tissue, thus greatly increasing the density of the tissue image on the radiograph. Water can be an excellent contrast agent with some seeds.

(2) Vaporous agents — different vapors can be used to penetrate either live or dead tissue; for example, 2 to 4 hours of exposure to chloroform ($CHC1_3$) (or other halogen derivatives of alk anes) produce high density at dead or damaged tissues.

d. Safety is important in seed radiography. Energy levels used in seed work are normally very low, but radiation exposure has a cumulative effect in cells. Equipment should have approved shielding, which must be checked periodically. Operators should wear monitoring devices.

3. **Special Techniques**

a. Stereoradiography —With two radiographs of the same seed, one taken with the seed shifted, a three-dimensional view can be obtained with a stereoscope. The ratio of object shift to **FFD** is generally 1:10.

b. Tomography — Radiographic images are taken of preselected planes within the object. The plane serves as the focal point; tube and film are above and below at fixed heights and are moved simultaneously during exposure. Only the target plane stays in focus. This method is widely used in medicine.

c. Xeroradiography — Xeroradiography combines radiography and xerography, a technique with exceptional image resolution. Instead of x-ray film, the image is captured on a photoreceptor plate sensitized with a charged, selenium surface.

d. Other techniques — Other special techniques are available, but none has yet found widespread use in seed radiography.

4. **Application in seed testing** —X rays were first used on seeds in 1903 in Sweden. Practical development for seed testing came in the 1950's in Sweden.

a. Currently x rays are used to test for:

(1) Determining seed anatomy, including embryo presence, size, and shape. This process is good for empty seed counts before germination tests.

(2) Determining insect damage, including the location and extent of damage and the growth of insect larvae.

(3) Determining internal mechanical damage, including seedcoat cracks invisible to the naked eye.

b. X rays have limited usefulness in determining viability or other physiological attributes; the standard germination test is better.

5. **Summary—Show** sample radiographs, if available, on a screen.

E. Leachate Conductivity

1. **Major Points**

a. Deterioration: As seeds deteriorate, substances can be leached in proportion to the degree of deterioration. Measurements of these substances can be correlated with seed quality.

b. Measurable materials:

(1) Sugars

(2) Amino acids

(3) Electrolytes (the easiest to measure, both in terms of time and expense.) (fig. 45) [no equivalent figure in Student Outline]

2. **Techniques**

a. Multiple-seed analyzer: Instruments made in the United States, the ASA-610 and ASAC-1000 meters, have the same circuits for measurement, but the ASAC-1000 has other features.

(1) Advantages

(a) Fast

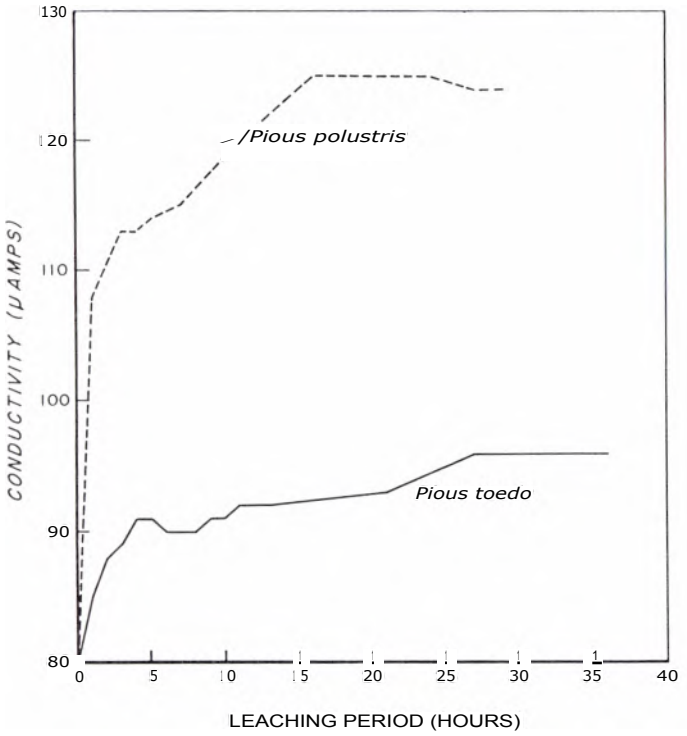

Figure 45. —*Release of electrolytes over time from seeds of* Pinus palustris *and R* taeda *(adapted from Bonner and Vozzo 1986) [no equivalent figure in Student Outline].*

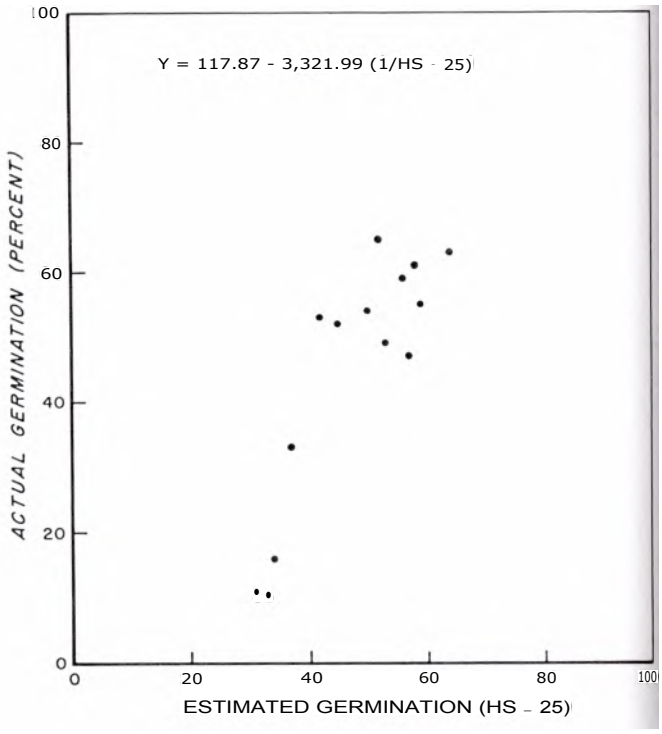

Figure 46. —*Relationship of actual germination to estimated germination in* Pinus palustris *as determined with the ASAC-1000 Analyzer (adapted from Bonner and Vozzo 1986) [no equivalent figure in Student Outline]*

(b) Receives input from individual seeds
(c) Data are printed on paper tape (ASA-610)
(d) The ASAC-1000 has a microprocessor that calculates some statistics, and the data can be stored in computer files.

(2) Disadvantages
(a) Expensive (US$6,500)
(b) Some equipment not reliable
(c) Conductivity/germination relationship not completely understood
(d) Manufacturer's support not adequate

b. Single probe techniques: Single probes measure conductivity in bulk samples with a simple conductivity cell.

(1) The ISTA handbook of vigor test methods (Perry 1981) includes this method for peas.

(2) Advantages
(a) Fast
(b) Equipment inexpensive and common in general laboratories
(c) Completely objective

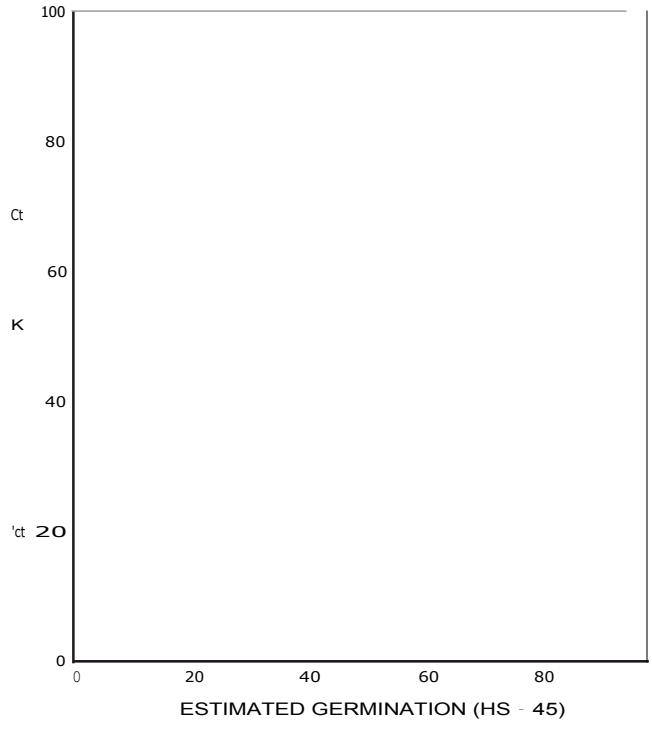

Figure 47. —*Relationship of actual germination to estimated germination in* Pinus taeda *as determined with the ASAC-1000 Analyzer (adapted from Bonner and Vozzo 1986) [no equivalent figure in Student Outline].*

(d) Accuracy (within 10 percent of germination for some species) equals or exceeds that of the ASAC-1000

(3) Disadvantage—As with the multiple-seed analyzers, some influences are not yet understood.

c. Results with United States species.

(1) ASAC-1000 — See table 27 [no equivalent table in Student Outline] and figures 46 and 47 [no equivalent figures in Student Outline] for examples of correlations with germination.

(2) Single probe — See figures 48 through 53 [no equivalent figures in Student Outline] for examples of correlations with germination.

3. **Current status—The** single-probe method is promising. More research is needed, but this method can already be used to group seeds into high, medium, or low quality classes. It is also easy to calibrate.

F. Sources

For additional information on x rays, see Vozzo 1978, 1988; Willan 1985, p. 224-226. For more information on leachate conductivity, see Bonner 1991a; Perry 1981, chapter 6.

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. Identify the types of seed vigor tests and which ones are most suitable for tree seeds.

C. Key Points

The following points are essential in conducting 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 during germination and seedling emergence" (Perry 1981).

Table 27. — *Correlations of conductivity data with laboratory germination and nursery emergence and calculated error limits for six pine species (Bonner and Vozzo 1986) [no equivalent table in Student Outline]*

Species	Number of seedlots	Laboratory germination			Nursery emergence	
		Best histogram segment*	Correlation coefficient	Error limit	Best histogram segment	Correlation coefficient
			r	*Percent*		r
Pinus echinata	9	35	0.7477	4.8		
P *elliottii*	24	40	0.7806	7.2	45	0.6974
P. palustris	14	25	0.9252	10.9	20	0.8145
P strobus	14	25	0.8546	13.6	25	0.7086
P. taeda	24	45	0.9775	6.5	40	0.7122
P virginiana	*11*	30	0.5342	3.1		

'Refers to that portion of the distribution frequency of current that indicates a good seed for that particular species (See Bonner and Vozzo 1986).

tN_o nursery tests with these species.

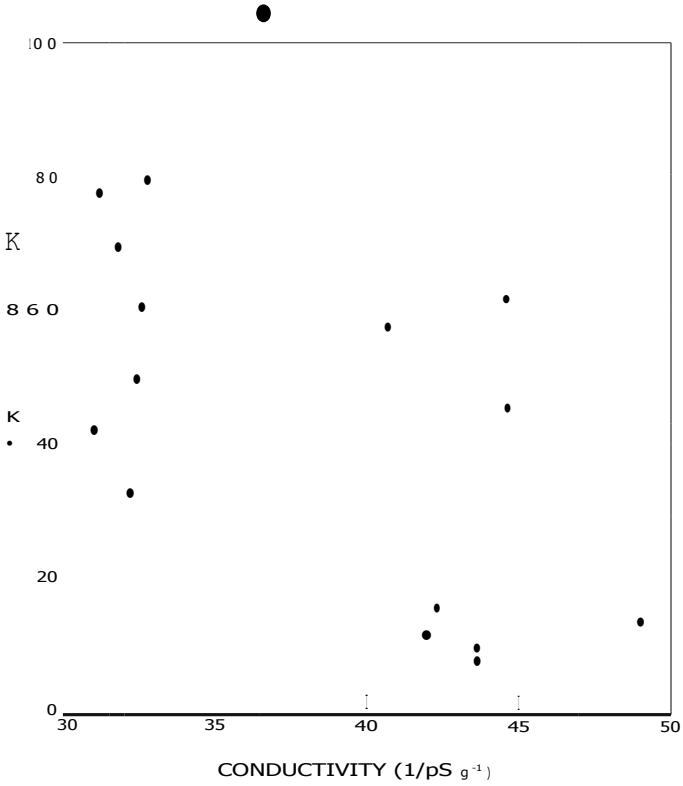

Figure 48. —*Relationship of germination to leachate conductivity for* Eleagnus angustifolia *[no equivalent figure in Student Outline].*

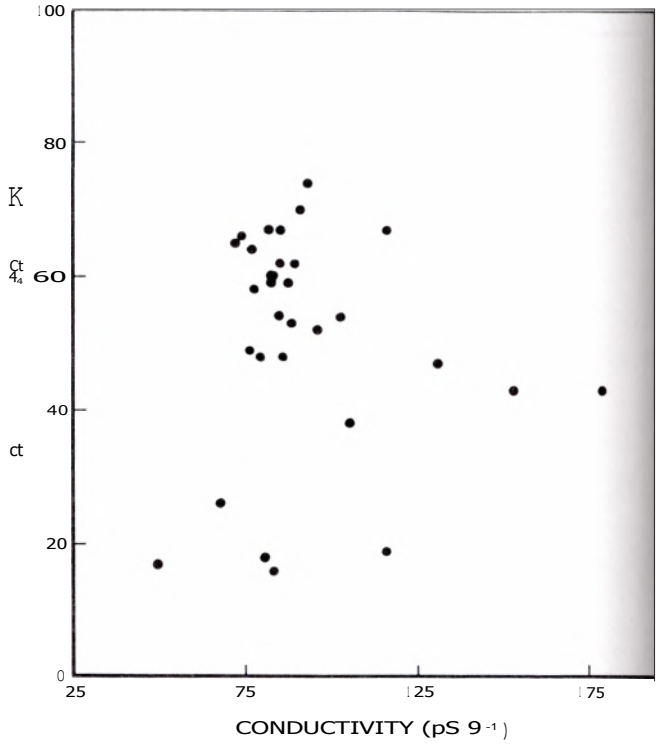

Figure 49. —*Relationship of germination to leachate conductivity for* Picea glauca *as measured with a bulked sample (Bonner and Agmata-Paliwal 1992) [no equivalent figure in Student Outline].*

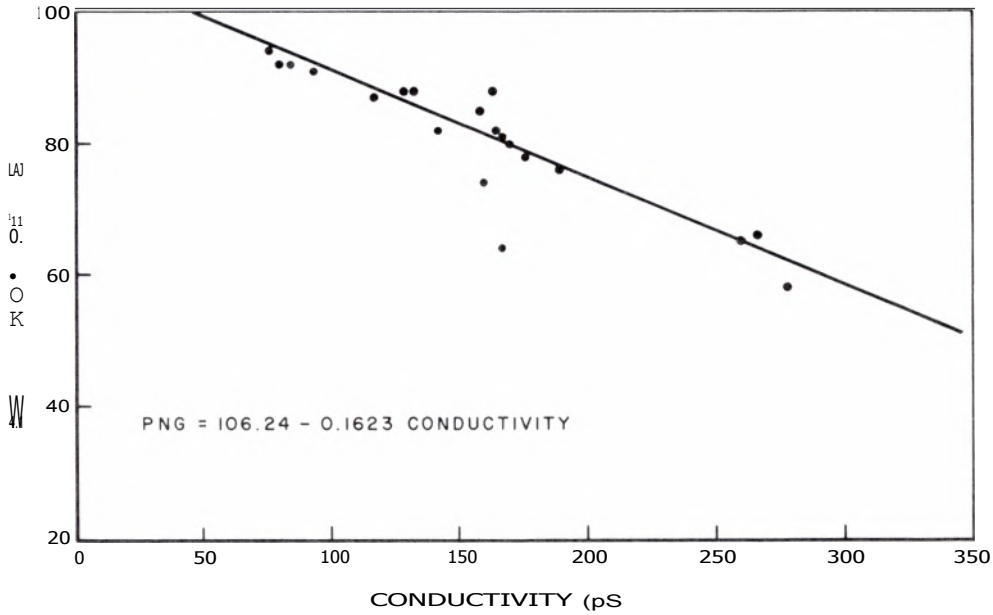

Figure 50. —*Relationship of germination to leachate conductivity for* Gleditsia triacanthos *samples that were given accelerated aging to produce varying levels of quality (Hooda and others, in press) [no equivalent figure in Student Outline].*

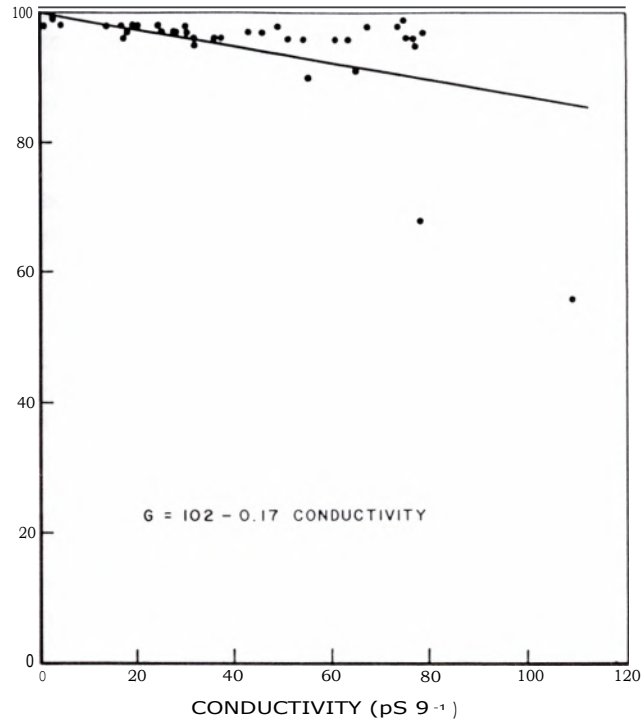

Figure 51 . —*Relationship of germination to leachate conductivity for 38 mixed seedlots of* Picea rubens *(Bonner and Agmata-Paliwal 1992) [no equivalent figure in Student Outline].*

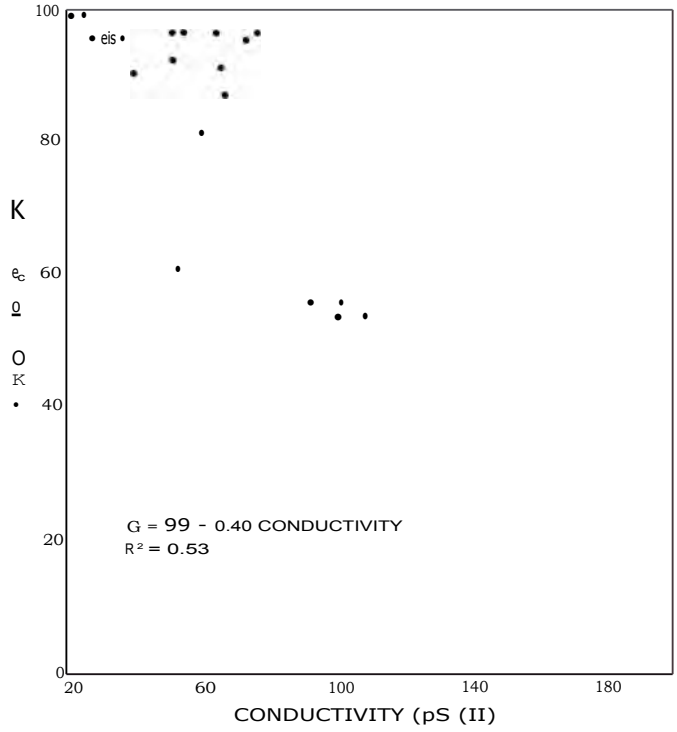

Figure *52.— Relationship of germination to leachate conductivity for 14 seedlots of* Picea rubens *that were given accelerated aging to produce varying levels of quality (Bonner and Agmata-Paliwal 1992) [no equivalent figure in Student Outline].*

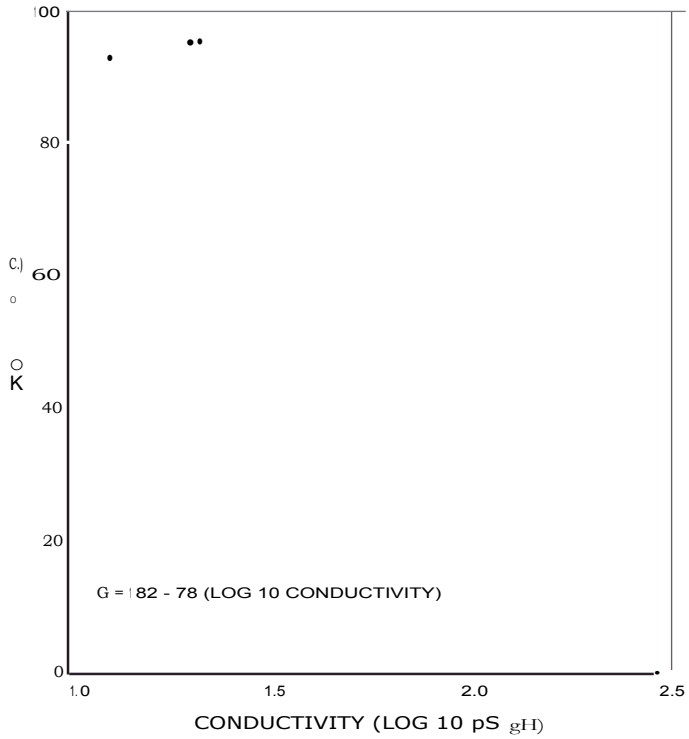

Figure 53. —*Relationship of germination to leachate conductivity for seeds from a single mother tree of* Picea rubens, *which were given accelerated aging (Bonner and Agmata-Paliwal 1992) [no equivalent figure in Student Outline].*

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 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 — Similar to the standard germination test, except that normal seedlings are further classified as strong or weak based on deficiencies of roots, shoots, or cotyledons that are symptomatic of reduced quality. Not commonly used with tree seeds, seedling vigor classification is closely related to incidence of abnormal seedlings.
   b. Seedling growth rate — Similar to the standard germination test, but at the end of the germination period, seedling growth is measured as either linear growth or weight. This method has been tested on tree seeds with mixed results.

2. **Stress tests**
   a. Accelerated aging— Seed germination is the criterion for evaluation.
      (1) In this test, seeds are subjected to 40 to 45 °C and nearly 100 percent relative humidity for various periods up to 6 days.
      (2) Developed at Mississippi State University on agricultural seeds, this test is now being used for tree seeds.
   b. Cold test — Seeds are placed in soil (high moisture and low temperature [10 °C]) for a specified period, then transferred to favorable temperature for germination. Used primarily for corn, it is probably the oldest vigor test used in the United States.
   c. Cool germination test — Used commonly with cotton. Seeds are germinated at nearly minimal temperature (15 °C) for 7 days, then seedling length is measured. Normal cotton seedlings of 4 cm or longer are considered high vigor seedlings.
   d. Osmotic stresses — Osmotic stresses have been used but less than the other stress tests.
   e. Methanol treatment — This chemical stress test was studied in Indonesia on *Heuea* seeds and in the United States on several *Pinus* species. It is still being developed.

3. **Biochemical tests**
   a. Tetrazolium chloride (TZ) staining is primarily used to rapidly estimate germination, but some analysts interpret it for seed vigor also. Location, rather than intensity of the stain, is most important. It is a subjective interpretation that takes years of practice to master.
   b. Adenosine triphosphate (ATP) activity is a laboratory test that has occasionally shown good correlation with seed vigor

but is not widely used. Some trials have been run with conifers in the United States.

c. Glutamic acid decarboxylase activity (GADA) is an enzyme activity test that measures carbon dioxide output. It was tested with hardwoods in the United States with poor results.

d. Oxygen uptake (respiration) is a laboratory test that requires respirometers; results have generally been mixed with tree seeds.

e. Leachate test —Deteriorating membranes allow many cellular substances to leach out when seeds are soaked in water. The amount of these substances can be related to seed vigor; for example:

(1) Sugars, tested with *Picea glauca* in Canada and *Pinus* in the United States.

(2) Amino acids, tested with *Pinus* in Canada.

(3) Electrolytes. This method is by far the easiest. It is probably more useful as a rapid test for estimating germination (see previous section).

4. **Germination data** — **Another** approach is to use germination test data, although more frequent counts than ISTA requires may be needed to achieve the required sensitivity.

a. Modeling. Mathematical modeling of the germination response allows quantitative comparisons of frequency distributions.

(1) Normal distribution — If there is no dormancy, frequency distribution of germination should be normal (bell-shaped curve). If dormancy is present, the curve will be skewed right (Janssen 1973).

(2) Polynomial regressions for curve fitting (Goodchild and Walker 1971).

(3) Logistic function (Schimpf and others 1977).

(4) Probit transformation — Cumulative germination percentages are transformed to probits and plotted against the germination rate instead of test period in days. This calculation should give a straight line if no dormancy is present. Some good results have been obtained with conifers (Campbell and Sorensen 1979).

(5) Weibull function — A three-parameter function that can quantitatively describe curve shape and beginning and ending of germination on a time scale. Research has studied both hardwood and conifer species (Bonner 1986b, Bonner and Dell 1976, Rink and others 1979).

b. Germination rate.

(1) Early counts —As prescribed by ISTA, the first (early) count can be used as a vigor indicator.

(2) Percentiles — Time required to reach 50 percent, 75 percent, etc., of germination is calculated. Similar to mean germination time (below).

(3) Mean germination time (MGT)— Can be useful in some cases. However, slow germination because of dormancy may inflate the value by giving equal weight to the very last seedlings to emerge; in the nursery, these seedlings may never germinate.

(4) Germination value (GV) and peak value (PV) (Czabator 1962):

$$GV = PV(MDG)$$

where, PV equals the largest quotient of cumulative germination at day x, divided by x, and *MDG* equals the mean daily germination, or final germination percentage divided by total test days. Germination value combines elements of rate and completeness of germination. These expressions, more than any other, are used worldwide. Peak value is a good germination-rate term to express vigor in temperate species.

G. Recommendations For Tree Seeds

1. Germination rate parameters are best overall. Peak value may be best, but others are good also.
2. Seedling growth tests are good if facilities are available.
3. Tetrazolium (TZ) staining can be good for large, tropical seeds, if interpretations can be standardized.
4. Accelerated aging shows promise, but general recommendations are not yet available for tree seeds.
5. Leachate conductivity also shows promise and deserves more attention. Its nondestructive nature and inexpensive equipment requirements are attractive.

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