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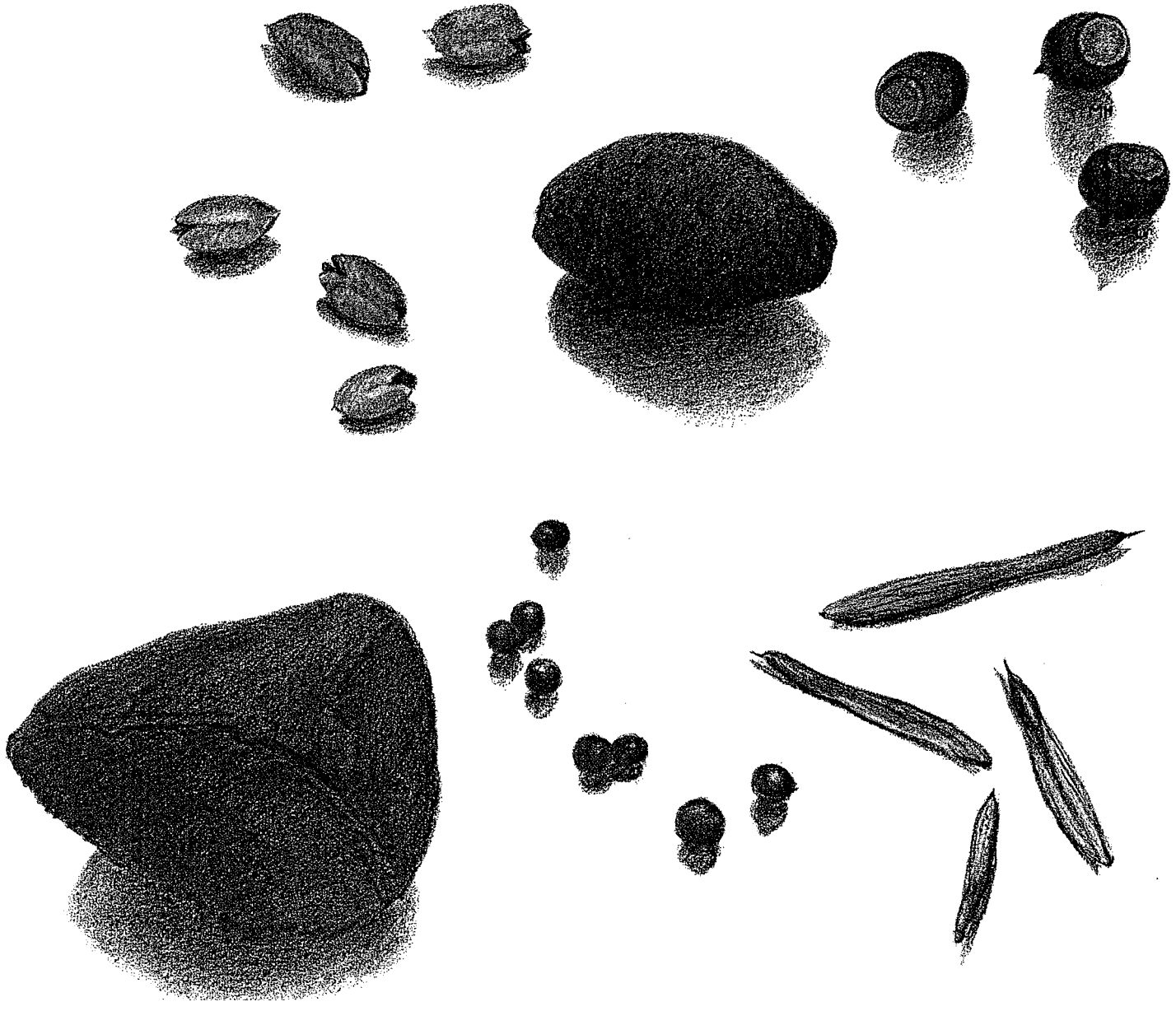
Tree Seed Technology Training Course

Instructor's Manual

F. T. Bonner, J. A. Vozzo, W. W. Elam, and S. B. Land, Jr.



SUMMARY

This manual is intended primarily to train seed collectors, seed-plant managers, seed analysts, and nursery managers, but it can serve as a resource for any training course in forest regeneration. It includes both temperate and tropical tree species of all intended uses. The manual covers the following topics: seed biology, seed collection, seed handling, seed-quality evaluation, seed protection, seed basics for nurseries, and seed programs. It also includes a course evaluation questionnaire and practical exercises.

All parts of the course will not be suitable for all groups. The instructor can choose the pertinent material for presentation. The scope of material is wide enough to serve many purposes.

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Introduction

OFFICIAL WELCOME

Introductory remarks and welcoming speeches from the host institution and government representatives come first. Take care of all administrative matters and housekeeping items at this time. Be sure to include an orientation to the site and facilities. A map handout is desirable.

INTRODUCTION OF INSTRUCTORS AND STUDENTS

First instructors, then students, stand and tell the group:

a. Name
b. Affiliation and title (if any).
c. Job assignment at home (e.g., agroforestry or traditional forestry).
d. What they hope to learn in this course (skills, species information, personal contacts, etc.).

GOALS OF THE COURSE

The objective of this course is to provide a basic understanding of the following topics:

A. Reproductive cycles, from flowering through seed germination
B. Seed origin
C. Seed collection
D. Seed maturity
E. Collection and postharvest care
F. Seed extracting, cleaning, and conditioning
G. Insect and disease problems
H. Seed storage
I. Seedlot sampling
J. Tests for moisture, purity, weight, germination, and vigor
K. Rapid viability estimates
L. Seed test results
M. Seed handling in nurseries
N. Seed programs
0. Seed labeling and certification
P. Germplasm conservation
Q. Seed center design and staffing
R. Applied seed research (fig. 1) [no equivalent figure in Student Outline1

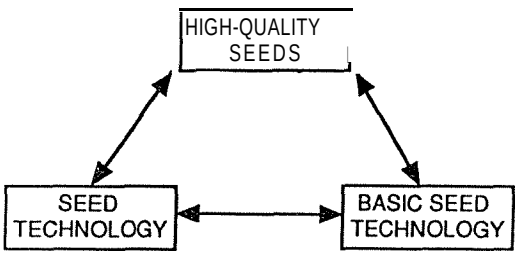


Figure 1. -*The relationship of seed technology,* basic *seed biology, and high-quality seeds [no equivalent figure in Student Outline],*

All figures and tables in the Instructor's Manual are not presented in the Student Outline. In order for the instructor to reference all figures and tables in the student book, these differences are noted throughout the manual.

SCOPE

The emphasis in this course will be on indigenous, multipurpose tree species suited for forestry and rural agroforestry. However, exotics are included, because fast-growing exotics have a definite place in forestry programs.

SOURCES OF INFORMATION AND TECHNICAL HELP

Sources of information and technical help include journals, reference books, international organizations, regional organizations, and research institutions.

Journals

Seed Science and Technology
International Seed Testing Associati(ISTA)
Reckenholz, P.O. Box 412, CH-8046
Zurich
Switzerland

Journal of Seed Technology
Association of Official Seed Analysts (AOSA)

Seed Abstracts
CAB International Information Services
Wallington, Oxon OX 10 8 DE
UK

Agroforestry Abstracts
(same as above)

New **Forests**
Dr. Mary Duryea, Editor-in-Chief
Department of Forestry
118 N-Z Hall
University of Florida
Gainesville, FL 32611
U.S.A.

Indian Forester
(includes seed technology articles)

Indian Journal of Forestry
(includes seed technology articles)

Journal of Tropical Forest Science
Business Manager
Forest Research Institute of Malaysia
P.O. Box 201
Kepong, 52109 Kuala Lumpur
Malaysia

Commonwealth Forestry Review
Commonwealth Forestry Institute
11 Keble Road
Oxford
UK

Pakistan Journal of Forestry
Editor
P.O. Pakistan Forestry Institute
Peshawar, N.W.F.P.
Pakistan

Canadian Journal of Forestry Research
Editor
Forestry Canada
P.O. Box 490
Sault Ste. Marie, Ontario
P6A 5M7
Canada

Forest Science
Society of American Foresters
5400 Grosevenor Lane
Bethesda, MD 20814-2198
U.S.A.

Reference Books

The following reference books provide information and technical assistance:

Bewley and Black 1982
Chin and Roberts 1980
Murray 1984a, b
Schopmeyer 1974
von Carlowitz 1986
Willan 1985

International Organizations

Food and Agriculture Organization (FAO) of the United Nations
Forest Resources Development Branch
Forest Resources Division
Via delle Terme di Caracalla
I-00100 Rome
Italy

International Union of Forestry Research Organizations (IUFRO)
Secretariat
Schonbrunn
A-1131 Vienna
Austria

IUFRO Seed Problems Project Group
Current Chair: Dr. D.G. Edwards
Forestry Canada
Pacific Forestry Centre
506 West Burnside Road
Victoria, BC
V8Z 1M5 Canada

International Seed Testing Association (ISTA)
ISTA Secretariat
Reckenholz, P.O. Box 412
CH-8046 Zurich
Switzerland

Regional Organizations

F/FRED Coordinating Unit
c/o Kasetsart University
Faculty of Forestry
P.O. Box 1038, Kasetsart Post Office
Bangkok 10903
Thailand

Organization for Economic Cooperation and Development (OECD)
Directorate for Agriculture and Food
Paris
France

Research Institutes

ASEAN-Canada Forest Tree Seed Centre
Mauk Lek, Saraburi
Thailand

Nitrogen Fixing Tree Association
P.O. Box 680
Waimanalo, HI 96734
U.S.A.

International Council for Research in Agroforestry (ICRAF)
P.O. Box 30677
Nairobi
Kenya

DANIDA Forest Seed Centre
Krogerupvej 3A
DK 3050, Humlebaek
Denmark

Forest Research Centre
P.O. Box HG 595
Highlands, Harare
Zimbabwe

Centre National de Semences Forestieres
PB 2682, Ouagadougo
Burkina Faso

CSIRO Division of Forest Research
P.O. Box 4008
Queen Victoria Terrace
ACT 2600, Canberra
Australia

Commonwealth Forestry Institute (CFI)
University of Oxford
Department of Forestry
Oxford
UK

USDA Forest Service
Tree Seed Research Unit
Forestry Sciences Laboratory
P.O. Box 906
Starkville, MS 39759
U.S.A.

USDA Forest Service
National Tree Seed Laboratory
Rt. 1, Box 182-B
Dry Branch, GA 31020
U.S.A.

USDA Forest Service
Institute of Tropical Forestry
University of Puerto Rico, Agricultural Experiment Station
P.O. Box 25000
Rio Piedras, PR 00928-2500
U.S.A.

Centro Agronomieo Tropical de Investigacion y Ensenanza (CATIE)
Turrialba
Costa Rica

Petawawa National Forestry Institute
Box 2000
Chalk River, Ontario
KOJ 1JO Canada

GROUP ASSIGNMENTS

If library and study facilities are available to the trainees, a short report should be assigned. A wide range of topics is possible, but those of a general nature are best. A lot may depend on the participants' level of education and training and the amount of free time available to them.

Suggested Topics

Species reports on how to collect, clean, store, and test seeds of a designated species. The information can be developed during the course and may encourage classroom participation by the trainees. This topic is appropriate for technicians.

Country or province status reports on the current status of tree seed needs and the biggest obstacles to filling them in the trainee's country, province, state, village, and so forth. This topic is appropriate for administrators.

Collection plans for a species or a group of species to supply seeds for a country or province planting program of a designated size.

PRESENTATIONS

Near the end of the course, reports should be presented to the class, four or five per day or all in a special period. Oral presentations (10 minutes maximum) should be emphasized, but written reports should be required with at least an outline of the material.

ADMINISTRATIVE MATTERS

Take time to explain administrative matters, such as currency exchange, fee payment, housing, meals, transportation, coffee/tea breaks, class schedules, and so on. The local administrator of the host agency or institution should convey this information. During this period, also preview any scheduled field trips, tours, or shopping trips. Tell when, where, what to wear, and so on. Finally, allow a short time for questions from the group on any topic related to the course. Give everyone a chance to understand how the course will be conducted.

Biology

I. Flowering, Pollination, and Seed Maturation

A. Introduction

Knowledge of the seed biology of a tree species is essential to successful seed production and handling. The sexual life cycle must be known to plan for genetic improvement, production, collection, conditioning, storage, and planting of the seeds.

B. Objectives

1. Define common terms used to describe life cycles of plants.
2. Describe the general sexual cycle, flower structure, seed structure, and origin of the fruit of gymnosperms.
3. Describe the general sexual cycle, flower structure, seed structure, and origin of the fruit of angiosperms.
4. Identify primary differences between angiosperm and gymnosperm sexual cycles.
5. Describe the general development of fruits and seeds.

C. Key Points

The following points are essential for understanding flowering, pollination, and seed maturation:

1. A plant's life cycle is the time required to grow from zygote to seed production; there are two developmental cycles- a sexual cycle and an asexual cycle.
2. Knowledge of the sexual cycle is required for:
   a. tree-breeding programs
   b. seed orchard management
   c. seed collection
   d. seed conditioning and storage
   e. nursery management
3. The gymnosperm life cycle follows this order:
   a. naked seed
   b. seedling
   c. mature sporophyte
   d. strobili (cones)
   e. microspore and megaspore mother cells
   f. meiosis
   g. microspores and megaspores
   h. male and female gametophytes
   i. pollination
   j. single fertilization
   k. zygote and gametophytic tissue
   1. embryo
   m. naked seed on ovulate cone scale
4. The angiosperm life cycle differs from the gymnosperm life cycle in having:
   a. seeds enclosed in fruit (ripened ovary)
   b. true flowers rather than strobili
   c. double fertilization
   d. triploid endosperm tissue rather than haploid female gametophytic tissue in the seed

D. Definition of Terms

1. **Life cycle-the** time required to progress from zygote to seed production. There are two possible developmental cycles-the sexual cycle involving seeds (used in regeneration of "high forest" systems) and the asexual cycle involving vegetative propagules of coppice forestry (used in regeneration of "low forest" systems).
2. **Genotype-** the genetic makeup of a cell nucleus or an individual.
3. **Phenotype-the** external appearance of an organism; it is an expression of the genotype interacting with the environment.
4. **Mitosis-nuclear** (and usually cellular) cell division in which the chromosomes duplicate and divide to produce two nuclei that are identical to the original nucleus.
5. **Meiosis-two** successive nuclear divisions in which the chromosome number is halved and genetic segregation occurs; it occurs in reproductive cells during sexual reproduction.
6. **Pollination-transfer** of pollen grains from the anther or microsporophyll to the stigma or ovule.
7. **Fertilization-fusion** of sperm and egg (and also sperm with two polar nuclei to form endosperm in angiosperms).
8. **Diploid** (2N) -two sets of chromosomes in a cell nucleus. Somatic cells have two sets and are diploid (except for polyploid plants).
9. **Haploid** (1N) – one set of chromosomes in a cell nucleus. Germ cells (egg and sperm cells) from a diploid plant are haploid.
10. **Fruit-a** ripened ovary, sometimes including accessory flower parts, that surrounds the seed in angiosperms. The term "fruit" is used to refer to any seed-bearing structure; thus, it can include the ovulate cone of fleshy aril of the conifers.
11. **Seed-** a ripened ovule that consists of an embryo, its stored food supply, and protective coverings. The term "seed" is also often used to include the fruit wall of one-seeded, dry, indehiscent fruits, such as achenes and nuts. The "seed of commerce" is the reproductive structure as bought and sold. It may be multiseeded in some species. Examples include *Tectona* and *Liriodendron.*

12. Mature seed-a seed that can be removed from the tree without impairing the seed's germination.

E. Life Cycles

An understanding of life cycles is needed because:

1. Sexual and asexual systems reproduce genetically different populations.
2. Knowledge of the asexual cycle is needed before vegetative propagation can be used for coppice systems, cloning of selected genotpes, conservation of germplasm, direct planting of propagules, or enhancement of flowering in clonal seed orchards.
3. Knowledge of the sexual cycle is needed for success in:
   a. tree breeding
   b. seed production
   c. seed orchard management
   d. seed harvesting and conditioning
   e. nursery management
   f. planting and subsequent management of forest stands

F. Angiosperm and Gymnosperm Sexual Cycles

1. **All tree species** are seed-producing plants (division, Spermatophyta) and belong to either the class Gymnospermae or Angiospermae.
   a. The true flowering plants of angiosperms have seeds enclosed in carpels.
   b. The coniferous gymnosperms have seeds borne naked on scales arranged spirally on a central axis to form a cone.
   c. Seeds of nonconiferous gymnosperms are borne singly, each enclosed in a fleshy, arillike covering.

2. Gymnosperm life cycle (fig. 2) [figure 1 in Student Outline]

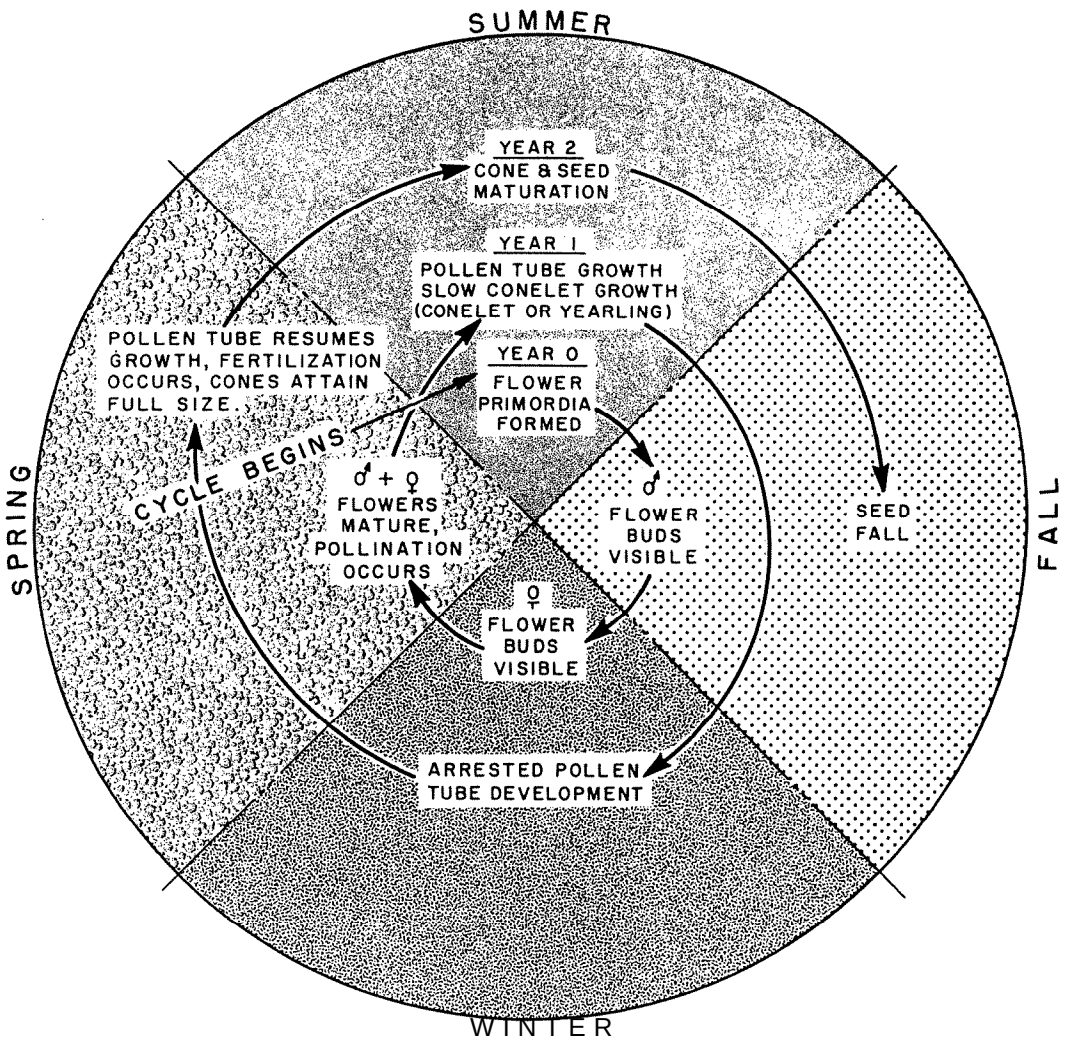


Figure 2. *-Life cycle of a gymnosperm* (Pinus *spp.) (Banner 1991b) [figure 1 in Student Outline].*

a. Sporophyte – The diploid plant (tree) that arises from the zygote.

b. Strobilus or cone- Using a pine from the Conifer-ales as an example:

(1) Reproductive short shoot (not a "flower"-a term that should be limited to angiosperms).

(2) Staminate cone (male) -axis with spirally arranged microsporophylls and two microsporangia on the bottom side of each sporophyll.

(3) Ovulate cone (female) -axis with spirally arranged bracts and ovuliferous scales, Two ovules are located on the upper side near the base of each scale.

(4) Gymnosperms may be either monoecious (female and male strobili on same tree) or dioecious (tree has only one sex).

c. Meiosis and gametophytes

(1) Diploid microspore mother cell in microsporangium undergoes meiosis to produce four haploid microspores (pollen grains). After pollination, the pollen grain germinates to produce a six-nucleate male gametophyte, two sperm nuclei, two generative nuclei, tube nucleus, and stalk nucleus).

(2) Diploid megaspore mother cell in the megasporangia of the ovule undergoes meiosis to produce four haploid megaspores, of which three deteriorate. The remaining megaspore undergoes many mitotic nuclear divisions, has a free nuclei stage, and finally forms cell walls to produce a haploid female gametophyte (archegonia with egg and gametophytic tissue).

d. Fertilization-A sperm cell from the male gametophyte unites with the egg cell to form the diploid zygote (single fertilization).

e. Seed (fig. 3) [figure 2 in Student Outline]

(1) Develops from the fertilized ovule.

(2) Contains an embryo (cotyledons, hypocotyl, radicle) (2N from sexual recombination), a seedcoat (2N of mother plant, from integuments), storage tissue (1N from female gametophyte), and sometimes a seed wing (2N from mother plant, from upper surface of ovuliferous scale>.

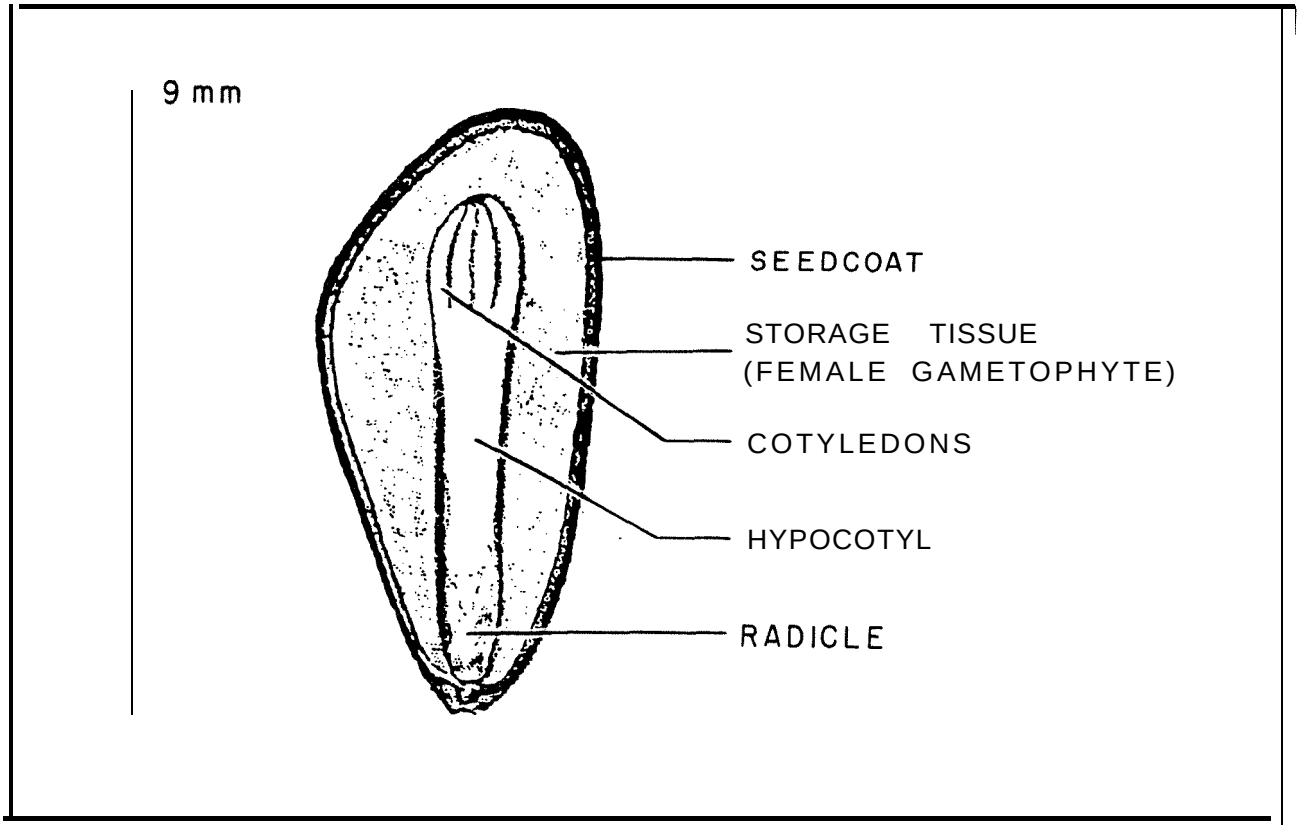


Figure 3. -*Cross section of a typical mature gymnosperm seed* (Pinus ponderosa) *(adapted from Krugman* and *Jenkinson 1974) [figure 2 in Student Outline].*

f. Fruit
(1) Gymnosperms do not have true "fruits" (matured ovaries).
(2) The types of structures that enclose gymnosperm seeds are:
(a) dry ovulate cones *(e.g., Abies, Araucaria, Cupressus, Pinus,* and *Tsuga)*
(b) fleshy, arillike structures enclosing single seeds (e.g., *Ginkgo, Taxus,* and *Torreya)*
(c) berrylike ovulate cones (e.g., *Juniperus* -one to two seeds per cone)

3. Angiosperm life cycle

a. Sporophyte — the diploid plant (tree) that arises from the zygote (same as gymnosperm).
b. Flower-a short shoot with sterile and reproductive leaves and a receptacle.
(1) Sterile leaves include:
(a) sepals (outer whorl), collectively, corolla
(b) petals (inner whorl), collectively, corolla
(c) perianth, a collective term for sepals and petals
(2) Reproductive leaves (sporophylls) include:
(a) stamen (male), composed of anther (a pollen sac containing microsporogenous tissue) and filament
(b) carpel (female), composed of stigma, style, and ovary. The ovary is the basal portion of a pistil that bears the ovules and its surrounding carpel wall. The ovule is composed of megasporogenous tissue surrounded by the nucellus, two integuments, and the micropyle.
(c) pistil, a collective term that describes visible female structures, whether a single carpel or a fusion of many carpels.
(3) Receptacle, a stem axis of the flower.
(4) Flowers may have one or both sexes.
(a) Perfect flowers contain both stamens and pistils.
(b) Imperfect flowers contain only one sex, and the tree may be either monoecious or dioecious.
(c) Polygamous includes some perfect and imperfect flowers on the same tree.
c. Meiosis and gametophytes
(1) Diploid microspore mother cell in microsporogenous tissue of anthers undergoes meiosis to produce four haploid microspores (pollen grains). After pollination, the pollen grain germinates to produce a six-nucleate male gametdphyte (same as for gymnosperms).
(2) Diploid megaspore mother cell in megasporogenous tissue of ovule undergoes meiosis to produce four haploid megaspores, of which three deteriorate. The remaining megaspore undergoes three mitotic divisions and forms cell walls to produce a haploid female gametophyte (eight-nucleate, seven-celled embryo sac containing three antipodal **cells,** two polar nuclei, two synergid cells, and the egg).
d. Fertilization
(1) A sperm cell unites with the egg cell to form the diploid zygote (one fertilization).
(2) The second sperm cell unites **with** the two polar nuclei (triple fusion) to form a triploid (3N) endosperm nucleus (second fertilization).
e. Seeds (figs. 4, 5) [no equivalent figures in Student Outline]
(1) Seeds develop from the double-fertilized ovule.
(2) They contain an embryo (2N from sexual recombination), seed coat, storage tissue (may be 2N cotyledons, hypocotyl from embryo, 3N endosperm from triple fusion, or 2N perisperm from nucellus of mother tree), and sometimes other seed coverings (2N of mother tree from remains of nucellus, 3N from remains of endosperm, or 2N of mother tree from attached parts of fruit).
(3) They may be classified as endospermic (embryo is reduced in size compared with the rest of the seed) or nonendospermic (embryo is dominant).
f. Fruits
(1) Fruits develop from the matured ovary (and sometimes from the receptacle or perianth); thus, the ovary has the mother tree's diploid genotype.
(2) They enclose the seed (matured ovule).

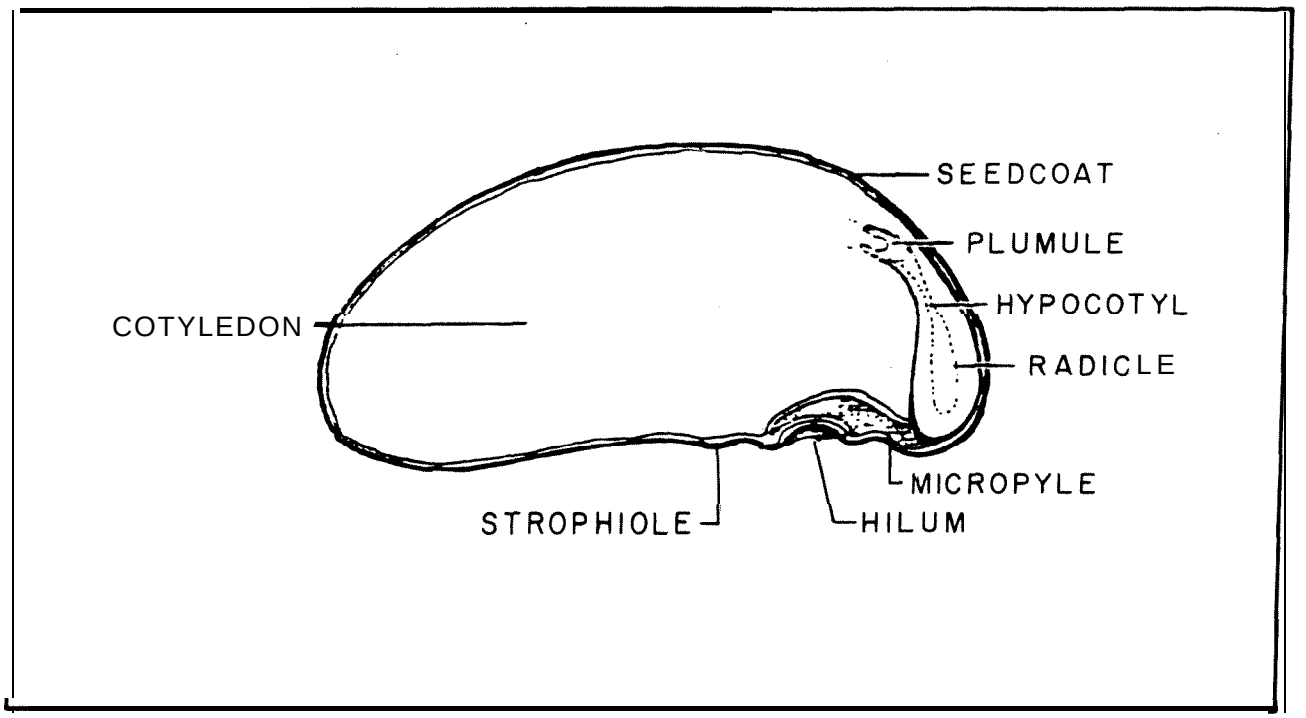


Figure 4. *-Cross section of a mature seed of* Centrolobium paraense *(adapted from Triviño and others 1990) [no equivalent figure in Student Outline].*

(3) It is not always possible to separate the fruit and the seed when the seedcoat and fruit are joined as a single unit. In this case, the fruit itself is referred to as the "seed."

4. **Sexual cycles** -The gymnosperm and angiosperm sexual cycles differ in four ways:
   a. In gymnosperms, seeds are not enclosed in the ovary, and flowers are unisexual; in angiosperms, seeds are borne in a closed ovary, and flowers are perfect or imperfect.
   b. Flowers are true flowers in angiosperms but are strobili (cones) in gymnosperms.
   c. Double fertilization takes place in angiosperms; single fertilization takes place in gymnosperms.
   d. In gymnosperms, the developing embryo is nourished by the haploid female gametophyte; in angiosperms, it is nourished from either diploid cotyledons, hypocotyl of the embryo, triploid endosperm, or diploid nucellar material.

G. Seed and Fruit Development

1. Physical development

a. Angiosperms

(1) Pollination and fertilization trigger:

(a) Formation of embryo and endosperm within ovules.

(b) Cell divisions and enlargements in ovary and peripheral tissues leading to production of fruit. Most angiosperms flower and ripen in one growing season (except for the red oak subgenus of ***Quercus).***

(2) Legumes have:

(a) simple pistil (one) with a superior ovary having one cavity (locule). When the pistil matures, a fruit (pod) is produced-a single, dry, dehiscent fruit. Seeds develop along the side of the pod where the carpel margins join. The other side is the midrib, where the pod splits open. A typical seed is dicotyledonous and lacks endosperm. Each seed is attached to the pod by the seedstalk (funiculus). As the seeds leave the pod, the seedstalk breaks off, leaving a scar (hilum). Above the hilum is the raphe; below is the micropyle (fig. 6) [figure 3 in Student Outline].

(b) Seedcoats are composed of a histologically dense cuticle, radial columnar cells, sclerenchy-

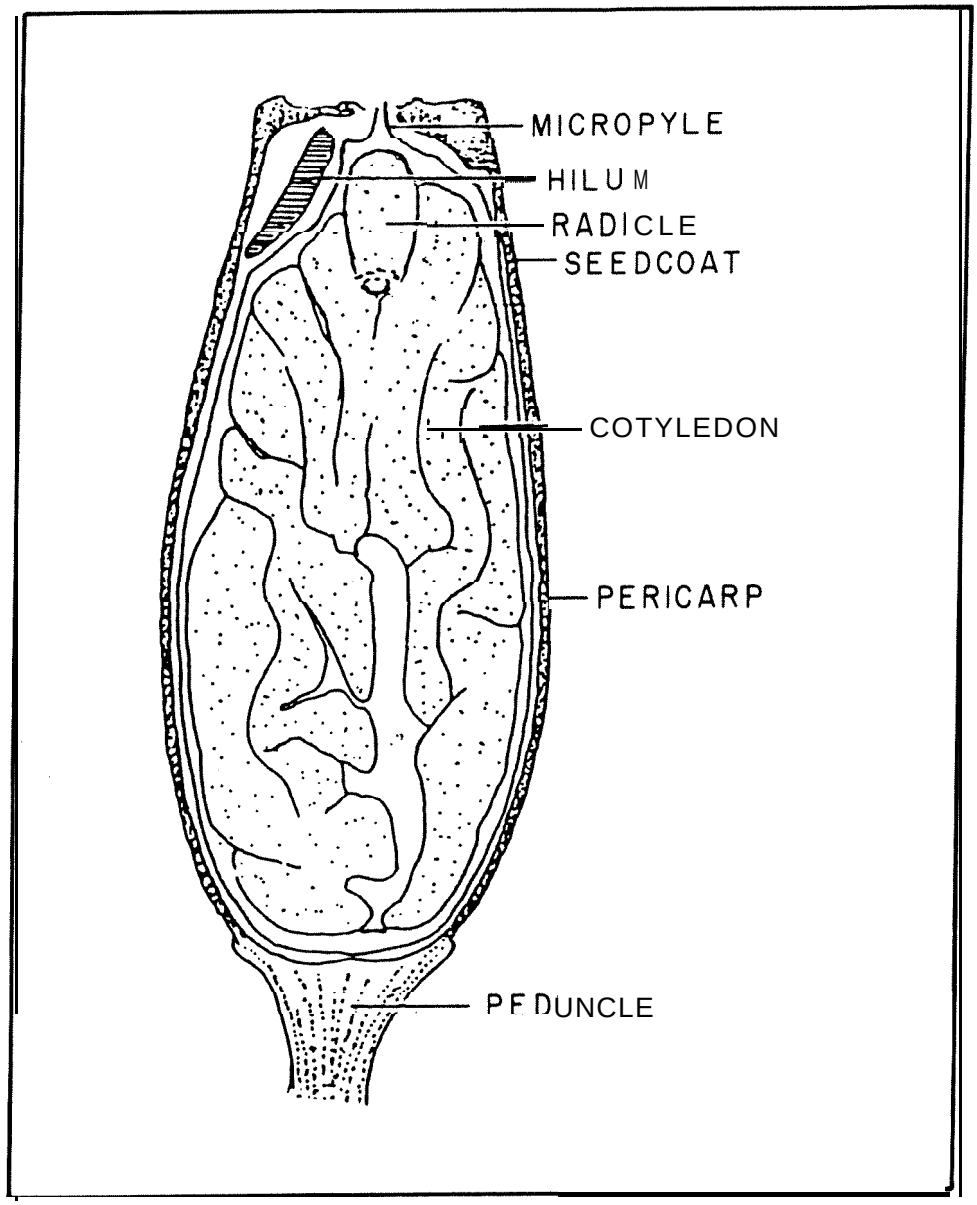


Figure *5. -Cross section of* **a** *mature seed of* **Cordia** alliodora *(adapted from Triviño and others 1990) [no equivalent* **figure** *in Student Outline].*

matous cells, lignin, and osteosclereid cells. These seedcoats are highly impervious to water and gases. Malpighian cells internal to the palisade layer are characterized as sclerenchyma without intercellular spaces -very dense cells, protecting the seed and denying water uptake. The "light line" in the palisade cells is caused by wax globules, also a water barrier (See fig. 7) [figure 4 in Student Outline].

(3) Definition of Terms
- (a) **cuticle-** waxy layer on outer walls of epidermal cells
- (b) **lignin –** organic component of cells associated with cellulose
- (c) **light line** -continuous thin layer of wax globules
- (d) **osteosclereid-** bone-shaped sclerenchyma
- (e) **palisade cells –** elongated cells perpendicular to the coat surface
- (f) **parenchyma-** undifferentiated, live cells

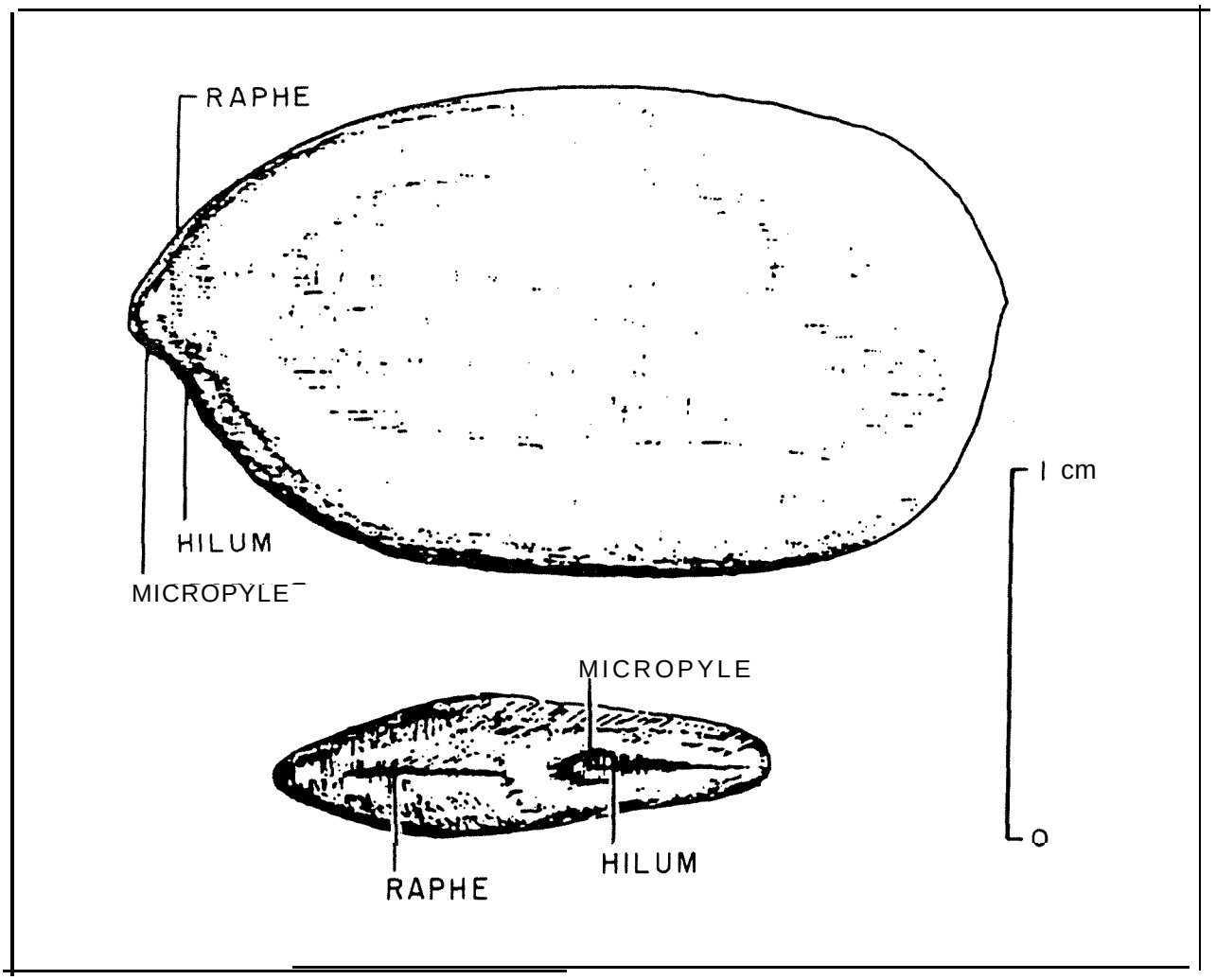


Figure *6. -External morphology of a typical legume seed of* **Schizolobium parahybium** *(adapted from Triviño and others 1990) [figure 3 in Student Outline].*

(g) sclerenchyma – thick, lignified cells

b. Gymnosperms – Fertilization stimulates growth of conelets and development of the embryo; female gametophytic tissue is already present when fertilization occurs. Many conifers flower and ripen seeds in one growing season, but some require two seasons, and a few require three seasons (pines require 2.25 years from formation of flower primordia to seed dispersal) (fig. 2) [figure 1 in Student Outline].

2. Physiological development

a. Moisture content increases rapidly after fertilization and decreases at maturity. There is evidence that a desiccation period is required before all germination and growth enzymes can be synthesized in orthodox seeds (fig. 8) [no equivalent figure in Student Outline]. In recalcitrant seeds there is only slight desiccation before maturity (fig. 9) [no equivalent figure in Student Outline].

b. Hormone contents are higher where meristematic activity is greater, that is in immature (maturing) seeds.

c. Metabolic changes are many; simple sugars, fatty acids, and amino acids are converted to proteins, oils, and lipids (fig. 10) [no equivalent figure in Student Outline].

3. Classification of mature fruits (see table 1) [table 1 in Student Outline]

H. Sources

For additional information, see Dogra 1983; Hardin 1960; Hartmann and others 1983, chap. 3, p. 59-65; Krugman and others 1974; Willan 1985, p. 7–10, 13–15.

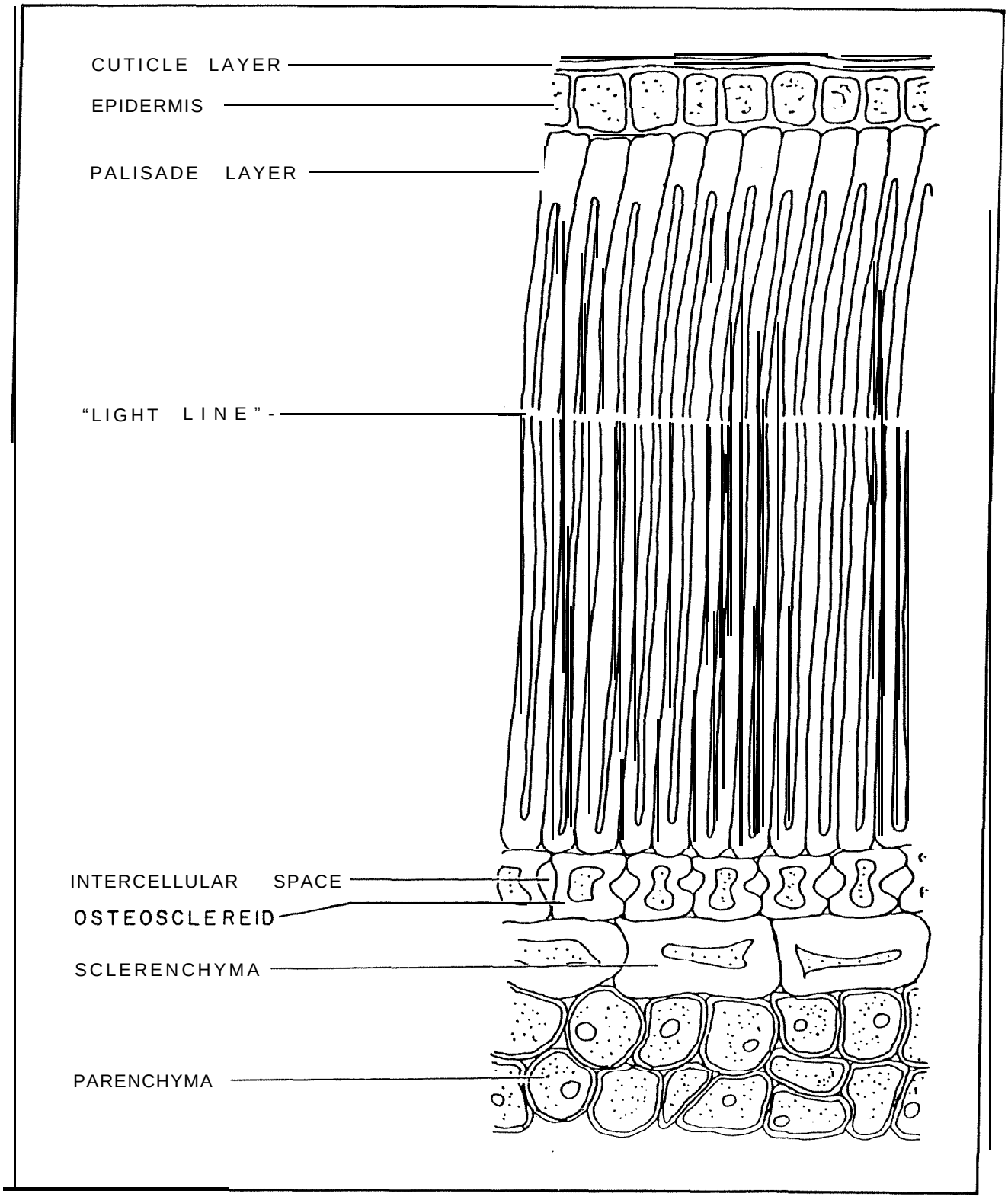


Figure '7. -Partial *section through the seedcoat of* a *hard seed (legume) [figure 4 in Student Outline].*

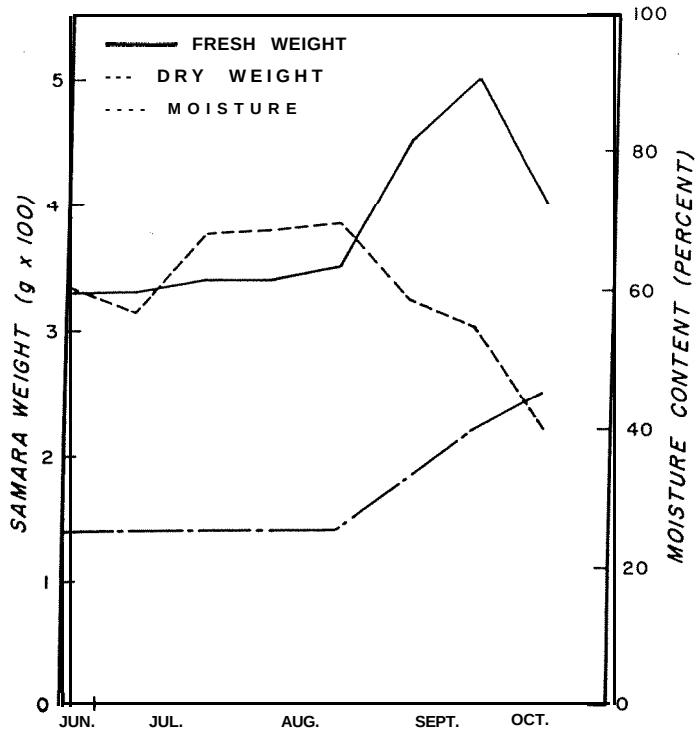


Figure *8. -Seasonal changes in fresh weight, dry weight, and moisture content during maturation of samaras of* Fraxinus pennsylvanica *(adapted from Bonner 1973) [no equivalent figure in Student Outline].*

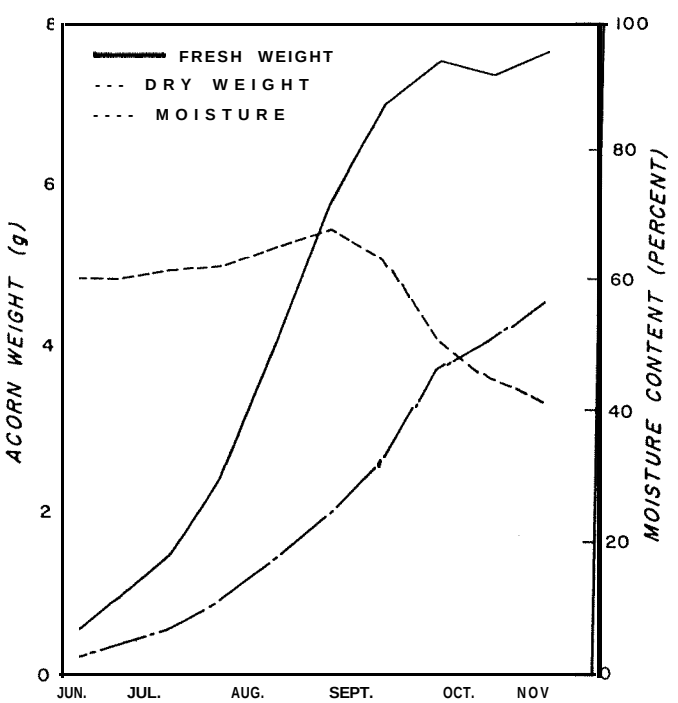


Figure 9.-Seasonal *changes in fresh weight, dry weight, and moisture content during maturation of* Quercus shumardii *acorns (adapted from Bonner 1976) [no equivalent figure in Student Outline].*

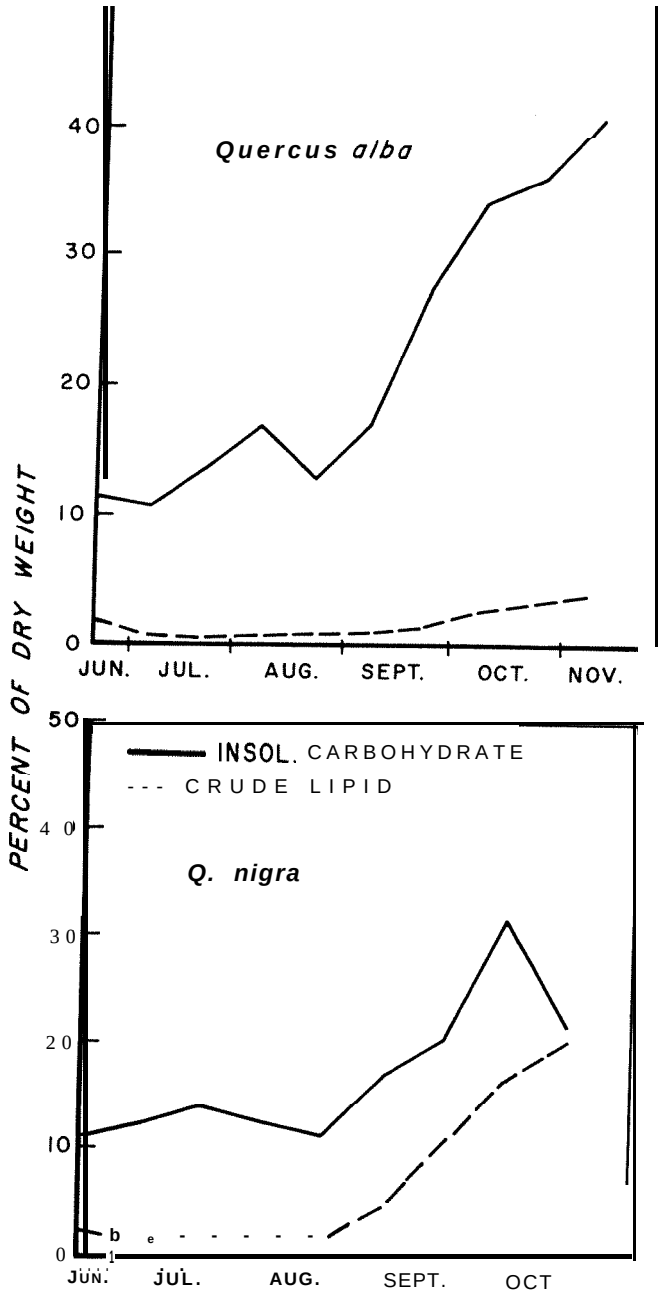


Figure 10. *-Changes in insoluble carbohydrate* and *crude lipid fractions in maturing acorns of* Quercus alba *and* Q. nigra *(adapted from Bonner 1974b, 1976) [no equivalent figure in Student Outline].*

Table 1.—*Common fruit types for woody trees (adapted from Hardin 1960) [table 1 in Student Outline]*

Description	Type	Example
Simple Fruit (product of single pistil)		
Dehiscent walls (splitting naturally)		
Product of one carpel		
Dehiscing by one suture	Follicle	*Zanthoxylum*
Dehiscing by two sutures	Legume	*Acacia, Prosopiq Robinia*
Product of two or more carpels	Capsule	*Eucalyptus, Populus*
Walls indehiscent (not splitting naturally)		
Exocarp fleshy or leathery		
Pericarp fleshy throughout	Berry	*Vaccinium, Diospyros*
Pericarp heterogeneous		
Exocarp leathery rind	Hesperidium	*Citrus*
Exocarp fleshy		
Endocarp a "stone"	Drupe	*Prunus, Vitex, Tectona*
Endocarp cartilaginous	Pome	*Malus, Crataegus*
Exocarp dry (papery, woody, or fibrous)		
Fruit winged	Samara	*Triplochiton, Terminalia, Acer*
Fruit without wings		
One-loculed ovary; thin wall; small seed	Achene	*Platanus,* Cordia
Several-loculed ovary; thick wall; large seed	Nut	*Quercus*
Compound Fruit (product of multiple pistils)		
Pistils of a single flower	Aggregate	*Magnolia*
Pistils from different flowers (inflorescence)	Multiple	*Platanus*

II. Seed Dormancy

A. Introduction

Once seeds have matured, survival of the species requires that they germinate at a time and place favorable for growth and survival of the seedlings. The mechanism that prevents germination at undesirable times is called dormancy. The mechanics of seed dormancy must be known before nursery practices for overcoming dormancy can be developed to ensure timely germination and uniform growth of the seedlings.

B. Objectives

1. Describe the different types of seed dormancy.
2. Discuss methods for overcoming seed dormancy, both for germination testing and for nursery operations.

c. Key Points

The following points are essential to understanding seed dormancy:

1. To a large degree, dormancy is under genetic control.
2. Environmental conditions during seed maturation can influence the degree of dormancy.
3. Seeds can have more than one type of dormancy mechanism.
4. Postharvest environment can create secondary dormancy.
5. The distinction between "dormancy" and "delayed germination" is not always clear.
6. The least severe treatment to overcome dormancy should be tested first to avoid damage to the seeds; then increasingly severe treatments can be tested as needed.

D. Definition of Terms (Bonner 1984a)

1. **Afterripening –** physiological process in seeds after harvest or abscission that occurs before, and is often necessary for, germination or resumption of growth under favorable environmental conditions.
2. **Dormancy-** a physiological state in which a seed disposed to germinate does not, even in the presence of favorable environmental conditions.
3. **Chilling-** subjection of seeds to cold and moisture to induce afterripening.
4. **Prechilling-** cold, moist treatment applied to seeds to hasten afterripening or to overcome dormancy before sowing in soil or germinating in the laboratory.
5. **Pretreatment** -any kind of treatment applied to seeds to overcome dormancy and hasten germination.
6. **Scarification-weakening** of seedcoats, usually by mechanical abrasion or by brief

soaks in strong acids, to increase their permeability to water and gases or to lower their mechanical resistance to swelling embryos.

7. Stratification-placing seeds in a moist medium, often in alternate layers, to hasten afterripening or to overcome dormancy. Commonly applied to any technique that keeps seeds in a cold, moist environment, it is sometimes used to describe warm stratification (warm incubation).

8. **Delayed germination-** a general term applied to seeds that do not germinate immediately but are not slow enough to be described as dormant.

E. Types of Dormancy

Many different classifications of dormancy have been used by seed scientists. Because there is no universal agreement on the subject, anyone can devise a system. However, most workers accept the following classifications:

1. Seedcoat (or external) dormancy

a. Impermeability to moisture or gases; *e.g., Acacia, Prosopis, Robinia,* and other legumes

b. Mechanical resistance to swelling embryo; e.g., *Pinus* and *Quercus.* (Mechanical resistance frequently contributes to seedcoat dormancy but is seldom the primary factor.)

2. Embryo (or internal) dormancy

a. Inhibiting substances usually within the embryo or surrounding tissues; e.g., *Fraxinus, Ilex,* and *Magnolia.*

b. Physiological immaturity — Some enzyme system or crucial metabolite may not be "in place"; e.g., *Juniperus virginiana.* (Supporting data are weak for this type of dormancy.)

3. Morphological dormancy results from the embryo not being completely developed when the seeds are disseminated; additional growth is required; *e.g., Ilex opaca* and some *Fraxinus* spp. and *Pinus* spp. in northern latitudes or high elevations. Morphological dormancy is similar to physiological immaturity.

4. Secondary dormancy results from some action, treatment, or injury to seeds during collecting, handling, or sowing; e.g.; *Pinus taeda* being exposed to high temperatures and moisture during storage.

5. Combined dormancy results from two or more primary factors, such as seedcoat dormancy and embryo dormancy; e.g., *Tilia,* which has a very hard seedcoat, plus embryo dormancy that requires stratification.

6. Double dormancy results from embryo dormancy in both the radicle and epicotyl. Double dormancy is difficult to demonstrate but apparently common in *Prunus.* In some *Quercus* species, radicles are nondormant, while epicotyls have a dormancy.

F. Overcoming Dormancy

1. **Seedcoat dormancy** — Treatment must increase moisture uptake and gas exchange and ease radicle emergence. Try the gentlest method first; then increase the severity of the method until success is achieved.

a. Cold water soak-Soak seeds in water at room temperature for 24 to 48 hours. This method softens seedcoats and may remove inhibiting substances in the outer coverings. Change the soak water every 12 to 24 hours if inhibitors are present.

b. Hot water soak-Bring water to a boil, put seeds in, remove from heat, and allow to stand until water cools. Hot water softens the seedcoat, and imbibition occurs as the water cools. Some workers prescribe a specific period for the hot water soak, but seed variation prohibits such specific recommendations for most species.

c. Hot wire-Use a heated needle or electric woodburner to burn a small hole through the seedcoats. Hot wire is a promising new method. Once "burned," seeds can be shipped or even stored without damage; the length of storage is still being tested, but at least several months are possible. The method works on all hard-seeded species, not just legumes.

d. Acid treatment -Pour a strong mineral acid over the seeds and mix. (Sulfuric acid is preferred.) Remove the seeds after a time determined by trials with samples, usually 15 to 60 minutes, and wash thoroughly to remove acid from the surface. Seeds scarified by water soaks or acid cannot be returned to storage. Acid treatment has three disadvantages:

(1) Excessive treatment can harm seeds.

(2) Acid is expensive.

(3) Unskilled workers may be injured.

e. Physical scarification — Crack or break the hard seedcoats.

(1) Use hand methods (nicking) with

files, knives, clippers, sandpaper, etc.

(2) Use mechanical methods for large quantities including motor-driven scarifiers, cement mixers with rocks, or the impact machine recently developed by DANIDA (Stubsgaard 1986) for *Prosopis* and Acacia.

2. **Embryo dormancy-** Treatment must overcome physiological barriers within the seeds.

a. Stratification (chilling and prechilling) -Refrigerate fully imbibed seeds at 1 to 5 °C for 1 to 6 months. Primarily for Temperate Zone species, this treatment simulates natural winter conditions after seeds fall. Many nondormant seeds respond to short stratification periods (1 to 2 weeks) with faster and often more complete germination. One benefit that nurseries often overlook is the delay of early germination by low temperature, which produces a big flush of germination when high temperature is encountered (table 2) [table 2 in Student Outline]. During stratification:

(1) Imbibition is completed.

(2) Enzyme systems are activated.

(3) Storage foods change to soluble forms.

(4) Inhibitor/promoter balances change.

b. Incubation/stratification — Some species respond to a short, warm incubation (15 to 20 °C), followed by cold stratification.

c. Chemical treatment-Some species respond to chemical stimuli well enough to justify treatment.

(1) Hydrogen peroxide- Soak for 48 hours in a l-percent hydrogen peroxide solution *(Pseudotsuga menziesii).*

(2) Citric acid-Soak for 48 hours in l-percent citric acid solution, followed by 90-day stratification *(Juniperus, Taxodium distichum).*

(3) Gibberellins -Many species respond in the laboratory, but field results are lacking or rare.

(4) Ethylene -Treat seeds for 48 hours with 0.021 M Ethrel solution (reported to help germination for *Ricirwdendron rautentenii* in South Africa).

d. Light-Provide light treatments (red/far-red mechanism) *(Betula).*

G. Significance

1. **Survival strategy-** Dormancy allows germination during favorable environmental conditions. It also permits seed survival in litter or soil for several years, even in the Tropics.

Table 2. ***-Recommended prechill periods for nursery sowing of some pines of the Southern United States (Bonner 1991b) [Table 2 in Student Outline]***

Pine species	Normal sowing*		Early sowing+	Seed conditions	
	Fresh seed	Stored seed		Deer, dormancy*	Low vigor
			Prechill *(Days)*		
Pinus strobus	30-60	60	60-90	60-90	30
P. taeda	30-60	30-60	60	60-90	20-30
P. palustris	0	0	...§	o-15	0
I? rigida	0	O-30			
P. serotina	0	O-30	...	...	...
P. clausa					
var. *immuginata*	o-15	o-21	...	...	...
var. clausa	0	0	...		...
P. echinata	O-15	O-30	15–30	30-60	0
I? elliottii					
var. elliottii	0	0 3 0		1530	0
var. *densa*	30	O-30			
P. glabra	30	30	...	...	...
P. oirginiana	O-30	30	30	...	...

'Spring sowing when mean minimum soil temperature at seed depth is at least 10 °C

†Early sowing when soil temperatures at seed depth may be below 10 °C.

*Dormancy demonstrated by paired tests or past performance of the seedlot.

§Conditions not encountered with this species.

2. **Genetic factor-Dormancy** in many seeds is under genetic control, particularly those with seedcoat dormancy because seedcoats are maternal tissue. Dormancy has been "bred out" of most agricultural crops.
3. **Multiple causes-** Many species have probably evolved with more than one dormancy mechanism; that is, if one fails, another is in place. *Quercus nigra is* a good example, with probably three separate mechanisms.
4. **Environmental influence -Hot,** dry weather during maturation may increase dormancy, particularly that associated with seedcoats. Research is just beginning to show this effect in trees.

H. Sources

For additional information, see Khan 1984; Krugman and others 1974; Murray 1984b; Nikolaeva 1967; Willan 1985, p. 17-19, chap. 8.

III. Germination

A. Introduction

The goals of seed technology are successful germination and seedling establishment. The two major considerations are the physiology of the seed and the condition of the environment. In the two preceding sections, seed maturation and dormancy were considered. In this section, environmental factors and how they control germination through their interactions with seed biology will be examined.

B. Objectives

1. Describe the two types of germination and their importance in woody plants.
2. Review environmental requirements for germination.
3. Review physiological changes within seeds that lead to germination.
4. Discuss how seed physiology and environmental factors interact in germination.

C. Key Points

The following points are essential to understanding germination:

1. The two types of germination are epigeous and hypogeous.
2. Moisture availability is the primary factor controlling germination.
3. The effects of temperature and light on germination are strongly related.
4. Constant and alternating temperature regimes may lead to similar total germination, but germination is usually faster under alternating regimes.
5. As germination begins, the key to internal processes is the change from insoluble to soluble metabolites. Details of such metabolism are beyond the scope of this course.

D. Types of Germination

1. **Epigeous (epigeal)** germination occurs when cotyledons are forced above the ground. by elongation of the hypocotyl (fig. 11) [figure 5 in Student Outline]; e.g., *Pinus, Acacia, Fraxinus,* and *Populus.*
2. **Hypogeous (hypogeal)** germination occurs when the cotyledons remain below ground while the epicotyl elongates (fig. 12) [figure 6 in Student Outline]; e.g., *Juglans, Quercus,* and *Shorea.*
3. In *Prunus,* both types of germination may be found (fig. 13) [no equivalent figure in Student Outline].

E . Environmental Requirements for Germination

The four primary environmental requirements for germination are moisture, temperature, light, and gases.

1. **Moisture**

a. Imbibition is usually considered the first step in germination; thus, availability of moisture is the first requirement for germination.

b. Uptake typically occurs in the following three phases:

(1) A rapid, mainly physical, initial phase that occurs in dead seeds as well as live ones.

(2) An extremely slow second phase, the "flat" portion of the uptake curve. The greater the dormancy, the longer this phase takes.

(3) **A** rapid third phase that occurs as metabolism becomes very active. Some evidence suggests that splitting of seedcoats by emerging radicles is required for the most rapid examples of this phase.

c. The first phase is imbibitional; it releases energy in the form of heat, displaces gases, and creates great imbibitional pressure via colloidal swelling (proteins).

d. A minimum state of hydration is needed within a seed for mobilization of food reserves and activation of enzyme systems.

e. Minimal requirements for germination are frequently studied with osmotic solutions of mannitol or polyethylene glycol.

(1) The best germination may occur at slight moisture stress (0.005 to

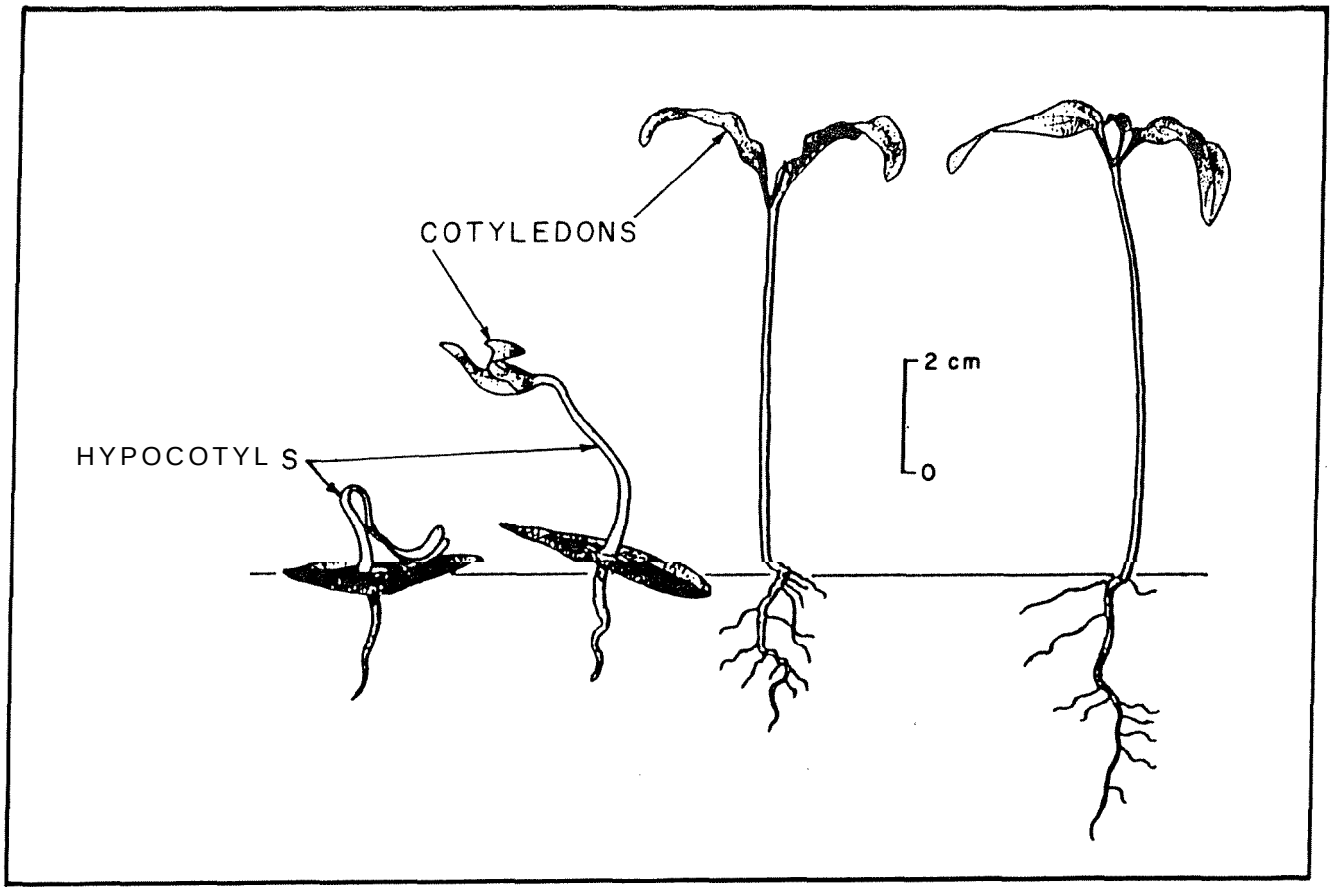


Figure 11. — *Epigeal germination sequence of* Fraxinus *spp. (adapted from Bonner 1974a) [figure 5 in Student Outline].*

0.500 bars); zero stress may form a film of water around the seed, inhibiting oxygen uptake.

(2) Even slightly lowered water potentials will slow, but not stop, germination.

(3) Critical levels of water potential vary by species (table 3) [no equivalent table in Student Outline]. Germination stops in 12 European conifers at − 8 to − 12 bars.

2. Temperature

a. It is difficult to separate the effects of temperature from those of light and moisture.

b. Woody species usually germinate over a wide range of temperatures; the germination rate is strongly temperature dependent. Some Temperate Zone species will eventually germinate in stratification.

c. The upper limit of temperature is around 45 "C. Many species begin germinating above 40 °C, but seedlings are abnormal.

d. The lower limit is around 3 to 5 °C because germination processes will occur near freezing with emergence of radicle and plumule; however, few species can produce normal seedlings under these conditions. *Picea mariana* (black spruce) is one that can.

e. Optimum temperatures are as follows:

(1) For Temperate Zone species, alternating regimes of 20 °C (night) and 30 °C (day) have proved best for many species; similar results can be obtained at constant temperatures of about 25 "C, but germination rates are almost always greater with alternating regimes. Amplitude (10 to 12 °C), not cardinal points, seems to be most important (within limits>.

(2) For tropical species, although few critical studies are available, constant temperatures may be best, although optimums are not necessarily higher than those of temperate species. Examples include: *Azadirachta indica, 25 "C; Bombax ceiba, 25 "C; Eucalyptus camaldulensis, 30* °C; *Leucaena leucocephala, 30* °C; *Prosopis cineraria,*

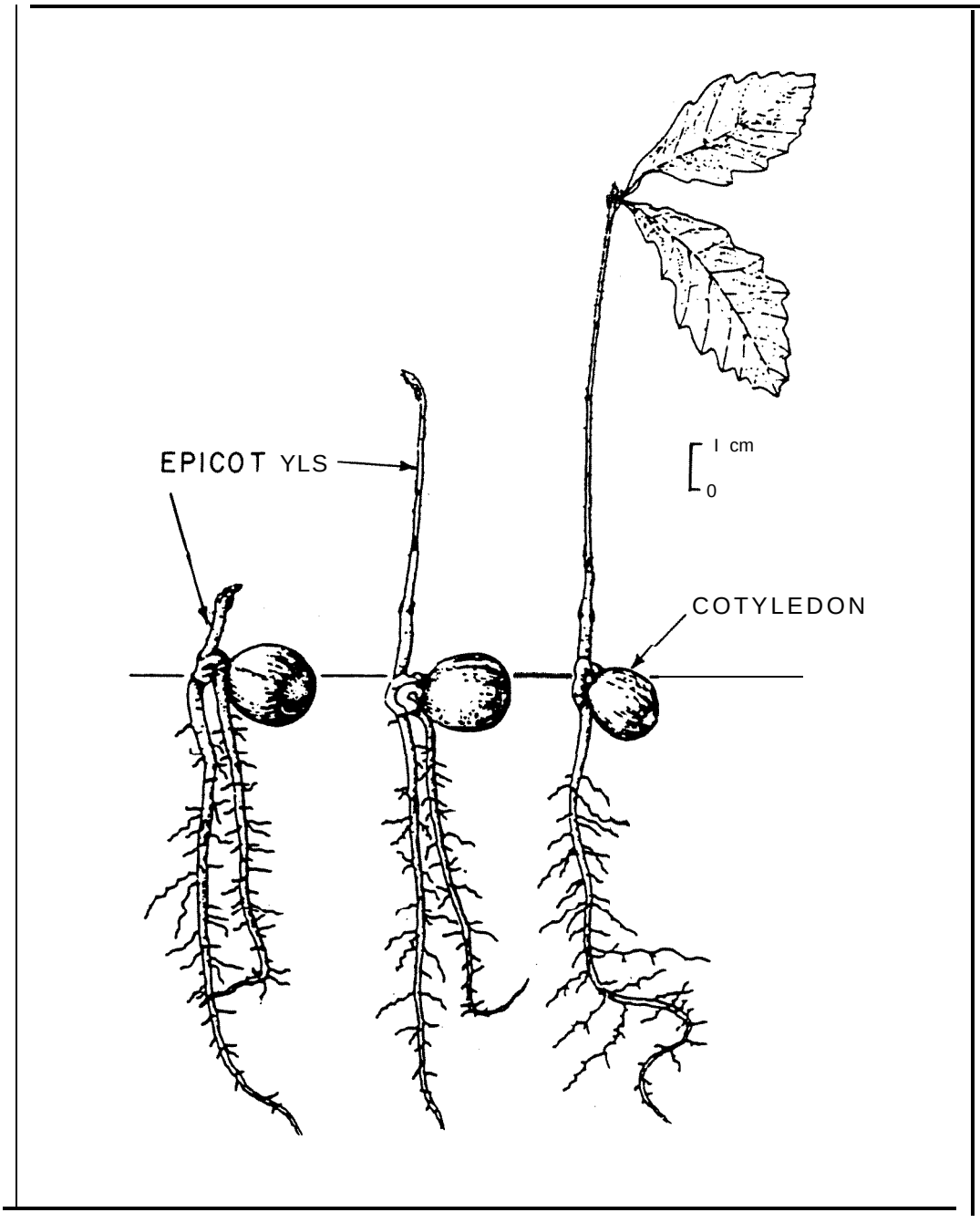


Figure *12. — Hypogeal germination sequence of* Quercus *spp. (adapted from Olson 1974) [figure 6 in Student Outline].*

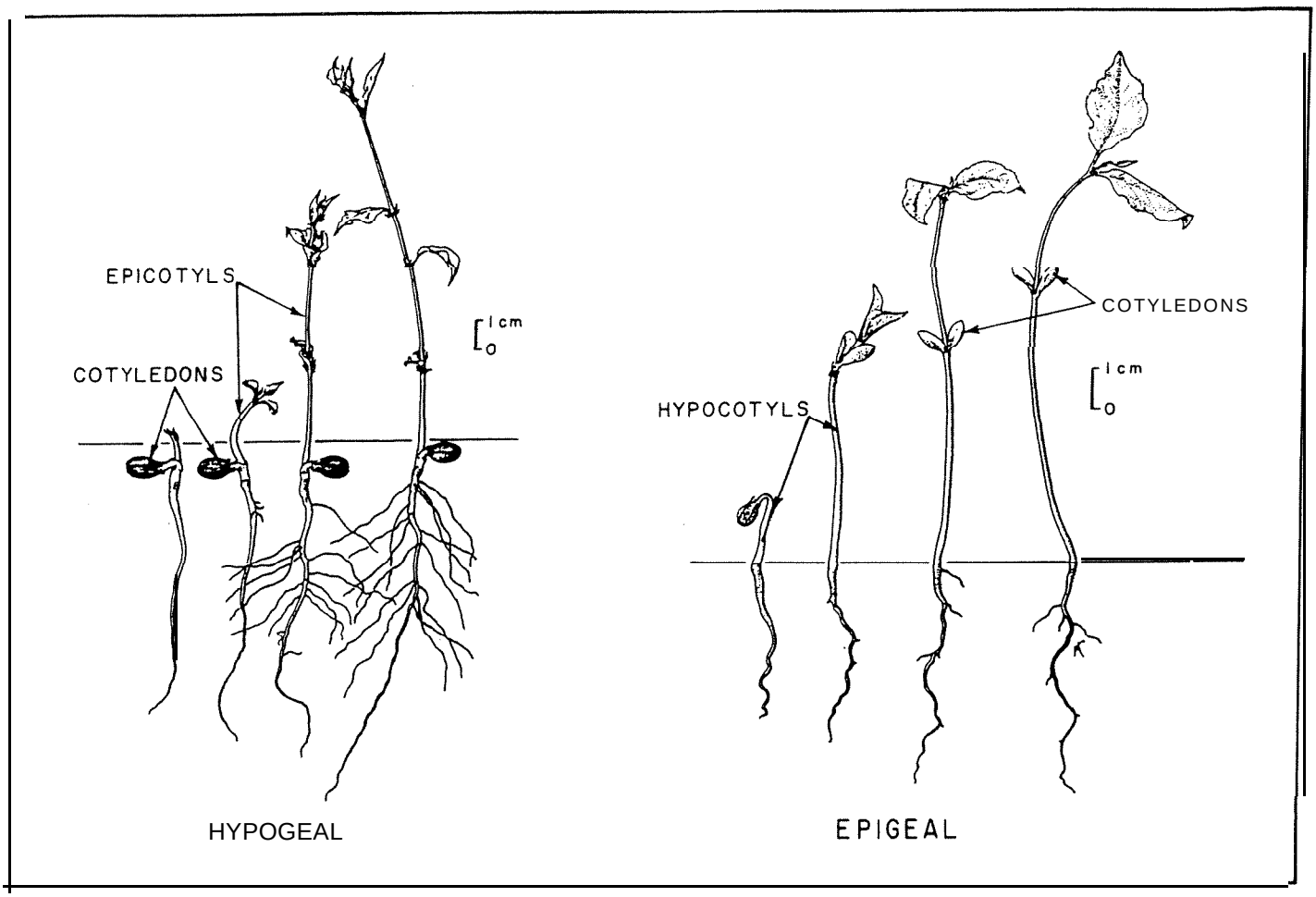


Figure 13. -*Both* epigeal *and hypogeal germination* as *they occur in* Prunus *spp. (adapted* ***from*** *Grisez 1974) [no equivalent* figure ***in*** *Student Outline].*

Table *3. -Critical levels* ***of*** *water potential* ***for*** *germination [no equivalent table in Student Outline1*

Species	Germination: Significantly decreased	Germination: Effectively stopped	Reference
	*Bars**		
Pinus ponderosa	- 4	- 8	Djavanshir and Reid 1975
P. eldarica	- 6	- 12	Djavanshir and Reid 1975
Populus ciliata	- *1*	- 3	Singh and Singh 1983
Quercus palustris	- 5	-20	Bonner 1968

'1 bar equals 0.1 MPa.

30 °C; and *Tectona grandis, 30 °C.* Other species do as well or even better under alternating temperatures; *e.g., Acacia* spp., *Cedrela* spp., and tropical *Pinus* species.

3. **Light**
   a. Light stimulates germination of many tree seeds but is necessary for few, if any. Stratification or high temperature can sometimes overcome dark inhibition, probably through the phytochrome system.
   b. Phytochrome is a pigment involved in the photocontrol of germination. It exists in two reversible forms. One form, Pr, has a maximum absorption at 6,600 Å, while the other, Pfr, has a maximum absorption at 7,300 Å. Less than a second of exposure to red light can supply the stimulation, which increases as seed moisture increases. Among trees, it has been shown to occur in *P. taeda* and *Betula pubescens.* The exact mechanisms of this action are still unknown. The general reaction is as follows:

$$Pr \underset{\text{far red light}}{\overset{\text{red light}}{\rightleftharpoons}} Pfr \rightarrow \text{dormancy removal}$$

   c. In germination testing, minimal light levels should be 75 to 125 fc (750 to 1,250 lux). Light is usually supplied during testing of all species, even to those that do not require it or to those that have been stratified.

4. **Gases**
   a. Respiration requires a certain supply of oxygen, and the carbon dioxide produced must be removed. High levels of carbon dioxide can inhibit germination of most species, while lowering oxygen can do the same. Excessive moisture on germination test blotters will retard germination of many species.
   b. Some species germinate well in anaerobic conditions, even under water.
   c. Oxygen uptake patterns in seeds are similar to those of moisture.
   d. Many aspects of the influences of gases on germination need to be studied.

F. Internal Physiological Changes

1. **Structural** changes – Imbibition is a precursor to necessary metabolism through its role in restoring structural integrity in membranes and organelles.
2. **Enzymes** – Some systems are present in dry seeds; others are synthesized as imbibition proceeds. In some species, a desiccation period at the end of maturity is necessary before rehydration to produce all the needed enzymes. No research on this subject has been done on tree seeds, but this finding can probably be assumed for orthodox species.
3. **Reserve food mobilization** – Generally, insoluble forms are converted to soluble forms (in some ways a reverse of maturation trends).
   a. Carbohydrates. Amylases are the primary enzyme systems to change starch to soluble sugars.
   b. Lipids. Lipase enzymes are important for these compounds; they separate fats into fatty acids and glycerol. Fatty acids then undergo beta-oxidation to acetyl coenzyme A, which enters the glyoxylate cycle, eventually showing up as carbohydrates.
   c. Proteins. Some proteins are important storage foods, but most tree seeds depend on carbohydrates and lipids.
4. **Nucleic acids-These** compounds are closely allied to the new enzymes formed during germination.
5. **Translocation** – The movement of materials within the embryo is crucial. In some plants, a stimulus originates from the radicle tip, which controls amylase activity. There are also other stimuli (possibly hormones) in cotyledons that are necessary for translocation in some species. These stimuli have been studied very little in tree seeds.

G. Sources

For additional information, see Bonner 1972, Mayer and Poljakoff-Mayber 1975, Murray 1984b, Stanwood and McDonald 1989, Willan 1985.

Collection

I. Genetics and Seed Source

A. Introduction

Seed quality involves both the genetic and the physiological quality of seeds. In this section, the general principles and methods for selection of seed source and improvement of seed quality through genetic selection are presented. Genetic improvement of seed quality is based on the seeds' ability to produce trees that are genetically well suited to the sites where planted and for the products desired. In later sections, the physiological quality of seeds will be considered. Good seeds are those that have both high physiological quality and genetic suitability.

B. Objectives

1. Recognize the importance of seed origin (provenance) and recommend general rules for seed movement.
2. Review the advantages and disadvantages of exotic tree species and interspecific hybrids for tree improvement.
3. Define factors that must be considered when a tree improvement program is initiated.
4. Identify the conditions required for genetic improvement of tree seeds (genetic gain concept).
5. Distinguish between a minimum initial strategy of genetic improvement and a maximum long-term strategy.
6. Identify some terms and concepts of new biotechnology for genetic improvement.

C. Key Points

The following points are essential to understanding seed source and genetic improvement:

1. A successful tree improvement program should not be tried in another country or region without considering desired products and available sites.
2. Knowledge of phenotype and genotype is necessary to understand genetic improvement of trees.
3. The genetic gain equation explains the advantages of one improvement method over another.
4. Genetic gains can be obtained from selections among species, provenances within species, and/or trees within provenances.
5. The primary risk of using exotics or nonlocal provenances is planting on unsuitable sites.
6. Test plantings are the only sure method to determine genetic quality of seeds.
7. Without results of test plantings, the safest rule is to use seeds from phenotypically selected stands or trees in the local provenance for native species or land race for exotic species.
8. The seed orchard concept has two parts—the breeding program and the production program.
9. Seed orchard breeding programs involve progeny tests and selection for the next advanced generation of genetic improvement.
10. Seed orchard production programs are managed to maximiie seed production through protection and cultural treatments.

D. Tree Improvement

1. **Tree improvement** begins with the decision to use artificial rather than natural regeneration. Tree improvement is the development and application of genetically improved trees and intensive cultural practices to increase forest productivity through artifical regeneration.
2. **Tree improvement programs** are plans of action to bring about desired objectives. The following factors should be considered when a tree improvement program is initiated:
   a. The products desired determine what species can be used and what traits should be emphasized when breeding for genetic improvement.
   b. The geographic location and physical and climatic characteristics of the sites to be regenerated determine what species and seed sources can be used.
   c. Only species and provenances that are adapted to the planting sites should be used to avoid failure or substandard performance.
   d. Conservation of forest gene resources should be planned from the beginning to maintain genetic diversity.

E. Strategies for Genetic Improvement

1. **Genetic gain**
   a. Genetic improvement (genetic gain) is accomplished by:
      (1) Having a population of trees with genetic differences
      (2) Selecting the genetically desirable trees to serve as parents for production of seeds
   b. The amount of genetic gain *(R)* to he captured from phenotypic selection of parent trees for a particular trait can be expressed as:

$$R = i V_P h^2$$

where i = the intensity of selection
h^2 = the heritability of the trait

V_P = the amount of phenotypic variation.

c. Gain can be obtained from selection among species (R_S), selection among provenances within species (R_P), or selection among individual trees within provenances (R_I). The total gain (R_T) is the sum:

$$R_T = R_S + R_P + R_I$$

2. Species selection

a. Species-site studies provide information about the relative performance of different species when planted together on various sites.

b. Exotic tree species should be used only when the desired product cannot be obtained with native species at a comparable cost.

c. Interspecific hybridization has been used to obtain unique combinations of valuable traits (combinational hybridization) and to obtain hybrid vigor (e.g., *Populus* and *Pinus*).

3. **Seed source**

a. Provenance refers to where the mother trees were growing and the seeds were collected. The stand may be indigenous or nonindigenous. Seed source is the same as provenance. Origin refers to where the original progenitors of nonindigenous stands were growing in natural forests and where their genetic characteristics developed through natural selection. For an indigenous stand of trees, origin, provenance, and seed source are the same. Some foresters in the United States use the term "provenance" in place of "origin" when referring to nonindigenous stands.

b. The largest, cheapest, and fastest gains in most tree improvement programs can be made by ensuring the use of adapted, productive provenances of the desired species. "Local" sources should be used until provenance test results are available.

c. Provenance tests should be conducted during the early stages of a tree improvement program so that geographic collection and planting zones can be delineated. Results of provenance tests include:

(1) Mapping patterns of geographic genetic variation

(2) Delineating provenance boundaries

(3) Determining provenances best adapted and most productive for specific geographic areas.

d. The general results of provenance testing are:

(1) Wide seed transfer is safer near the center of a species' range than near its edge.

(2) Where environmental gradients are steep, movement of material must be restricted.

(3) Provenances from harsh climates (cold or dry) are slower growing but more hardy because of the stress factor than provenances from milder climates.

4. Improvement strategies

a. The initial strategies for a new program are:

(1) Collect available information.

(2) Select among indigenous tree species.

(3) Select seed production areas within the "local" seed sources near the planting site.

(4) Remove phenotypically inferior trees from seed production areas.

b. The long-term strategies for maximum and continued gains are:

(1) Collect all existing information.

(2) Select several species for the program, and begin supplemental tests (species-site, exotic, or interspecific hybridization) to obtain missing information on these and other promising species.

(3) Conduct provenance tests or use existing provenance tests in the area to delineate optimal provenances for the planting sites.

(4) Select the phenotypically "best" trees from the best provenances.

(5) Establish a first-generation seed orchard from the phenotypically selected trees.

(6) Test the progeny of the selected trees.

(7) Remove genetically poor trees from the orchard.

(8) Select the best individuals from the best families in the progeny tests and place these in a second-generation seed orchard.

(9) Test the progeny of these second-generation selections and repeat steps (6), (7), and (8) above for subsequent generations. Search con-

tinually for new first-generation selections or for selected clones from other programs to incorporate into the program (enrichment).

c. New strategies for genetic improvement are:

(1) Gene transfer, using recombinant DNA technology, to insert a desired gene into a species where it does not occur.

(2) Cell selection in a cell-suspension culture to screen for resistant cells to a pathogen, toxin, or herbicide, and developing these cells into mature plants by tissue culture techniques.

(3) Fusion of protoplasts '(without cell walls) to create a new hybrid cell with two sets of chromosomes.

(4) Somaclonal variation, which is genetic variation among individual propagules regenerated from cell and tissue culture of the clone. It provides a new source of genetic variation for selection.

F. The Seed Production Program

The seed production program may be combined with the breeding program or may be kept separate. The objective of a seed production program is to produce sufficient quantities of genetically high-quality seeds to meet seed needs.

1. **Seed Production Areas**

a. Seed production areas (SPA's), or seed stands, are existing stands of natural or plantation origin that are selected for phenotypic superiority and managed for production of seeds.

b. SPA's are used on an interim basis until seed orchards come into production.

c. SPA's can use genetic improvement from superior provenances.

d. SPA's can provide seeds for minor species having small planting programs.

e. Practices to improve genetic quality of seeds include:

(1) Removing phenotypically undesirable trees

(2) Establishing a pollen dilution zone around the SPA

f. Practices to increase seed production include:

(1) Thinning the stand

(2) Fertilizing

(3) Establishing access roads (for protection and for seed collection)

2. **Seed orchards-A** seed orchard is a collection of selected trees established and grown together under intensive management for production of genetically improved seeds.

a. There are two types of orchards: seedling seed orchards and clonal seed orchards.

(1) Seedling seed orchards involve progeny tests that are rogued (poor families and individuals removed) so that the remaining trees can cross-pollinate and produce seeds.

(2) Clonal seed orchards are collections of vegetative propagules (usually grafts) of select trees. The propagules are establised together, progeny tested when they flower, and then rogued based on progeny test results.

b. The genetic quality of seeds is increased by:

(1) Reducing inbreeding through nonrandom assignment of clones or familes to the orchard.

(2) Establishing a pollen dilution zone around the orchard.

(3) Separating provenances into differentorchards.

c. Seed production from orchards is increased by:

(1) Establishing the orchard where soil and climatic conditions are most favorable for seed production.

(2) Spacing wide enough for maintenance of a full crown but not so wide that cross-pollination is reduced.

(3) Applying fertilizers to increase the number of flowers and the portion of the crown bearing flowers.

(4) Irrigating all year during dry periods to stimulate flower production. (Irrigation has not provided consistent results.)

(5) Subsoiling to improve health and wind firmness of orchard trees and to stimulate flower production.

(6) Protecting flowers, fruits, cones, and seeds from insects by periodically applying insecticides.

(7) Protecting flowers from late spring freezes through cold water irrigation.

(8) Ensuring supplemental mass pollination.

G. Sources

For additional information, see Burley and Styles 1976, Khosla 1982, Nienstadt and Snyder 1974, Rudolf and others 1974, Wright 1976, Zobel and Talbert 1984, Zobel and others 1987.

II. Production

A. Introduction

Most tree-planting programs are begun by collecting seeds from in-country sources, both natural stands and plantations. To plan these collections effectively, seed managers should understand the factors that affect tree seed crops and generally know what seed yields may be expected. With this basic information, opportunities may arise to stimulate seed production in key areas, such as seed orchards or managed seed stands.

B. Objectives

1. Recognize the problem of periodicity of seed production in trees.
2. Learn how environmental factors affect seed production.
3. Learn how seed production can be stimulated in trees.

C. Key Points

The following points are essential to understanding seed production:

1. Many tree species bear good crops in cycles.
2. Production is less frequent in high latitudes and high altitudes and among heavy predator populations.
3. Environmental factors influence flower production, pollination, and seed maturation.
4. Several options are available to stimulate seed production.
5. Except for seed orchards of a few species, production data are extremely variable.

D. Periodicity of Seed Crops

1. **Temperate secies**
   a. Many conifers bear in cycles, producing good crops every 3 to 4 years.
   b. Some trees, mainly angiosperms, produce good seed crops every year (e.g., *Acer, Betula,* and *Fraxinus).*
   c. As latitude or altitude increases, the interval between good crops and the frequency of crop failure increase.
2. **Tropical species**
   a. Periodicity may depend on wet/dry cycles. In Nigeria, good seed years occur after very dry weather in August.
   b. Some species *(e.g., Tectona grandis)* usually flower each year, with bumper crops every 3 to 4 years. Other species *(e.g., Pinus kesiya, Cassia siamea, Cupressus lusitanica,* and *Delonix regia)* produce good crops most years. Bawa and Webb (1984) found 93 percent flower abortion in seven Costa Rican tropical hardwoods within 2 days, but the reasons for the abortion are largely unknown.
   c. Dipterocarps in Malaysia bear irregular heavy seed crops at 1- to 6-year intervals.
   d. Some *Eucalyptus* species have large crops more regularly when grown in plantations perhaps because of the concentration of pollen in a pure stand. In the United States, the same finding may be true for many genera, especially *Liriodendron* and *Platanus.*
3. **Genetics-** Fecundity is an inherited trait.
4. **Documentation-There** have been many observations but few detailed physiological studies on periodicity of seed crops.

E. Effects of Environment During Flowering

1. **Temperature**
   a. During hot summers, trees usually produce many floral buds and thus large crops of seeds the next year, but the reason is not known. It may be related to partitioning of carbon within the tree.
   b. Late freezes can destroy flowers; sprinkler irrigation can provide some protection.
   c. The combination of hot summers and late freezes suggests that orchards should be moved to warmer climates (also to escape insects). However, *Pinus taeda* orchards were once moved to south Texas, but without success.
2. **Light** -The effect of light has not been studied extensively. In the Northern Temperate Zone, southern and western sides of crowns have the largest flower and fruit crops. The reason could be light, temperature, or pollen supply. A study of *Acer pennsylvanicum* found an increase in female-to-male flower ratio as crowns closed in the stand. The reason could be environment or internal physiology.
3. **Photoperiod** does not appear to have a direct effect on trees.
4. **Moisture**
   a. Drought, coupled with high temperatures, seems to stimulate flower production the following year.
   b. Excessive rain during pollination leads

to low seed yields in wind-pollinated species.

5. **Mineral nutrients** — Some general observations and weak study results suggest that trees on fertile sites flower and produce more fruit than trees on infertile sites. Nitrogen and phosphorous are most important for flowering, but the reasons are still unknown.
6. **Biotic agents** — Insects, birds, mammals, and micro-organisms can destroy flowers, especially in the following tropical species:
   a. *Triplochiton scleroxylon;* attacked by *Apion ghanaense* (weevil)
   b. *Tectona grandis;* attacked by *Pagyda salvaris* larvae
   c. *Pinus merkusii;* attacked by *Dioryctria* spp. (cone worms)

F. Pollination Agents

1. **Wind pollination** occurs among all conifers and most Temperate Zone hardwoods of commercial value.
   a. Wind pollination requires:
      (1) Lots of pollen
      (2) Pollen shed coinciding with receptivity
      (3) Relatively close spacing of plants
      (4) Good weather-low rainfall, low humidity, and good winds
   b. Supplemental mass pollination (SMP) has been used in United States southern pine orchards.
   c. Contamination in orchards is a concern. The degree of contamination can be determined by isoenzyme analyses.

2. Animal pollination

   a. Insects pollinate many temperate hardwoods. *(e.g., Liriodendron* and *Prunus).*
   b. Bats and birds pollinate many tropical hardwoods, such as honeyeaters in *Acacia.*
   c. Animal pollination is usually common in tropical forests with:
      (1) High species diversity and wide spacing
      (2) Abundant foliage to filter out pollen
      (3) High humidity and frequent rainfall
      (4) Absence of strong stimuli to coordinate flowering (day-length and temperature changes)
      (5) Abundant animal vectors

G. Stimulation of Flowering

Flowering can be stimulated in seed production areas and seed orchards by fertilizing, girdling and other wounding, thinning, growth regulator treatment, and supplemental mass pollination.

1. **Fertilizing**
   a. Use primarily nitrogen and phosphorous, but frequently potassium. Spring and summer application in pines of the Southern United States usually increases flowering and cone production. Zobel and Talbert (1984) recommend the following annual fertilizer levels for loblolly pine orchards: 400 kg/ha nitrogen, 80 kg/ha potassium, 40 kg/ha phosphorous, and 50 kg/ha magnesium.
   b. Irrigation at the same time as fertilizing may also help; however, success has not been universal. Many orchards use drip-irrigation techniques.
   c. Hardwoods may react favorably; e.g., *Acer, Fagus,* and *Juglans.* In Hungary, 200 kg/ha nitrogen and 240 kg/ha phosphoric acid more than doubled nut production and tripled the number of sound seeds.

2. Girdling and other wounding

   a. Girdling and other wounding are used to produce the so-called "stress crops."
   b. Girdling is supposed to inhibit downward translocation of carbohydrates, thus improving the carbon/nitrogen ratio. The optimum time to girdle Douglas-fir is 1 month before vegetative bud burst.
   c. Girdling has increased production somewhat in hardwoods; *e.g., Castanea* and *Fraxinus.*

3. Thinning-In pine orchards, the benefits of thinning are apparent 3 to 4 years after thinning. The goal is full crown development but not so much open space as to reduce cross-pollination.

4. Growth regulator treatment -Great strides have been made with gibberellins (GA) applied to conifers by Ross and Pharis (1976) in Canada.
   a. Water-based foliar spray is best.
   b. A GA 4/7 mixture is most effective.
   c. Both pollen and seed cones are induced.
   d. Apply at bud determination.
   e. Mode of action is still not known.
   f. Treatments are most successful when applied with girdling, root pruning, or moisture stress.

5. **Supplemental mass pollination (SMP)** has been used in pine orchards in the Southern United States.

H. Postfertilization Problems

1. **Insect damage to cones-A** major cause of losses in conifers.

2. **Drought-Extremely** dry weather during seed development and maturation may cause cone drop (e.g., *Pinus monticola*) and losses in seed weight. Russian data show lower seed weight in *Robinia;* the same reaction occurs with *Pinus* and *Quercus.*
3. **Cone** drop-Postfertilization cone drop in *Pinus* is not common except for insect loss.
4. **High winds-** Late summer storms can cause great losses of large cones (e.g., *P. palustris* and *P. elliottii* in the Southern United States).

I. Production Data

Production data include the following published yield figures:

1. ***Pinus* seed orchard production-** In 15- to 20-year-old orchards in the Southern United States, *Pinus* produced these crops: *P. taeda, 98* kg/ha; *I? elliottii, 86* kg/ha; and *P. strobus,* 24 kg/ha. These values should increase as the orchards get older. If 1 kg of seeds produces 17,000 plantable seedlings, 1 ha of orchards yielding 50 kg should produce enough seeds annually to plant 600 ha.
2. ***Pinus elliottii* in Brazil** produced up to 94 kg/ha of seeds with 500 trees per hectre in a natural stand, not an orchard.
3. **Hardwoods**
   a. *Cecropia obtusifolia,* a Mexican dioecious pioneer, produced 2 to 3 kg/ha of seeds in a mature forest. The average was 900,000 plus seeds per tree per fruiting (Estrada and others 1984).
   b. *Quercus* spp., in the North Carolina mountains, produced 16,500 to 236,500 seeds per hectre and 3,700 kg/ha (5 species).
   c. Liquidambar styraciflua, in the Mississippi River floodplain, had twice the seeds per fruit head as did trees in Coastal Plain sites; the former were almost twice as "fruitful" also.
   d. *Acacia albida,* in Sudan, produced 0.5 million seeds from mature trees (Doran and others 1983); and in South Africa, large trees produced several million seeds. In the Sahel, 125 to 135 kg of pods per tree, 400 to 600 kg of pods/hectre and ± 20 seeds per pod were produced, but 95 percent may have been lost to insects.
   e. Tectona grandis, in Thailand, produced 1 kg per tree at age 10 with 8- by 8-m spacing (1,000 seeds per kilogram).
4. **Trade-off-In** Temperate Zone species, radial growth could be decreased 30 to 40 percent in good seed years because of carbon allocation. This means that the current year's carbohydrates are used in cone growth. For *Picea abies* in Poland, growth was less in good seed years. The researchers concluded that selection for fast growth is also a positive selection for fecundity. It may be more important in other species; foresters must decide whether to select for growth or seeds. The agroforestry aspect must also be considered.

J. Sources

For additional information, see Franklin 1982; Owens and Blake 1985; Rudolf and others 1974; Sedgley and Griffin 1989; Whitehead 1983; Willan 1985, chap. 3; Zobel and Talbert 1984.

III. Collection Operations

A. Introduction

Successful collection of tree seeds is usually the result of detailed early planning. Ample time must be allowed to plan an efficient and practical collection strategy and to assemble the resources necessary for its implementation. Key elements include a good estimate of crop size, proper equipment, and a well-trained crew. Comprehensive collections for research certainly require more detailed planning than mutine bulk collections and may require a lead time of 1 to several years depending on the circumstances.

B. Objectives

1. Identify simple techniques for seed crop estimation.
2. Determine the factors that should be considered when collections are planned.
3. Understand the importance of documentation.

C. Key Points

The following points are essential for planning collection operations:

1. The best seed sources available must be selected.
2. Good planning requires advance estimates of the seed crop and, at a later date, estimates of seed yield per fruit.
3. Large collection planning must include choice of personnel, training, transportation, collection equipment, safety of workers, labeling of seedlots, description of sites and stands, etc.

D. Seed Source

Seed source is extremely vital for all seed supplies; this point must never be underestimated. (See "Genetics and Seed Source.") Terms used in genetics are:

1. **Origin-the** natural stand location of the original mother tree.
2. **Provenance-** the place where mother trees that produced the seeds are growing. Seed source and provenance are the same. (Origin and provenance are usually reversed in the United States.)
3. **Laud** race-exotics that adapt to develop improved sources.
4. **Seed zone maps** -necessary for a good program to eventually develop seed zone maps (figs. 14 through 16) [no equivalent figures in Student Outline].

E. Estimating Seed Crops

If seeds are in short supply, all possible seeds should be collected, stretching crews and equipment. If the location of large crops is known, more seeds can be collected for a given amount of cost and effort. Seed crops can be estimated by any of the following five methods:

1. **Flower counts-** only feasible with large, showy flowers. *Pinus* spp. strobili are about the minimum size.
2. **Immature fruit and seed counts**– useful in the collection zone before seed maturity because many flowers may abort.
3. **Fruit counts on standing trees-** work best when seeds are nearly mature.
   a. Total counts are feasible only for light seed crops.
   b. Crown sampling is most common and uses portions of the crown (10 or 25 percent). [Do the exercise in exercise 2 if there is time.] A 5-percent sample of orchard trees in midsummer gave a good estimate in *I? taeda* in Virginia. Some people use a two-person system (one on each side of the tree).
4. **Rating** systems-Examples of rating systems are the Tanzania rating system (table 4) [no equivalent table in Student Outline] and a North American rating system for conifers (table 5) [no equivalent table in Student Outline].
5. **Cross-section seed counts-Cones** are cut lengthwise (or perpendicular to the axis for round fruits), and exposed filled seeds are counted (table 6) [table 3 in Student Outline]. This number can be related to total good seeds per fruit. Enough seeds must be counted to calculate a simple regression. [Do the exercise in exercise 2 if fruits are available.]

F. Planning Considerations

The steps of planning a collection are:

1. **Define the objectives**
   a. For effective planning, the coordinator of collecting operations needs a clear statement of objectives. Prior knowledge of species and provenance priorities, sampling strategy, the standard of documentation, and amount of seeds required is important to assemble the resources needed for the type of collection proposed.
   b. Flexibility should be built into the planning to allow collectors to make decisions about unforeseen circumstances. For example, it would be highly wasteful for collectors to return empty handed from remote areas because of restricted or narrowly defined aims and procedures when an alternative and potentially valuable seed harvest was available for collection.

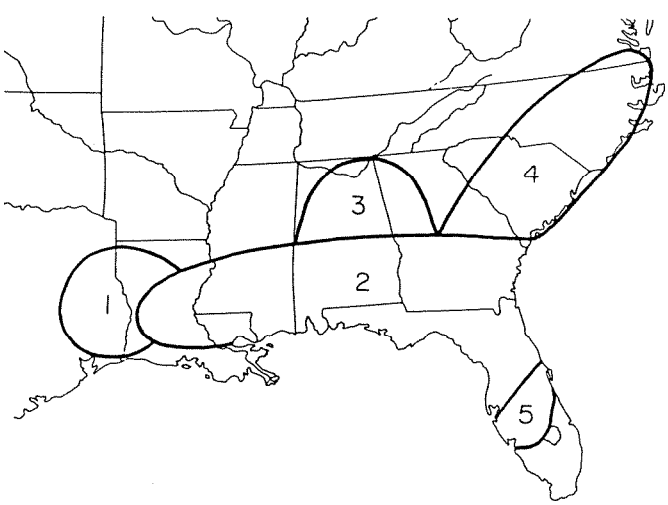


Figure 14.–*Recommended seed collection and planting zones for* Pinus palustris *in the United States (Lantz* and *Kraus 1987) [no equivalent figure in Student Outline].*

Table 4.–*Tanzania rating system for seed crops (adapted from Willan 1985) [no equivalent table in Student Outline]*

Crop rating		
Numerical	Wording	Criteria
0	None	Trees without flowers and fruits
1	Weak	Flowering and medium-sized seed crops on free-growing trees and on trees on free borders of stands
2	Medium	Flowering and very good seed crops on free-growing trees and on free borders of stands; trees within stands bearing seed crops at top of crowns
3	Very good	Flowering and very good seed crops on most trees

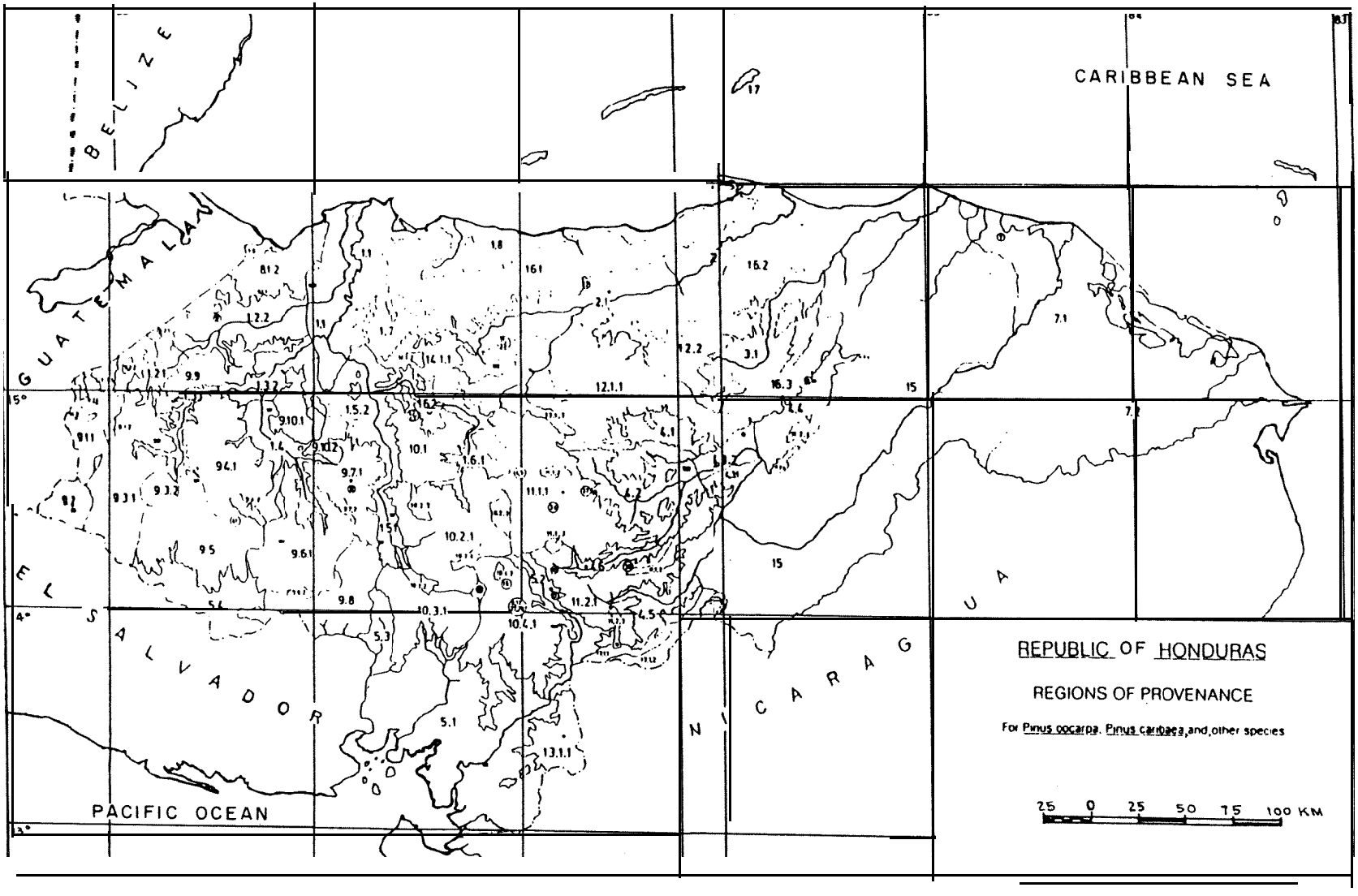
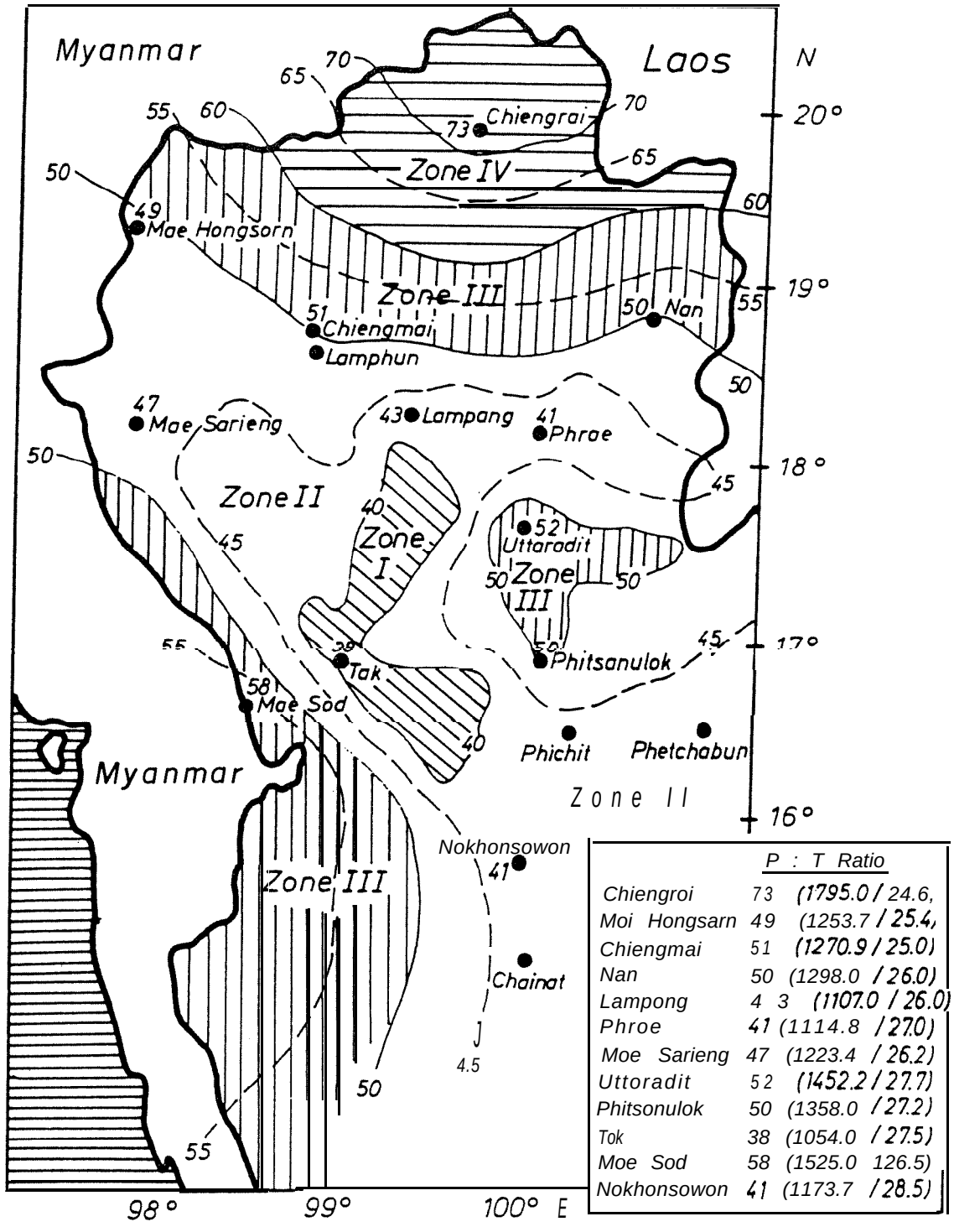


Figure 15. *and other species in Honduras (Robbins and Hughes 1983) [no equivalent figure in Student Outline].*



	P : T Ratio
Chiengroi	73 (1795.0 / 24.6,
Moi Hongsarn	49 (1253.7 / 25.4,
Chiengmai	51 (1270.9 / 25.0)
Nan	50 (1298.0 / 26.0)
Lampong	43 (1107.0 / 26.0)
Phroe	41 (1114.8 / 27.0)
Moe Sarieng	47 (1223.4 / 26.2)
Uttoradit	52 (1452.2 / 27.7)
Phitsonulok	50 (1358.0 / 27.2)
Tok	38 (1054.0 / 27.5)
Moe Sod	58 (1525.0 126.5)
Nokhonsowon	41 (1173.7 / 28.5)

Thailand teak seed collection zones :

Zone I : dry-humid zone
Zone II : medium-humid zone
Zone III : moist zone
Zone IV : wet zone

Figure 16. -*A proposed seed zone map for teak* (Tectona grandis) *in Thailand based on the annual precipitation/mean annual temperature ratio moisture index value for 1951-1975 (Kaosa-ard 1983) [no equivalent figure in Student Outline].*

Table 5. -*North American rating system for conifer seed crops (adapted from Willan 1985) [no equivalent table in Student Outline]*

Crop rating*		
Numerical	Condition	Criteria
1	Failure	No cones to a few scattered on a few trees
2	Very light	Some cones on some trees
3	Light	Good to fair cone crop on 50 percent of exposed crown of 50 percent of trees
4	Medium	Good to medium cone crop on 75 percent of exposed crown on most trees
5	Heavy	Good cone crop on all exposed crowns of most trees.

'A numerical rating of 4 or 5 is a good prospect for all collectors. A rating of 3 has possibilities for more experienced collectors. A rating of 1 or 2 is a poor prospect for all collectors.

Table 6.—*Sound seed yield per cone for four Pinus species as estimated from the number of sound seeds exposed when cones are bisected longitudinally (Derr and Mann 1971) [Table 3 in Student Outline]*

Sound seeds exposed	*P. palustris* (Louisiana)	*P. taeda* (Louisiana)	*P. elliottii* (Louisiana)	*I? elliottii* (Georgia-Florida)	*P. echinata* (Virginia)
	Sound seeds per cone				
2	23	31	20	31	12
4	35	44	35	50	22
6	47	57	50	69	31
8	59	70	65	a7	41
10	71	a3	80	106	51
12	83	96	95	124	60
14	95	109	110	143	70

2. **Gather background data**

a. A literature search may provide information on the natural distribution of a species -its habitat, ecology, and genetic variability — and on fruiting and flowering times in different parts of its range. A visit to regional herbaria to examine relevant herbarium specimens may add significantly to the published information on species distribution, variability, and reproductive phenology. Personal contact with botanists, field foresters, rural people, and others who study or use a species can also help.

b. If the collections are wide ranging and cross political boundaries, early official contacts with the forest services in the States or countries concerned are essential to develop a good working relationship of mutual benefit to all parties.

c. All available information should be collected and summarized. Data on natural occurrence are best plotted on maps detailed enough to show the main transportation systems (e.g., roads, rivers, railways, and airstrips), topography, and other information that may assist in selecting collection sites. Seed-zone overlays for these maps are very useful.

d. Field reconnaissance is essential, preferably including adequate seed crop estimates and samples of fruits and seeds.

e. The coordinator of collecting operations must determine the number of person-

nel needed for the collecting team and must verify that they have the skills to meet the stated objectives. Key personnel should be selected early so that they can familiarize themselves with the project and species and, if necessary, be trained in the techniques they will be using in the field. Collection team leaders are responsible for the discipline, morale, and safety of personnel and the success of the operation.

3. **Collect field data**

The field data provide the information for relocating the site in future years for further work and provide the background essential for interpreting the results of the experiments. The data to be gathered at each collection site must be specified, and systems must be developed to make their collection as reliable and easy as possible for the worker in the field. Specially prepared field data sheets are recommended to ensure that uniform records are obtained from all sites. The standardized "Seed Collection Report" sheet adopted for the Fuelwood Project of the FAO and the International Board of Plant Genetic Resources and the "Seed Data Sheet" of CSIRO are good examples (figs. 17, 18) [no equivalent figures in Student Outline]. Significant features of these documents are:

a. Locality as indicated by latitude and altitude is essential to define the provenance area. They should be stated accurately and concisely so that future collectors can return with certainty to the same collection site. Distances from the nearest town, village, or geographical feature (river or mountain), the forest district, and any other facts that will assist future field parties should be recorded. Maps (hand drawn if others are not available) and aerial photographs showing the stands and the position of seed trees are useful and should be filed with the data sheets for ease of reference.

b. Aspect, slope, climate, soils, and associated species help to build a picture of the environment and ecology in which the trees grow and may help in interpreting experimental results. The collector should encourage relevant comments from local people on the history of the area and its climate. Recording tolerance to such features as alkalinity or salinity in the soil, seasonal inundation, etc. may be important later.

c. Individual tree descriptions (e.g., height, diameter, stem form, and branching habit) and the number of trees in a provenance are of obvious importance to any future tree-breeding work. Photographs are a useful adjunct to written descriptions.

d. Collecting herbarium specimens of little known or variable species allows for later scrutiny by botanical experts. Care is needed in handling the specimens, and it is best, after drying, to dispatch them to the base at the earliest opportunity to prevent damage.

e. Other data and notes that may be of great value to future collectors are crop and seed details, collection methods, etc.

f. It is paramount in provenance work to adopt foolproof systems for maintaining the identity and purity of each collection. Systems must prevent any seedlot from being contaminated with the seeds of another lot through all steps, from collection to registration in the seed store. Careful labeling through each process is essential and can be facilitated by using preprinted durable labels. A well-proven method of numbering collections used by CSIRO is based on party leaders' assigning numbers to the collections in numerical sequence, prefixed by their initials. The number assigned to a particular tree appears on and in the collection sacks and seed bags, on the herbarium voucher specimen, and on the photograph, and it identifies any other sample or recording of that tree. The provenance number is not assigned until the seeds are ready for storage.

4. **Plan the itinerary:**

a. Reaching the collection region well in advance of the proposed date for beginning the collection is important. In developing an itinerary for the collection team, team leaders must be aware of this point.

b. Organizing the sequence of operations in a particular region may require 2 to 4 weeks. Extra permits may have to be obtained, labor recruited and trained, and reliable transportation arranged.

c. Make the schedule flexible. The itinerary is important as a guide, but it must be flexible to account for the unexpected

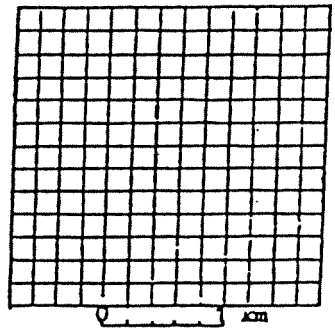
FAO PROJECT ON GENETIC RESOURCES OF ARID/SEMI-ARID ZONE ARBOREAL SPECIES:

SEED COLLECTION REPORT

Location

N

Sketchmap



Collection No. .....

Species: .....

Country: .....

Province: ..... District: .....

Lat: ..... ° ..... ' Long: ..... ° ..... ' Elev: .....

Topography: Blat/hilly

Slope: steep/medium/gentle

Soil: Deep/shallow/intermediate

Drainage: .....

.....

Stoniness: .....

Texture : .....

.....

pH: Acid/neutral/alkaline

Rainfall: Mean annual ..... mm; Wet months: ..... Dry months: .....

Temperature: Mean annual: ..... °C; Xean max: ..... °C; Mean min: ..... °C

Frost: .....days/year

Stand: Natural: Groups/open Thin/dense

Young/middle-aged/old

Plantation: Age . ..... years Height ..... m Diam .....cm

Original source: .....

Associated species: .....

Form: *lea: Single/multiple Straight/fair/poor

Crowns: Flat/narrow/average/wide

Seed crop: Light/medium/heavy

Seed collection: No, of trees ..... Min. distance apart: .....m; Kg: .....

Remarks: .....

.....

.....

Date of collection: .....

.....

Officer in Charge

Figure 17.-*Seed collection report form used in the FAO Project on Genetic Resources of Arid/Semi-arid Species (Willan 1988)* *[no equivalent figure in Student Outline].*

CSIRO Division of Forest Research
PO Box 4008
Canberra ACT 2600

SEED DATA SHEET

Species: _____ Seedlot No. _____

Collection locality: _____

_____

'Latitude: ° '_S Longitude: ° '_E Altitude:(m) _____ Aspect _____ Slope _____

Climatic zone: _____ Met. Station: _____

Association includes: _____

Geology and Soil: _____ pH: _____

Collection no.	Bot. spec.	Photo. no.	tit (m)	dbh (cm)	Tree description	Seed wt (g)	No. of viable seed /10 g

Work supervised by: _____ Date: _____

Figure 18. -Seed data sheet used by CSIRO (Willan 1985) [no equivalent figure in Student Outline].

problems that invariably arise in the field. Overly restrictive schedules may lead to loss of accuracy and attention to detail, and to lower worker morale.

5. Organize equipment permits and transportation

a. Team leaders must specify at an early stage what equipment is to be used if long delays in purchase or delivery are anticipated.

b. Identify applicable government regulations. Most countries have regulations governing collection, export, introduction, and, perhaps, movement of seeds. Official permits may be required for any of these procedures, and, in some cases, separate permits are needed for each collection site and for each individual seedlot exported or introduced. Customs officials and plant. health authorities may seriously delay the operation or even destroy seeds if the full entry procedures are not followed. For some seeds, extra delays of even a few days in transmission can be fatal.

c. Use care between the collection of the seeds and their arrival in the seed laboratories. This period is critical to their viability and vigor. Transportation must be where it is wanted when it is needed; thus, prior organization is essential to minimize transit time.

G. Collection Equipment -A Comprehensive List
The following items are necessary for collection operations:

1. **Administrative items:**
   a. Movement approvals
   b. Collection authorities
   c. Radio transmission permits
   d. Drivers' licenses
   e. Firearm permits
   f. Facilities for purchasing stores; e.g., gasoline (petrol) and oil

2. Literature:
   a. Road, topographic, and soil maps to cover the collection route itinerary
   b. Literature on the genera and species to be collected

3. Collection equipment:
   a. Notebooks, recording forms, pens, and pencils
   b. Binoculars
   c. Markers; e.g., colored plastic ribbon
   d. Camera and accessories
   e. Tree-measuring instruments; e.g., diameter tape, height-measuring instrument, and length tape
   f. Soil sampler, pH testing kit, and soil charts
   g. Compass
   h. Altimeter
   i. Hand lens
   j. Large collecting sheets; e.g., 4 by 4 m of heavy duty plastic or canvas
   k. Small collecting sheets; e.g., 2 by 2 m of calico or other finely woven cloth
   l. Seed bags made of finely woven cloth of various sizes; e.g., 100 by 100 cm to 10 by 20 cm for small seed samples, all with ties
   m. Large grain bags for dispatching seeds
   n. Cutting equipment; e.g., secateurs, long-handle pruning saws, shears, ladders, chain saws with fuel and accessories, bowsaws, flexible saws, throwing ropes, axes, rifles with ammunition, and rakes
   o. Safety gear; e.g., steel-capped boots, leather gloves, safety helmets, and safety belts
   p. Weatherproof tags for labeling each seedlot, to prevent the markings from becoming illegible when wet or abraded
   q. Tags for botanical specimens; e.g., white cardboard "jewelers" tags
   r. Plant presses for botanical specimens
   s. Papers to dry specimens in the plant presses
   t. Plastic bags
   u. Specimen bottles with preservative fluid
   v. Containers for soil samples
   w. String

H. Sources
For additional information, see Barner and Olesen 1984; Bramlett and others 1977; Doran and others 1983; Ontario Ministry of Natural Resources 1983; Willan 1985, chaps. 3, 4, 5 + appendices 1, 5, 6.

IV. Maturity

A. Introduction
Choosing good stands and trees for seed collection means nothing if fruit or seed maturity cannot be easily identified on the trees by unskilled workers. If seeds are disseminated immediately at maturity, workers must know how much in advance of maturity seeds can be collected without collecting seeds that will not germinate. If predators inflict large losses on mature seed crops, a similar problem exists. Good maturity indices are often the keys to successful collection.

B. Objectives
1. Learn the common indices of maturity employed in tree seed collections.
2. Recognize how these techniques can be adapted for new species.

C. Key Points
The following points are essential to recognizing seed maturity:
1. Seed moisture content is very important, but direct measurement in the field is impractical; indirect estimates may be substituted.
2. Color changes are the most common indices.
3. Chemical indices are possible but impracticed.
4. Artificial maturation of immature seeds is an option for some species.

D. Successful Collection
The following points are essential to successful collection:
1. **Biological ideal-to** collect seeds at the peak of their physiological maturity; however, this ideal is not possible for large-scale collections. First, physiological maturity, maximum food reserves, minimum moisture content, and ideal regulator balance must be defined. For example, ideal regulator balance is expected at abscission/dispersal, but it may not occur then.
2. **Practical collection** – In most collection operations, fruits and seeds are:
   a. Collected from the ground after dissemination (good for large seeds only).
   b. Collected from logging operations.
   c. Collected from standing trees as close to full maturity as possible before dissemination.
   d. Collected from standing trees well in advance of maturity and ripen artificially.
   e. Both c and d require climbing and/or the use of special equipment.

E. Collection after Dissemination
Seeds that can be collected after dissemination are primarily large, single-seeded fruits; e.g., those of *Quercus, Carya, Juglans,* and *Aesculus* (temperate), and dipterocarps, and other large seeds (tropical). However, the first seeds to fall are usually bad because of insect damage or an immature embryo. Workers must quickly collect the seeds before animals destroy them, especially in the Tropics.

F. Other Collection Strategies
Other collection strategies involve collection of seeds before dissemination. These collections would usually be from standing trees, although collection from logging slash would fall into this category also. These strategies depend greatly on the ability to judge seed maturity.

G. Maturity Indices
Maturity indices include physical and chemical characteristics. They are needed for all collection strategies.
1. **Physical characteristics**
   a. Color changes from green to yellow to brown or black (e.g., *Pterocarpus);* from green to red to purple or black (e.g., *Prunus);* from green to yellow; from green to yellow to purple (e.g., *Gleditsia triacanthos);* and from green to brown (e.g., conifers)
   b. Moisture content
      (1) There are three moisture trends during ripening:
         (a) In dry, orthodox seeds and fruits, moisture decreases slowly as seeds mature (fig. 19) [no equivalent figure in Student Outline].
         (b) In pulpy, orthodox fruits, moisture decreases at first, then increases mainly in the pulp (fig. 20) [no equivalent figure in Student Outline].
         (c) In recalcitrant seeds, moisture increases early, then slightly decreases (fig. 21) [no equivalent figure in Student Outline].
      (2) Moisture content is related to protein synthesis (see Rosenberg and Rinne 119861). Seed moisture must drop below 60 percent to trigger protein synthesis. Without this happening, seedling growth is arrested. This may be true for all orthodox seeds. Ellis and others (1987) found a critical level of 45 to 50 percent moisture for six grain legumes.
      (3) Moisture content can be measured directly by oven methods; that is, cut cones, large fruits, or seeds; weigh; dry for 17 hours at 103 °C; and then weigh again.
      (4) Specific gravity is usually discussed separately, but it really is just an estimate of moisture content. Specific gravity has been measured in:
         (a) Conifers-See table 7 [table 4 in Student Outline] and fig. 22 [figure 7 in Student Outline].
         (b) *Quercus* data from Russian sources found significant changes in specific gravity in

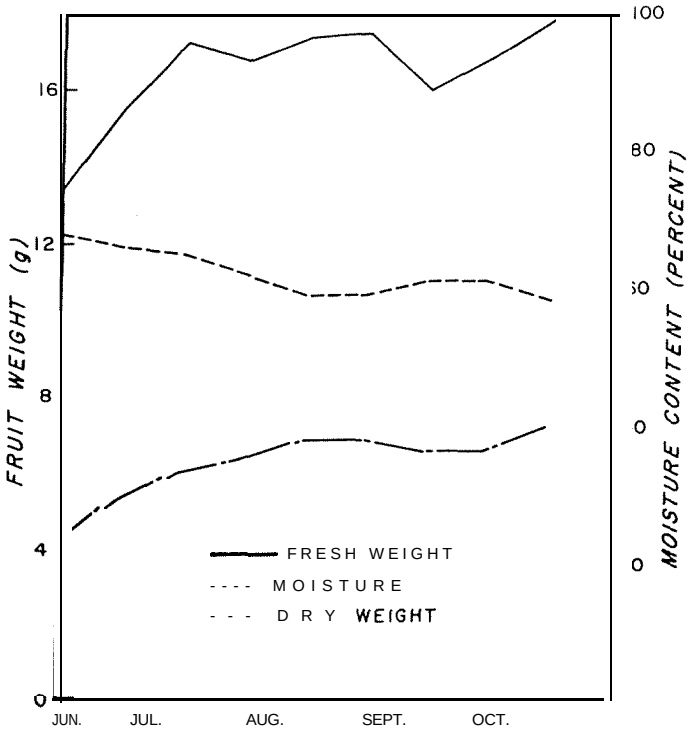


Figure 19. -Seasonal *changes in fresh weight, dry weight, and moisture content during maturation of fruits of* Platanus occidentalis *(adapted from Bonner 1972a) [no equivalent figure in Student Outline].*

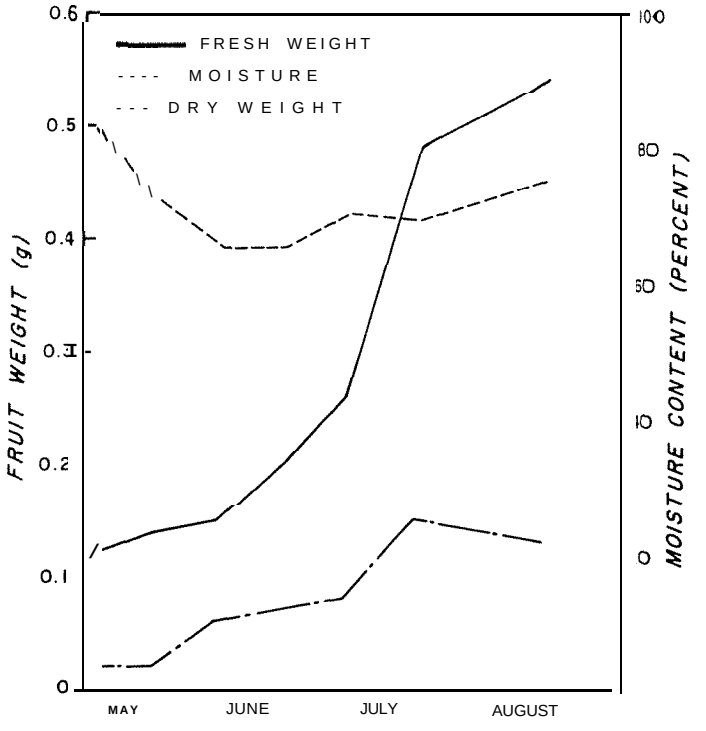


Figure 20. -Seasonal *changes in fresh weight, dry weight, and moisture content during maturation of* Prunus serotina *(adapted from Bonner 1975) [no equivalent figure in Student Outline].*

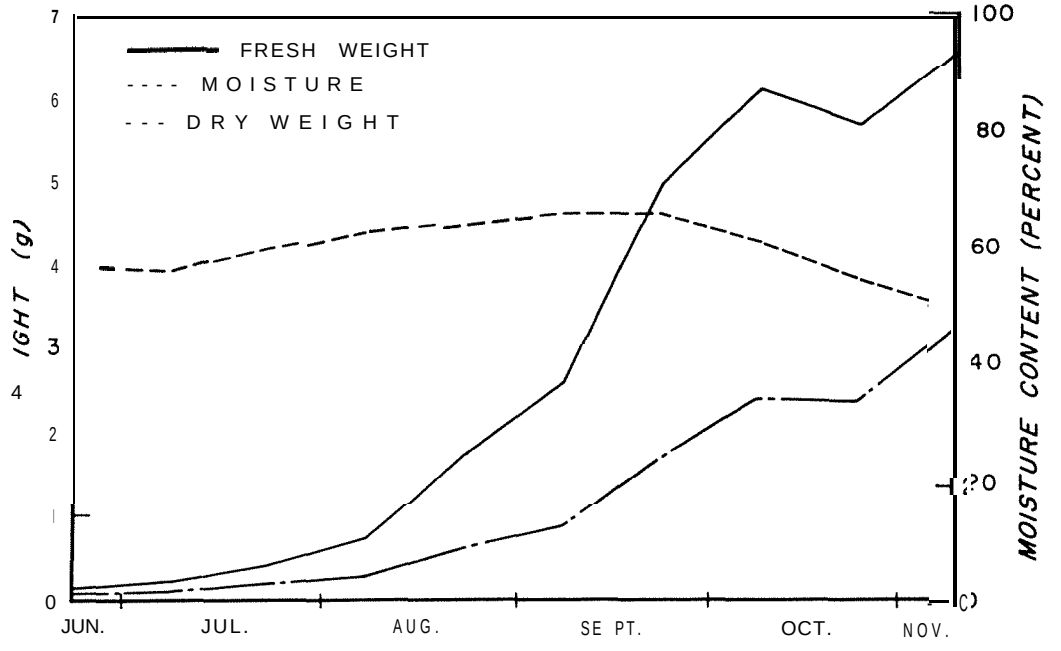


Figure 21.—*Seasonal* changes *in fresh weight, dry weight, and moisture content during maturation of* Quercus alba *(adapted from Bonner 1976) [no equivalent figure in Student Outline].*

Table 7. -*Cone specific gravity values that indicate seed maturity in some conifers [Table 4 in Student Outline]*

Species	Specific gravity	Reference
Abies grandis	0.90	Pfister 1967
Cunninghamia lanceolata	0.95	Jian and Peipei 1988
Pinus elliottii	0.95	Barnett 1976
P. merkusii	1.00	Daryono and others 1979
P. palustris	0.90	Barnett 1976
I? strobus	0.90	Bonner 1986a
P. taeda	0.90	Barnett 1976
P. virginiana	1.00	Fenton and Sucoff 1965

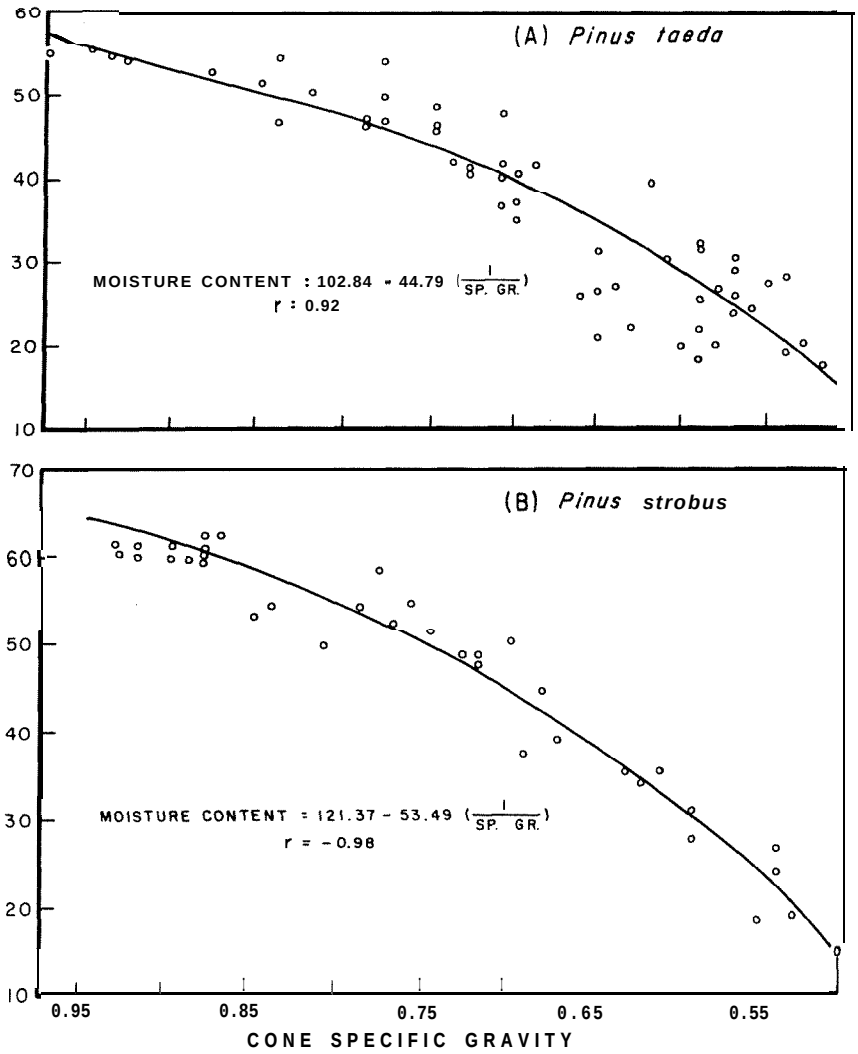


Figure 22. -*The relationship of moisture content to specific gravity for cones of* Pinus taeda *and* P. strobus *(Bonner 1991b) [figure 7 in Student Outline].*

acorns of some *Quercus* species as they matured, but results with North American species have been inconsistent.

(c) Other angiosperms-there is usually a flat line over maturation period (little success).

(d) Barnett (1979) developed a simple procedure for determining specific gravity using water flotation that is easy to perform (fig. 23) [figure 8 in Student Outline].

c. Other physical indices:

(1) Acorn cup release in *Quercus* is good; easy release signifies maturity.

(2) If bending *Pinus strobus* cones causes scales to "open," they are mature.

(3) When the white, brittle embryo in *Fraxinus* (and some other genera) is excised and bent, mature embryos will break.

(4) A minimum percentage of the embryo cavity must be filled; 75 percent is a general guideline for *Fraxinus excelsior* and *Pinus sylvestris* in northern Europe.

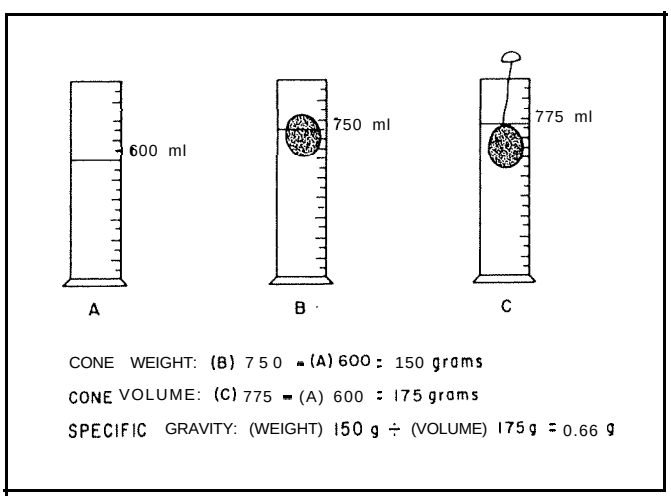


Figure 23. *-A simple technique for determining specific gravity of pine cones in the field using a graduated cylinder. (A) Fill the cylinder with water to the 600 mL mark. (B) Float the cone in the water and record the water level. (C) Using a pin or needle, submerge the cone enough to completely cover the cone with water, but no more. Record the new water level (adapted from Burnett 1979) [figure 8 in Student Outline].*

2. Chemical characteristics

Chemical indices are biologically sound but are not practical, even in sophisticated collection situations.

a. Accumulation of storage foods can indicate full maturity.

(1) Angiosperms of the Southern United States: *Liquidambar styraciflua,* 25 percent crude fat; *Fraxinus pennsylvanica,* 10 percent fat and 14 percent insoluble carbohydrates; *Quercus* spp. (red oaks), 20 percent fat and 25 percent insoluble carbohydrates; *Quercus alba* (white oak), 40 percent insoluble carbohydrates (figs. 24 through 27) [no equivalent figures in Student Outline].

(2) Western conifers: *Abies procera, 25* percent crude fat; *Pseudotsuga menziesii,* 23 percent crude fat or 1.3 percent reducing sugars.

b. Elemental analyses of calcium, magnesium, and phosphorus have shown some good relationships in angiosperms of the Southern United States. Relative amounts will decrease in acorns and increase in small seeds; absolute amounts increase in all seeds as they mature (fig. 28) [no equivalent figure in Student Outline].

c. Growth substances. In Poland, auxins decrease in maturing *Quercus robur* according to Michalski (1969).

(1) Predicting *Picea abies* germination by level of indoleacetic acid (IAA) has even been proposed by several European workers.

(2) Gibberellin changes have been followed, and levels usually increase with maturation.

H. Artificial Maturation

Artificial maturation is an option for some situations. Immature seeds can be artificially ripened by storing them in cool, moist conditions. Krugman (1966) (Western United States) and Lantz (1979) (Southern United States) successfully used artificial maturation with *Pinus* spp. Bonner (1972c) (Southern United States) was successful with *Liquidambar styraciflua* and *Liriodendron tulipifera* but not with species of *Fraxinus* or *Quercus.*

1. **Single-seed versus multiple-seed fruits**

Single-seed fruits are cut off from all nutrition when picked, but multiple-seed fruits

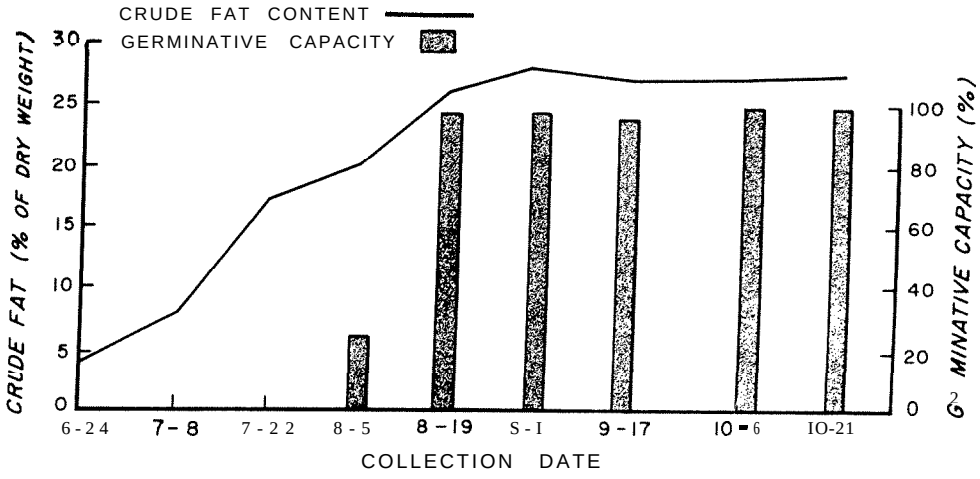


Figure 24. -*The relationship of crude fat content and germination in maturing seeds of* Liquidambar styraciflua *(Bonner 1972a) [no equivalent figure in Student Outline].*

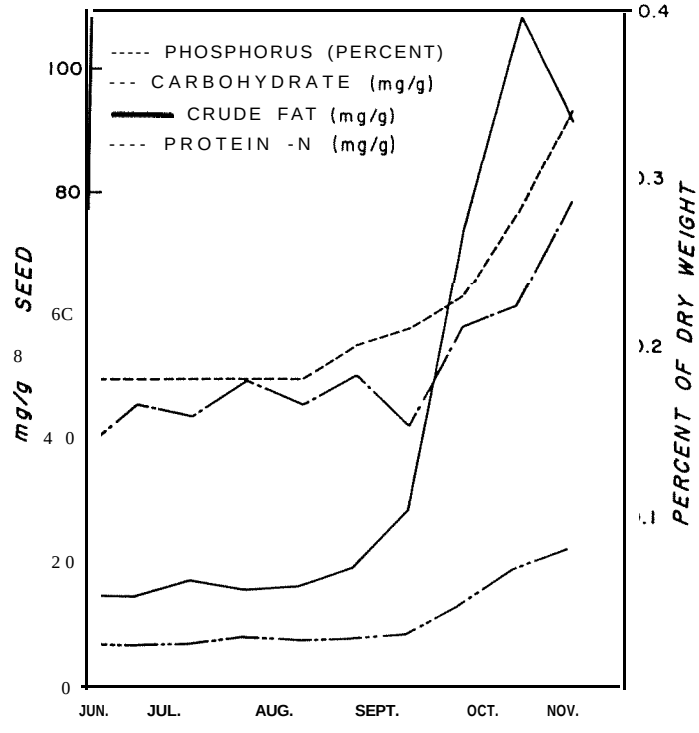


Figure *25. -Seasonal changes in phosphorus, soluble carbohydrates, protein-nitrogen, and crude fat in samaras of* Fraxinus pennsylvanica *(adapted from Bonner 1973) [no equivalent figure in Student Outline].*

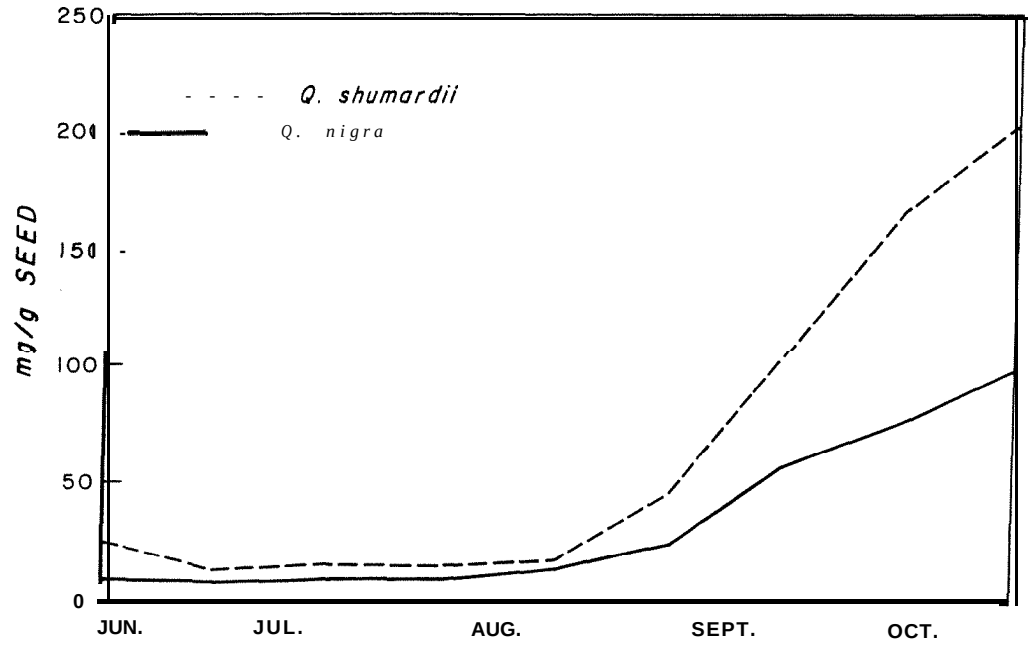


Figure 26. -*Seasonal changes in crude fat content of fruits of* Quercus nigra and Q. shumardii *(adapted from Bonner 19743, 3976) [no equivalent figure in Student Outline].*

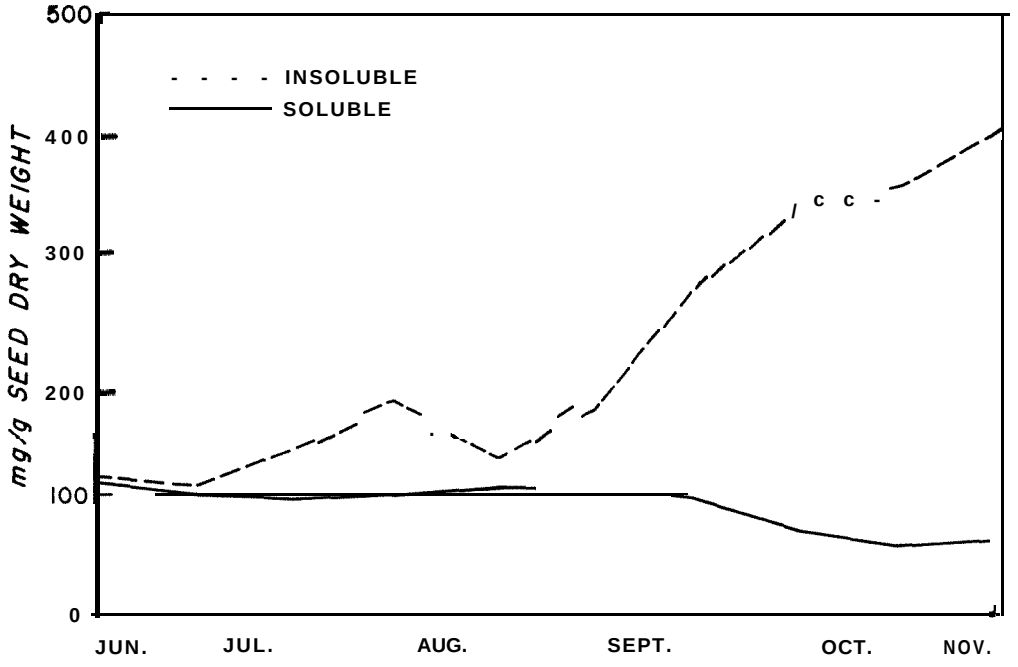


Figure 27.—*Seasonal changes in insoluble and soluble carbohydrates in fruits of* Quercus alba *(Bonner 1976) [no equivalent figure in Student Outline].*

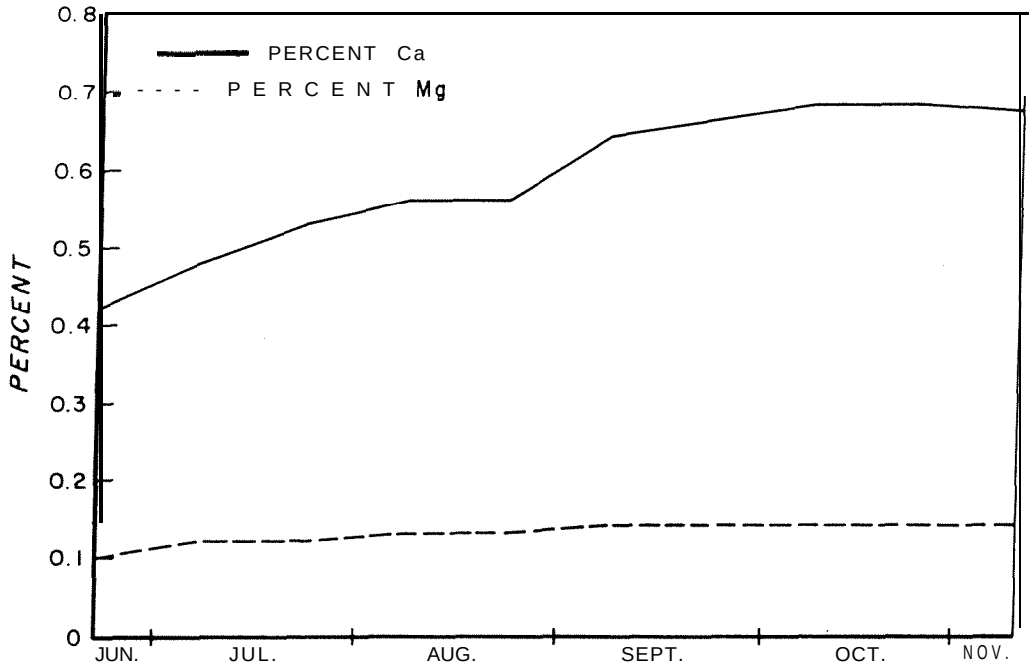


Figure *28. -Seasonal changes in calcium and magnesium in samaras* ***of*** Fraxinus pennsylvanica *(adapted* ***from*** *Bonner 1973) [no equivalent figure in Student Outline].*

can obtain some nutrition or growth factors from cone or fruit tissues to complete maturation.

2. Avoiding dormancy

a. *Tilia americana-This* species can be picked before full maturity when the seedcoat is not completely dried.

b. *Acacia* spp. -Early collections were reported by Doran and others (1983).

c. Gleditsia triacanthos -Early collection also works with this legume; the pod is picked while it still shows some yellow. Hard-seeded dormancy is not so strong, but resistance to disease is low.

3. Usefulness-Artificial maturation is useful when collecting on remote or expensive sites. However, seed yield and quality usually suffer. In one case, *Liquidambar styraciflua* yield was 20 percent lower than with natural maturation; germination was equal to that of seeds from later collections.

I. Delayed Collections

For certain species, there is no rush to collect the fruits; they do not disperse their seeds right away (orthodox seeds only>.

1. **Serotinous cones** ***(Pinus*** **and** ***Picea)*** **–** Resins prevent cones from opening until they melt in wildfires. In cool climates, seeds can remain viable for several years in the cones.

2. Delay in abscission- *Platanus* spp. are good examples of a fruit that stays intact on the tree for several months past maturity without decreasing seed quality.

J. Sources

For additional information, see Bonner 1972a, 1972c, 1976; Nautiyal and Purohit 1985; Rediske 1961; Willan 1985, p. 33-38.

V. Postharvest Care

A. Introduction

The time between collection and extraction is often overlooked as a crucial segment of seed acquisition. Fruits and seeds, often high in moisture content, must be stored and/or transported for extraction and cleaning. Special care must be taken during this period to avoid loss of seed quality, especially in tropical and subtropical areas where transportation systems do not allow immediate delivery to extraction centers.

B. Objectives

1. Recognize the crucial times when seed quality may be lost.
2. Plan storage and transportation systems to minimize the danger to seed quality.

C. Key Points

The following points are essential to postharvest care:

1. High moisture contents and high temperatures are dangerous for orthodox species.
2. High moisture levels must be maintained in recalcitrant seeds, but excessive heat is a problem with these seeds.
3. Fruit storage can be advantageous for some species because of the afterripening processes that occur in the seeds.

D. Storage Before Extraction

1. **Operation schedules-Time** does not permit seeds from all trees or families to be collected at peak maturity; therefore, some must be picked and stored. Extractories could not handle them all at once, anyway, at least for pines in the United States.
2. **Predrying-** When artificial heat can be used, drying during storage can remove much of the moisture and thus lower drying costs. In pines of the Southern United States, two-thirds of the moisture removal required for cone opening can be done in predrying storage. Moisture should decrease from about 70 to 30 percent.
3. **Completion of maturation-** Completion of maturation is an often overlooked benefit, but it is just as important as predrying.
   a. *Abies is* a well-known example; 5 or 6 months in cool, moist conditions complete maturation. In natural habitats, seeds mature as cones disintegrate. *Picea glauca* in Canada needs 6 weeks of storage in the cone to achieve the best germination.
   b. New data show similar benefits to seed quality from storage before extraction for some pines of the Southern United States.
   c. Premature collection is suitable for some multiseed angiosperms; e.g., *Liquidambar styraciflua, Liriodendron tulipifera,* and *Platanus occidentalis.* This is done to lengthen collection season or, in northern climates (Scandinavia and Russia), to complete ripening in conifers in short growing seasons. It is usually not recommended in the United States because yield per cone and seed quality generally suffer.

E. Southern Pines

1. **Storage** is usually related to operation schedules.
2. **Outdoor storage** is better than indoor storage because:
   a. Drying is faster outdoors.
   b. Cones open better when wet/dry/wet/dry cycles occur.
3. **Containers**
   a. Containers must provide air circulation.
   b. Loose-weave burlap bags (about one-third hectoliter) and 7-hectoliter, wooden crates are effective.
      (1) Burlap bags of *Pinus taeda* cones left in the seed orchard in the shade did well in one study, providing an extra 5 weeks of collection time. Shaded concentration points were no better than sunny ones.
      (2) Temperature profiles of the 7-hectoliter crate show no overheating in the middle.
   c. Plastic bags or sacks should not be used.
   d. Paper sacks are effective for small lots if their tops are left open.
4. **Time**
   a. Cone storage can improve germination rate.
   b. Maximum length of storage depends on the species:
      (1) *4* weeks for *Pinus elliottii*
      (2) 5 to *7* weeks for *I? taeda*
      (3) *3* weeks for *P. palustris*
      (4) *5* to *7* days for *I? strobus* in the Southern United States
      (5) *7* to *9* weeks for *P. strobus* in Ontario
5. **Other factors**
   a. Original maturity-More mature cones may have shorter optimum storage periods (very important), although this cannot be quantified as yet.
   b. Local conditions (weather, equipment, etc.) are also important; warm, rainy conditions increase the risk of cone molds.
6. **Immaturity/Dormancy**
   a. Seed ripening in cones increases germination rate and thus decreases dormancy.
   b. Past a certain point, cone storage will decrease germination – first, germination rate, then total germination. This point cannot be accurately defined as yet.
7. **Heat and Molds**
   a. Green cones can generate enough heat to char cones black.
   b. External molds are common in 7-hectoliter crates, but studies have found no evidence of damage to seed quality. *Pinus echinata* data from Pollock, Louisiana, and *Abies alba* from Yugoslavia demonstrated better germination in seeds from moldy cones.

c. Good aeration is essential to prevent mold growth on cones during drying.

F. Serotinous Cones

1. Storage is not a major problem for *Pinus glauca, P. contorta,* or *P. patula.*
2. **Maturity-** Some pine seeds need to stay in the cone because 3 years may be needed to reach maturity (*Pinus torreyana* in the Western United States).
3. **Viability-Seeds** can retain viability for several years in cones, but seed quality may be reduced. However, Canadian workers found that seeds from lo-year-old *P. contorta* cones in Canada can be very good.

G. Other Conifers

1. ***Abies*** (true firs) -Seeds of true firs must complete ripening in the cones. They should be stored in burlap bags in sheds with plenty of aeration around the bags for weeks to 6 months, depending on the species.
2. ***Picea*** (spruce) -Seeds of most spruce species should be extracted as early after collections as possible. Winston and Haddon (1981) in Canada found that 4 weeks of cone storage at 5 °C of *I? glauca* were enough, but Wang[1] reported that 6 weeks were needed. These seeds had excellent germination and no dormancy; no stratification was needed. Immature *P. rubens* cones can be stored for several weeks, then the seeds are "teased" out with wet/dry cycles.
3. ***Pseudotsuga*** - Extended storage of 3 to 4 months is possible in dry, well-ventilated conditions. Too much moisture leads to pathogen problems. Some artificial ripening is possible.
4. **Tropical pines**
   a. Treatment of tropical pines is similar to that for Southern United States pines, but the tropical environment is more stressful. Cones should be stored under a roof or cover with good ventilation, and temperature should be kept between 20 and 35 °C.
   b. Rodents and fungi can be big problems, so special efforts should be made to protect the seeds.
   c. In Honduras, *Pinus caribaea* is precured until all of the cone changes from green to brown.
   d. In New Zealand, immature cones of *P. radiata* are stored for 10 weeks at 20 to 24 °C to complete ripening and to fit the planting schedule (Willan 1985).
   e. In Indonesia, green and green/brown cones of *l? merkusii* had improved opening, yield, and seed quality when stored for 2 to 4 weeks before extraction (Arisman and Powell 1986).

H. Hardwoods

1. **Artificial ripening-Many** hardwoods of the Southern United States respond to artificial ripening of immature fruits; *Liguidambar styraciflua* can be collected 4 weeks *early; Liriodendron tulipifera* can be collected 4 to 6 weeks early. However, seed yields and quality will suffer. This effort has not been as successful in pines.
2. **Regular storage** -The above species should be stored in loose-weave bags and for as short a period as possible. They should be spread one or two fruits deep for drying and stirred two or three times daily to avoid overheating. Other orthodox species include:
   a. *Eucalyptus* -Tight-weave cloth or plastic can be used, but overheating is a danger with plastic; use with caution.
   b. *Legumes* -Storage is not difficult, but overheating is possible if moisture is high; they should be spread to dry.
   c. Drupes -Short storage to complete ripening is possible for some species, but they should be spread in a single layer and shaded, preferably indoors. As soon as the color changes, they should be cleaned. For *Prunus* species drupes should be cleaned within 3 days of collection.

I. Summary

Seeds of most species fit into one of three groups:

1. **Harvest dry, keep** dry-Usually start fruits drying immediately and keep dry after extraction *(e.g., Pinus, Liquidambar, Liriodendron, Acacia,* and *Eucalyptus).*
   a. Dry slowly.
   b. Provide good aeration; do not use plastic bags.
   c. Use suitable containers, including:
      (1) Burlap bags
      (2) Racks
      (3) Wooden crates
      (4) Canvas or plastic sheets
2. **Harvest moist, then** dry-Keep moist when collecting and during extraction to avoid formation of tough, impermeable

[1]Wang, B.S.P. 1985. Personal communication with the author. On file with: U.S. Department of Agriculture, Forest Service, Southern Forest Experiment Station, Starkville, MS 39759.

covers (drupes); extract, then dry seeds for storage (e.g., Nyssa and *Prunus).*

a. Spread to avoid heat and (in most cases) fermentation.
b. Use trays or bags; do not use plastic.
c. Avoid outer coat toughness.
d. Extract, wash, and dry for storage.

3. Moist forever-Recalcitrant seeds are kept moist forever because drying will decrease quality; seeds are not stored when collected, but are extracted immediately *e.g., Quercus, Aesculus, Shorea,* and *Hopea.*

a. Never dry.
b. Keep moisture ≥30 percent.
c. Refrigerate to a safe temperature:
(1) 1 to 3 °C for temperate recalcitrants *(e.g., Quercus).*
(2) 15 to 20 °C for tropical recalcitrants.
d. Use polyethylene-lined containers or polyethylene bags 4 to 10 mil thick.

J. Sources

For additional information, see Bonner 1987a; Willan 1985, p. 78–86.

Handling

I. Drying and Extracting

A. Introduction

Like agricultural seeds, many tree fruits dry as they mature, and the seeds are extracted best at low moisture contents. Other tree seeds are still very moist at maturity, and special considerations are needed for extraction. No matter what type of fruit is involved, however, the objective of extraction is to obtain the maximum amount of seeds in the best physiological condition in an economically efficient operation. During extraction, seed quality can be greatly reduced by excessively heating the fruit to force opening or by extracting by hand or machine.

B. Objectives

1. Recognize potential problems of seed extraction related to the type of fruit.
2. Identify the basic techniques of tree seed drying and extraction.

C. Key Points

The following points are essential to seed drying and extracting:

1. For species that require drying, excessive heat in the presence of high moisture content can be deadly.
2. Seed damage can occur during mechanical separation.
3. Good training of workers is essential.
4. Extraction strategy depends on the type of fruitinvolved.

D. Multiseed Fruits

Multiseed fruits include pods; moist, fleshy fruits; cones and capsules; and other multiple fruits. The steps in drying and extracting seeds are:

1. **For pods, Leguminosae**
   a. Dry the pods. Solar drying is fine, but supplemental heating can be used also.
   b. Thresh manually by:
      (1) Flailing with poles
      (2) Crushing by trampling
      (3) Hitting with heavy mallets
   c. Thresh mechanically with:
      (1) Slow, rotating drums (cement mixer)
      (2) CSIRO flailing thresher (Willan 1985)
      (3) Dybvig macerator
      (4) Hammer mills
   d. Use a series of steps for difficult species *(e.g., Prosopis):* break the pods open; soak in 0.1 N hydrochloric acid for 24 hours, wash and dry, and pound the dried material with a hammer. For species that have gummy material in the pods (e.g., *P. cineraria):* break the pods and run them through a coarse meat grinder to extract the seeds; or break up the pods, redry, and run them through the thresher again.
2. **For moist, fleshy fruits-** In general, pulp removal will help germination. But, if fleshy coverings are thin, removal may not be required (e.g., *Vitex parviflora).*
   a. Start quickly to avoid fermentation. (Fermentation may help some species; *e.g., Maclura pomifera* in the United States.) If this is not possible, spread in thin layers and stir occasionally.
   b. Soak the fruits in water until the pulp is soft if the fruits cannot be squeezed away easily with the fingers. The pulp must be soft for complete removal. Change the soak water to avoid fermentation.
   c. Extract with macerators, mixers, coffee depulpers *(e.g., Gmelina arborea),* feed grinders, hammer mills, etc.; use anything that can tear up the fruits without hurting the seeds.
   d. Run small fruits *(e.g., Rubus* and *Morus)* in blenders at slow speeds with lots of water to extract the seeds.
   e. Use a high-pressure stream of water against mesh bags of fruit (e.g., *Prunus* and *Vitis).*
   f. Float pulp fragments and small underdeveloped seeds away with running water.
3. **For cones, capsules, and other multiple fruits-Drying** for extraction assumes successful predrying.
   a. Air-dry on flat surfaces.
      (1) Canvas is best for large quantities. Use a tarpaulin (5 by 7 m or 5 by 10 m) to handle up to 12 to 16 hectoliters of *Pinus caribaea* cones in a layer one cone thick. In good drying weather, the cones will open in 3 days. *Pinus caribaea* should yield 4 to 6 kg of pine seeds from 16 hectoliters of cones. Agitate the cones every 2 to 3 hours to speed drying. Double the tarpaulin over to cover the cones at night. Remove seeds that have dropped out each morning. There will be some problems if it rains during drying (common in Central America).
      (2) Use screen trays for smaller lots, such as single-tree collections. Use larger trays to handle nearly 1 hec-

toliter at a time. Spread one cone deep, stir, and protect from rain. Elevate the trays to speed drying; catch extracted seeds on a sheet or cloth.

(3) Plastic sheets are not strong enough for *Pinus* cones (1 hL of cones = 35 kg). Plastic also encourages condensation when the cones are covered. Plastic sheets can be used indoors for lighter fruits, *(e.g., Liquidambar, Alnus, Casuarina,* or *Populus).*

(4) Some common principles: protect fruits from rain and predators, spread them thin and stir frequently, and collect seeds on the drying surface as they fall. Protection from rain is not needed if the fruits will be artificially dried later.

(5) Dry some species under shade (e.g., *Hopea* spp., *Triplochiton schelroxylon,* and *Pinus oocarpa*); dry others in direct sunlight *(e.g., P. caribaea* and *P. elliottii).*

b. Use solar kilns.

(1) One simple type of solar kiln is clear polyethylene stretched over the top of screen trays; this method is common in east Africa.

(2) Solar heat storage units are more sophisticated. Small buildings can contain solar panels and heat storage facilities, such as water barrels. Plans for one that can handle 15 to 18 hectoliters of cones at a time are available. Such units are good for easy-to-open cones of *Pinus,* such as *P. strobus,* which open in 1 week with good sun.

c. Use heated kilns. These kilns are most efficient for large quantities of cones, but capital investment and fuel costs may be too high. There are several types of heated kilns:

(1) Progressive kilns-Cone containers move along a gradient of increasing temperature inside the kiln. Because they are slow, they are not used much. They can be vertical or horizontal. One company in the United States has a tunnel progressive kiln with multitrayed, wheeled carts and computerized environmental controls. A few have been installed in Canada and the Northwest United States.

(2) Large-batch kilns -Large, heated chambers in which trays hold cones and rotate positions. Typical drying capacity is 300 to 350 hectoliters per day. There is no provision for small lots, and fuel costs are high. Natural gas is used in the United States, but almost all these kilns have been phased out.

(3) Small-batch kilns-Wire-bottom drawers hold about one-third hectoliter of cones each. They are inserted into a chamber in which a fan and gas-fired heater push heated air through the drawers. The standard kiln holds about 16 hectoliters at a time, but the design is modular for possible expansion. One big advantage is that small lots can be kept separate.

(4) Stack-tray system -Developed in the Southern United States for *Pinus,* it is a flexible and portable system. Wooden trays with perforated sheet-metal bottoms (not wire screens> fit together in stacks of six; eight stacks are heated by one heating system. Heated air is blown up through the trays from a bottom plenum and recirculated to the heater. One such unit can hold 100 hectoliters at a time and should process 7,000 hectoliters in a season. Fuel costs are high, but flexibility is an advantage.

(5) Tumbler driers-New cylindrical batch kilns that rotate while drying to remove seeds as soon as possible. Humidity sensors and humidifiers allow moisture and temperature control. Many sizes are available to fit individual needs.

(6) Other batch kilns -Many local designs are available. The Bobbins kiln, built in Honduras, seems to be a good, low-cost, forced-draft kiln. It has a capacity of 32 hectoliters, a drying period of 12 to 18 hours under good conditions, and uses old cones or wood as furnace fuel.

d. Set temperature and humidity parameters. The object is to remove moisture; high temperatures do this by creating a greater vapor pressure gradient. For conifers, 29 to 50 °C may be used, but initial temperatures should always be on the lower end of the scale. Pine cones of the Southern United States are predried

to well below 50-percent cone moisture before going into the kiln. Good predrying decreases cone moisture to 25 percent. Cones of *F! contortu* should be predried to 20-percent moisture. Kiln temperatures should be 43 to 49 °C, for tough seeds, such as *P. taeda* and *P. elliottii,* but 30 to 35 °C for "sensitive" seeds, such as *I? strobus* and *P. palustris.* Cones should be dried to 10-percent moisture content in the kiln for best opening.

(1) Serotinous cones (e.g., *P. banksiana, P. patula,* and *P. clausa*) need 63 to 65 °C or a 15-second dip in boiling water to break the resin bonds before going into the kiln. Live steam can also be used to open these cones. *Pinus contorta* cones can be "scorched" by heating 2 minutes at 220 °C to melt resin, then opened by heating 24 hours at 60 "C.

(2) Eucalyptus saligna seeds, in Brazil, need 8 hours at 60 °C to dry capsules to below 20-percent moisture for best extraction.

(3) Populus tremuloides seeds, in Canada, release at catkin moisture content below 70 percent; they are picked 1 week early and air-dried for 3 to 6 days.

e. Extraction after drying-Once the cones are open, a simple tumbling action will extract the seeds.

(1) Tumbler driers were described previously.

(2) Cement mixers are versatile machines that can also crush fruits and extract and scarify seeds. Extraction can be aided by placing a wire cage inside the drum.

(3) Homemade tumblers are easy to construct. Wire-screen boxes that turn on a shaft can be tilted so that seeds fall out.

E. Single-Seed Fruits

Single-seed fruits include drupes and fleshy fruits and nuts with husks.

1. **Drupes and fleshy fruits (*Prunus* and *Vitis*)** -Use macerators, mixers, etc.; use water to soften the contact. (See "Moist, fleshy fruits.")
2. **Nuts with husks (*Juglans*)** -Use macerators or hand rubbing.

F. Sources

For additional information, see Willan 1985, p. 87-111.

II. Cleaning and Upgrading

A. Introduction

Cleaning seedlots is a basic step in proper seed utilization. Cleaning should remove wings or other seed appendages, empty seeds, damaged seeds, and nonseed trash. This cleaning should also provide dramatic decreases in insect and disease problems. Many seedlots can be upgraded by removing immature, damaged, and dead seeds after the initial cleaning. Many people view large mechanical operations as the only way to clean and upgrade seedlots, but seedlot quality can be improved with simple equipment and techniques.

B. Objectives

1. Learn the advantages of cleaned and upgraded seedlots.
2. Become familiar with the principles of seed-cleaning equipment and techniques and expected results.
3. Apply these principles when seed cleaning and upgrading are planned.

C. Key Points

The following points are essential for seed cleaning and upgrading:

1. Liquid flotation can be an essential aid for many species, especially recalcitrant ones.
2. Screen cleaning is the basic seed-cleaning method.
3. Air separation, including winnowing, is a valuable technique.
4. Cleaning small lots for testing or research may be very different from cleaning large lots.
5. Upgrading seedlots offers potential improvements in eight areas.
6. Seed sizing can be useful for some species or sources but not for others.

D. Cleaning

1. **Flotation-The** simplest technique of all. Good seeds sink and bad ones float.

a. Initial moisture content is crucial because it determines whether good seeds sink or float. Long soaks (up to 24 hours) may be needed to make good seeds sink if they are extremely dry when collected.

b. Orthodox seeds are redried after flotation, unless they are sown immediately, but recalcitrant seeds are not.

c. Flotation

(1) Removes light trash.

(2) Removes many empty, broken, diseased, or insect-damaged seeds.

(3) Is very good for large seeds with high moisture contents (e.g.,

Quercus, Carya, and *Juglans*); and for some small seeds *(e.g., Liquidambar, Pinus, Juniperus, Robinia, Gleditsia,* and other legumes).

2. **Aspirators-Any** machine that uses air to clean and separate.
   a. Large machines are found only in large-scale, seed-cleaning plants.
   b. Small-lot models are available for testing laboratories, research, or valuable small collections. Devices include:
      (1) General ER – a model with good air control, but low capacity (75 to 100 mL).
      (2) South Dakota-an old standby in testing laboratories, with enough capacity for small lots.
      (3) Stults-a new blower with less capacity than the South Dakota, but much better air control. It has a vacuum gage to help standardization. It has worked well with pines and small legumes.
      (4) Barnes-a laboratory blower that is seldom seen anymore. It works well with small conifers (e.g., spruce and Douglas-fir) but lacks power to separate Southern United States pines.
      (5) Other models – (see Willan 1985)
      (6) Homemade fan devices –" winnowing" type cleaning is fine, but weight separations usually are not good.
      (7) Carter Day Duo Aspirator -a machine that can handle a wide range of seedlot sizes in continuous flow.

3. **Screens and sieves**
   a. Hand screens -a good set of hand screens is extremely helpful, especially for testing and research laboratories and for small seedlots.
   b. Mechanical screens -some small "flat-screen" cleaners use screen agitation only; they are essential for large quantities.

4. **Airscreen cleaners-use** both aspiration and screening. These are the basic seed-cleaning machines in most seed plants.
   a. Air screens perform the following three functions:
      (1) Scalping – removes large materials (twigs, leaves, etc.) with the top screen.
      (2) Sizing- drops small particles through the second screen and allows the seeds to remain.
      (3) Aspiration -removes very light material, including some empty seeds.
   b. These machines are more efficient for cleaning, not sizing. Large machines (for tree-seed plants) have five screens and two air systems. They can clean 650 to *700* kg of *Pinus* seeds per hour. Most plants use one machine to scalp (two to three screens and one air system) and another for final cleaning and sizing (four to five screens and two air systems).
   c. Small tabletop cleaners are great for small lots; they can clean 30 to 40 kg of small seeds per hour.

5. **Electrostatic cleaners-The** Helmuth machine is good for small. seeds (e.g., *Eucalyptus).* It is expensive, however, for such a limited function.

6. **Dewinging-** A special type of cleaning that reduces storage volume, makes upgrading possible for winged seeds (e.g., *Pinus* and *Liriodendron),* makes sowing easier in nurseries, and removes sources of pathogens. There are two basic methods of dewinging – wet and dry.
   a. The dry method is recommended for tough seeds because of the damage potential to thin seed coats. *Pinus palustris* is an exception.
      (1) The popcorn polisher (Crippen EP-26) is an old method that is rarely used now because of damage to seeds.
      (2) USDA Forest Service dewinger from Missoula Equipment Development Center flails seeds with rubber fingers in a cylinder with soft rubber on the walls to provide gentle action and little damage.
      (3) Dybvig macerators are good for *Liriodendron* and *Tilia* bracts.
      (4) Electric drum scarifiers can clean *Populus, Salix,* or other tiny seeds with hairy appendages.
      (5) New conifer dry dewingers have soft rubber walls and 90 kg per hour capacity in continuous flow.
   b. The wet method is usually preferred for pine and spruce seeds. It is quick enough to avoid much moisture uptake, but some redrying is usually needed for most species.

(1) Cement mixers are good for medium lots; seeds are sprayed with a mist while mixing.
(2) Commercial dewingers are available with capacities up to 90 kg per hour.
(3) Kitchen blenders can dewing small lots; water is added to the seeds at low speed for 10 seconds.
(4) Any cylinder with gentle agitation will dewing; even rapid stirring by hand will work for some species.

E . Upgrading

1. **Upgrading** is improving the potential performance of a seedlot by removing empty, damaged, weak, immature, or odd-sized seeds. Many people clean their seeds but never upgrade. Upgrading does not make good seeds from bad but can certainly improve the seedlot.

2. Upgrading will:

a. Remove weak seeds
b. Remove empty seeds
c. Reduce chances of insect and disease damage by removing damaged seeds
d. Improve control of density in nursery or germination beds
e. Reduce planting time in the nursery
f. Facilitate nursery operations with uniform seedings
g. Reduce costs and improve uniformity in container operations
h. Reduce storage space requirements

3. Methods and equipment

a. Specific gravity by flotation removes empty and some damaged seeds.
(1) Water is used for some *Pinus, Quercus,* and other large seeds.
(2) Organic solvents (usually alcohols) of different densities work on some small seeds but may damage the seeds, especially if they are stored for long periods. Seeds floated in organic solvents should be sown soon. The solution must always be stirred vigorously to give every seed a chance to float.

b. Air-screen cleaners, the basic cleaning machines, can also be used to upgrade.
(1) They can separate by three physical properties: size, shape, and density.
(2) They can upgrade some seedlots *(e.g., Platanus, Liquidambar,* and some *Pinus* spp.) by sizing or by removing empties with the air system.
(3) Screen pattern, feed rate, airflow, screen oscillation (pulleys), and screen pitch (in some models) can be regulated.

c. Air separators include large air-column separators, fractionating aspirators, and small laboratory blowers.
(1) Large air-column separators are not common in tree-seed plants.
(2) Fractionating aspirators are now widely *used* for *Pinus* in the United States.
(3) Small laboratory blowers are suitable for small research or testing lots.

d. Gravity separators were originally built to remove ore from clay. Heavy particles stay in contact with the shaking deck and "walk up" the high side. Light particles "float" on the air cushion and come off the low side.
(1) Separators can separate seeds of the same size and different densities, or different sizes and the same density; they cannot separate a mixture of densities and sizes.
(2) They are widely used to upgrade conifer seeds in North America.
(3) The operator can regulate feed rate, air stream through deck, deck pitch (side and end), and eccentric thrust.

e. Electrostatic separators create a charge that adheres to the seed surfaces.
(1) The Helmuth cleaner can handle *Eucalyptus, Platanus,* and small conifer seeds. Falling seeds receive negative charges and are deflected toward a positively charged plate; deflection varies according to the weight of the particle.
(2) Static electricity is used for very small seeds. The sides of a plastic beaker are wiped with a nylon cloth, and the seeds and chaff are poured into the beaker. The seeds are then poured into a noncharged glass beaker, and the chaff clings to the sides of the first beaker.

f. X-ray radiography is typically used for valuable research lots only. With Kodak paper or Polaroid film, production is good. One United States company has studied mechanization of x-ray sorting.

g. Color separators remove light-colored seeds. There is an application for high-priced lots, and one European company has a color sorter in its production line.

h. The incubation, drying, and separation (IDS) method is used on some *Pinus* and

Picea species in Sweden (Simak 1984). The IDS method:

(1) Incubates seeds for 3 days in 100 percent relative humidity, 15 "C, and light.

(2) Dries them for 12 hours at 15 "C, 35 percent relative humidity, and light.

(3) Separates dead from live seeds by floating in water; in 5 minutes, viable seeds sink. Aspirators or gravity tables can also be used to separate the seeds.

(4) Redries the live seeds to 6- to 7-percent seed moisture.

(5) Can substitute stratification for 30 days at 4 °C for the incubation step in (1) above for some species.

4. **Sizing** helps with some species or seedlots, but not with others (table 8) [no equivalent table in Student Outline]; e.g., clonal mixes vs. single family seedlots.

Table *8. -Correlation of seed size with germination rate and seedling size (adapted from Bonner 1987b) [no equivalent table in Student Outline]*

Species	Germination rate	Seedling size
	---------- *Correlation* ---------	
Acacia ***albida***	*No*	***Yes***
A. nilotica	*No*	***Yes***
Acer saccharum	***Yes***	. *
Araucaria angustifolia	***Yes***	***Yes***
Azadirachta indica	*No*	***Yes***
Carya ***illinoensis***	-	***Yes***
Picea abies†	*No*	*No*
P. abies†	*No*	***Yes***
P. ***glauca***	...	***Yes***
Pinus ***elliottii***	*No*	***Yes***
P. ***koraiensis***	-	***Yes***
P. ***roxburghii***	-	***Yes***
P. ***sylvestrist***	***Yes***	*No*
I? sylvestris†	*No*	***Yes***
P. taeda†	***Yes***	***Yes***
I? taeda†	-	*No*
Quercus alba	...	Yes
Q. ilex	*No*	.
Q. petraea		***Yes***
Q. prinus	-	***Yes***
Q. robur		***Yes***
Q. rubra	-	***Yes***
Q. velutina		***Yes***
Shorea contorta	***Yes***	***Yes***
Tsuga ***heterophylla***	..	*No*
Theobroma cacao	*No*	***Yes***

*Data not available.

†More than one experiment reported for these species.

F. Summary-See figure 29 Cno equivalent figure in Student Outline] for a flow chart for extracting and conditioning *Pinus* seeds.

G. Sources

For additional information, see Bonner 1987b; Doran and others 1983, chap. 5; Willan 1985, p. 87-128.

III. Storage Principles

A. Introduction

The primary purpose of storing seeds is to have a viable seed supply when it is needed for regeneration. Successful storage of woody plant seeds must be carefully planned, and good planning depends on an understanding of the purposes of storage, seed deterioration, and the effects of the storage environment on the deterioration processes.

B. Objectives

1. Learn the objectives and rationale of seed storage.
2. Identify factors that affect seed longevity in storage.
3. Review the general process of seed deterioration.

C. Key Points

The following points are essential to understanding seed storage principles:

1. Longevity of seeds is a species characteristic.
2. Prestorage factors affect longevity in storage.
3. The most important factors in storage are seed moisture content and temperature,
4. Seed deterioration begins at abscission and involves complex physiological changes.

D. Objective of Storage

Once mature seeds are collected, deterioration starts; deterioration is first manifested as slower germination and then as death of seeds and sometimes an' increase in abnormal germination. The objective of seed storage is to delay deterioration or decrease its rate until seeds are used.

E. Rationale for Storage

Seed storage includes short- and long-term storage; it may be extended for long periods for germplasm conservation.

1. **Short-term storage:**
   a. Is used for immediate operations
   b. Typically lasts less than 5 years
   c. Allows carry-over of surplus production in good seed years
   d. Allows minimum storage environment for most species

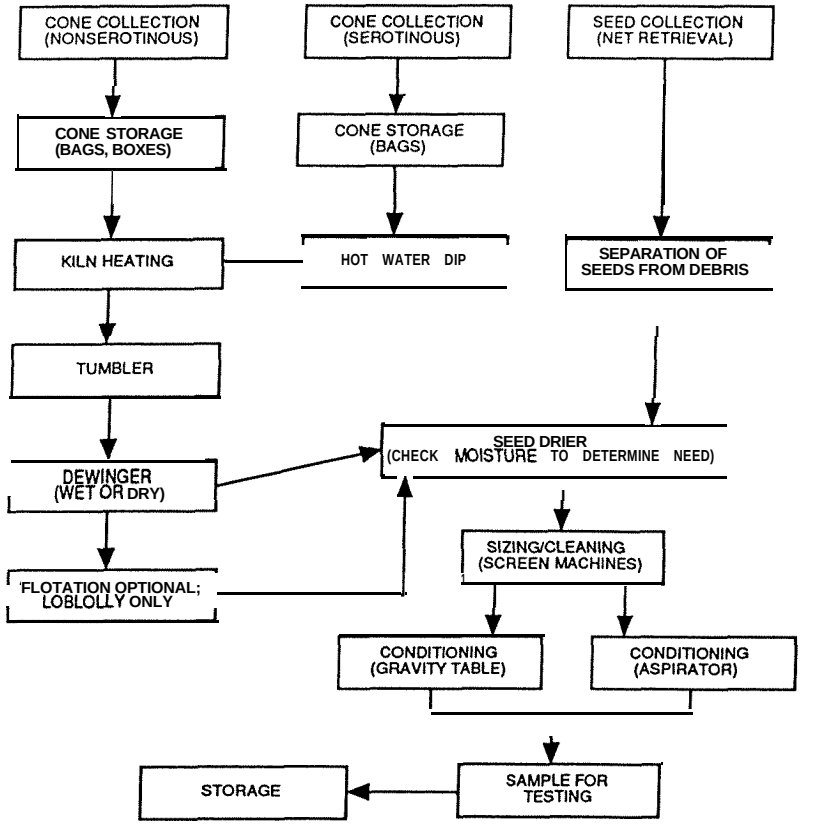


Figure 29. *-Flow chart for extracting and conditioning seeds of* Pinus *in a typical extractory in the Southern United States (Bonner 1991b) [no equivalent figure in Student Outline].*

2. Long-term storage:

a. Typically lasts from 5 to 10 years

b. Ensures constant seed supply for species that are irregular producers

c. Is used to save special lots that will not be collected annually; e.g., in geographically remote areas

d. Requires very good storage environments

3. Germplasm conservation requires:

a. Very long-term storage with a goal of 50 years or more

b. The best storage environment or perhaps special technology

F. Longevity in Storage

Seed characteristics, seed handling before storage, genetics, and the storage environment affect longevity in storage.

1. **Seed characteristics**

a. Basic physiology

(1) Orthodox seeds are tolerant of desiccation to low moisture contents (usually less than 10 percent); dried to this level, they may be stored at subfreezing temperatures.

(2) Recalcitrant seeds are intolerant of desiccation, usually dying if dried below moisture levels of 25 to 30 percent or even 50 percent for some tropical species; at these levels, subfreezing temperatures are lethal.

(3) These definitions were originated by Roberts (1973). They are not "biologically logical," but they are now well established.

b. Seed structure-Thick or hard seedcoats restrict moisture uptake and gas exchange; thin seedcoats allow too much of both.

c. Seed chemistry – Oily seeds tend to be harder to store than starchy seeds.

d. Stage of maturity-Immature seeds usually will not store as well as seeds that were fully mature when collected. Therefore, maturity indices and time of collection become very important.

e. Environmental stress during maturation -Drought, high temperatures, and perhaps nutrient deficiencies may decrease seed quality on the mother tree and thus decrease storage potential.

2. **Seed handling before storage**
   a. Physiological mistreatment, such as allowing overheating at high moisture levels, will damage storage potential.
   b. Processing can cause seedcoat cracks and bruised tissue that can lower seed quality, primarily by allowing invasion of pathogens (table 9) [no equivalent table in Student Outline].

3. Genetics- Good seed quality, and thus greater storage potential, seems to be inherited to some degree. There are also genetic differences among species; some are longer lived than others.

4. Storage environment
   a. Moisture content
      (1) Moisture content is the most important factor.
      (2) Potential damage thresholds are outlined in table 10 [table 5 in Student Outlinel.
      (3) The best range for orthodox seeds is 5 to 10 percent.
      (4) The best range for recalcitrant seeds is full imbibition.
      (5) The equilibrium moisture content is defined as the seed moisture content when seed moisture is in equilibrium with the moisture in the storage atmosphere (table 11) [table 6 in Student Outline].
         (a) Equilibrium moisture content is influenced by seed chemistry; proteins are most hydroscopic, then carbohydrates, then lipids (figs. 30, 31) [no equivalent figure in Student Outline for figure 30; figure 31 is figure 9 in Student Outline].
         (b) Equilibrium is rarely reached with recalcitrant seeds because metabolism rapidly changes seed weight and chemical condition.
         (c) Sorption and desorption difference must be recognized.
   b. Temperature
      (1) Generally, the cooler the seeds, the slower the deterioration rate for both orthodox and recalcitrant seeds.
      (2) The safe temperature range for orthodox seeds is related to the moisture content of the seeds; 20 percent may be the critical upper limit for 0 °C or lower (free water and ice crystals), 15 percent for

Table 9. *-Germination of unscarified and scarified seeds stored under different conditions for 2 months (Lauridsen and Stubsgaard 1987) [no equivalent table in Student* Outline]

Species	Seed storage condition	Scarification method: Seed gun	Scarification method: Seed burner
		percent	
Acacia farnesiana	Unscarifkd, 0 to 4 °C	51 (±11)	98 (±2)
	Scarified, 30 "C, and 80 percent RH	47 (±11)	97 (±3)
Prosopis cineraria	Unscarified, 0 to 4 °C	75 (±3)	94 (±3)
	Scarified, 30 °C, and 80 percent RH'	78 (±5)	†

'RH = relative humidity.
†Process not used for this species.

Table 10. *-Moisture content thresholds andpotential effects on stored seeds [table 5 in Student Outline]*

Moisture content	Effects
Percent	
>30	Germination begins
18 to 20	Overheating from respiration
10 to 18	Seed fungi become active
>9	Insect activity
5 to 8	Best range for sealed storage
<5	Desiccation damage possible in some species

Table *ll.-Equilibrium moisture contents at 4 to 5 °C and three relative humidities (Bonner 1981b, Justice and Bass 1978) [table 6 in Student Outline]*

Species	Relative humidity: 20	45	95
	Percent		
	Moisture content: Percent		
Orthodox trees			
Carya *ovata*	...*	10	15
Juglans nigra	...	11	20
Liquidambar styraciflua	.	8	20
Liriodendron tulipifera	...	10	19
Picea abies	6	8	..
Pinus sylvestris	6	8	..
P. taeda		10	17
Prunus serotina	.	9	17
Orthodox crops			
Glycine max	6	8	19
Zea nays	8	12	20
Recalcitrant trees			
Quercus *alba*	...	37	50
Q. nigra	...	13	29
Shorea robusta		...	35

*Data not available.

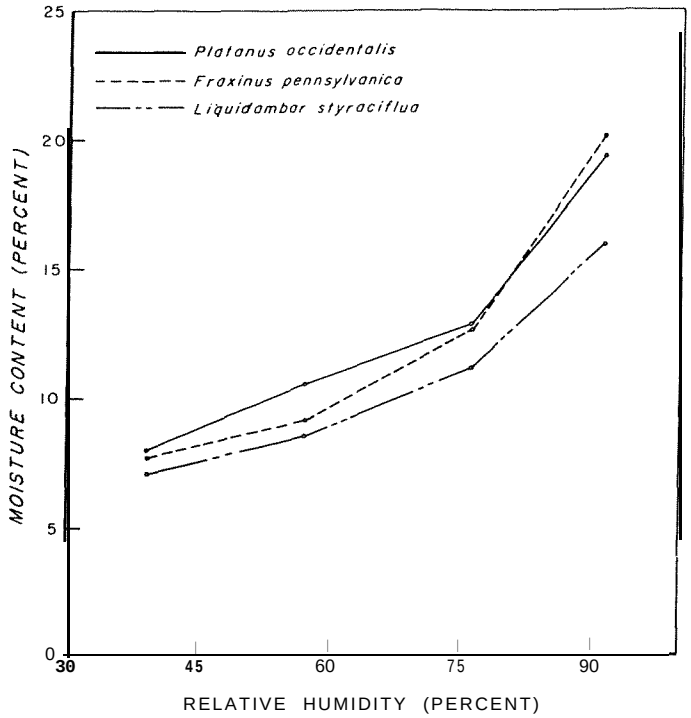


Figure 30.—*Equilibrium moisture content at 25 °C for three orthodox species (adapted from Bonner 1972b) [no equivalent figure in Student Outline].*

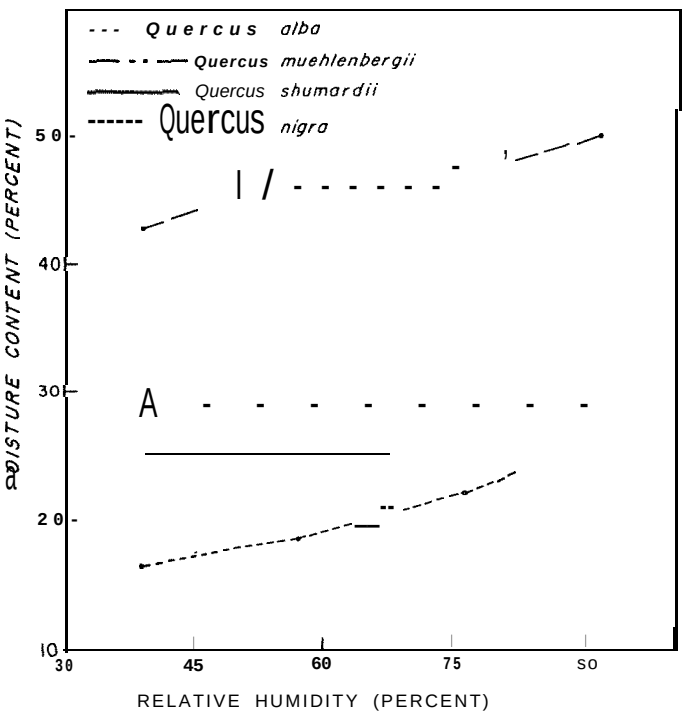


Figure 31.—*Equilibrium moisture content at 25 °C for four recalcitrant* Quercus *species (adapted from Willan 1985) [figure 9 in Student Outlinel.*

– 15 "C, and 13 percent for – 196 "C.

(a) Orthodox seeds at 5- to 10-percent moisture can be stored at most temperatures.

(b) Between 50 and 0 °C, every 5 °C lowering of storage temperature doubles the life of the seeds (Harrington 1972).

(3) The safe temperature ranges for recalcitrant seeds are:

(a) Temperate Zone species: – 1 to 3 "c!.

(b) Tropical species, usually above 12 to 15 °C because of chilling injury.

c. Storage atmosphere

(1) Reduced oxygen slows metabolism and increases longevity, but it is not usually practical to regulate oxygen level.

(2) Inert gases have been tested for storage. They show no advantage in long-term storage, but they may help in short-term storage:

(3) In sealed containers, the CO_2/O_2 ratio changes because seeds absorb oxygen in metabolism.

(4) Recent work shows that *Pinus radiata* stored in nitrogen or carbon dioxide retained viability long enough for transport in the Tropics.

(5) Research in Indonesia showed that coating *Shorea pinanga* seeds with wax retained close to 50-percent viability for 4 weeks; uncoated seeds died during this period. The coating reduced gas exchange.

G. Cells and Tissues During Seed Aging

The following changes occur in cells and tissues during aging:

1. Loss of food reserves by respiration.
2. Accumulation of metabolic byproducts from respiration. Some are toxic.
3. Irreversible enzyme deactivation because dried protein molecules lose their ability to form active protoplasms on rehydration.
4. Deterioration of cell membranes: endoplasmic reticulum and mitochondria.
5. Lipid peroxidation forming damaging free radicals. (There is some question as to whether peroxidation is the cause or an effect.)
6. Alterations of DNA, causing genetic mutations and physiological damage.

H. Sources

For additional information, see Bonner and Vozzo 1990; Warrington 1972; Justice and Bass

1978; Tang and Tamari 1973; Willan 1985, p. 129-160.

IV. Storage Applications

A. Introduction

The previous section introduced the principles and critical factors that influence seed longevity. This section discusses how these principles are applied in practice to store tree seeds.

B. Objectives

1. Relate seed storage principles to prescriptions for each species group.
2. Learn features of cold storage units.
3. Discuss storage constants and their application.
4. Learn basic principles of seed storage management.

C. Key Points

The following points are essential to storage applications:

1. There are four classes of seed storage behavior.
2. Cold storage is best but not always necessary for successful seed storage.
3. Each species, and perhaps individual populations within a species, will nearly always respond identically to a given set of storage conditions.
4. Good facilities and good seeds are not enough; there must be good management for optimum seed storage.

D. Seed Storage Classes

Experience in storing tree seeds suggests an expansion of Robert's definitions of seed storage classes (Bonner 1990). There are four classes of tree seed storage behavior (table 12) [table 7 in Student Outline].

1. **True orthodox**

a. True orthodox seeds are tolerant of desiccation (table 13) [table 8 in Student Outline], and:

(1) Can be dried to 5- to lo-percent moisture levels.

(2) Can be stored at subfreezing temperatures.

(3) Are easily stored for one rotation under these conditions.

(4) Have generally unknown upper limits of storage.

b. Examples include most of the valuable temperate genera (*Pinus, Picea, Betula, Prunus)* and many tropical genera *(Acacia, Eucalyptus,* and *Casuarina).*

2. Suborthodox

a. Suborthodox seeds require the same conditions as true orthodox seeds but are limited to shorter periods (table 14) [table 9 in Student Outline].

(1) They are stored under the same conditions.

(2) Some limits to storage potential are high lipid contents, thin seedcoats, and genetic makeup.

b. Examples include:

(1) Seeds with high lipid content (*Juglans, Carya,* some *Abies,* and *Pinus*).

(2) Seeds with thin seedcoats (*Populus* and *Salix*).

(3) Seeds that must be dried slowly *(Fagus* and *Citrus).*

(4) Genetics; e.g., [Have the students suggest some].

c. These species could be classed as "true orthodox" if the limitations were overcome. *Gmelina arborea* is one borderline species.

Table 12.-*Storage conditions for four* storage classes *of tree seeds [table 7 in Student Outline]*

Storage class	Storage period	Seed moisture	Temperature	Container type
	Years	*Percent*	°C	
True orthodox	<5	6–10	o-5	Airtight
	>5	6-10	-18	Airtight
Suborthodox	<5	6-10	o-5	Airtight
	>5	6-10	−18	Airtight
Temperate recalcitrant	<3	30-45	−1 to -3	4-mil* plastic, unsealed
Tropical recalcitrant	<1	30-45	12-20	4-mil plastic, unsealed

*mil = 1/1,000 inch = 0.025 mm.

Table 13. -*Storage test results for true orthodox species* (adapted *from Bonner 1990) [table 8 in Student Outline]*

Species	Storage conditions		Storage results	
	Temperature	Seed moisture	Time stored	Viability loss
	°C	*Percent*	*Years*	*Percent*
Abies procera	*0*	*9*	7.0	11
Acacia leptopetala	20-25	...'	18.0	1
A. mangium	4-8	...	1.2	6
A. pruinocarpa	20-25		16.0	20
Acer saccharum	-10	10	5.5	5
Albizia falcataria	4-8	...	1.5	10
Araucaria *cunninghamii*	-15	16-23	8.0	few+
A. cunninghamii	19	7	0.1	0
Casuarina equisetifolia	-3	6-16	2.0	O-5
C. torulosa	20-25	8-12	18.0	6
Liquidambar styraciflua	3	5-10	9.0	3
Pinus caribaea var. hondurensis	8	...	2.1	±16
P. elliottii	4	10	50.0	30
P. merkusii	4-5	<8	4.0	0
l? ponderosa	0	8	7.0	0
Tectona grandis	0 4	≃12	7.0	0
Tsuga heterophylla	5	8	2.0	0
T. heterophylla	-18	8	2.0	0

'Data not available.
†Exact value not available from original source.

Table 14. -*Storage test results for suborthodox species (adapted from Bonner 1990) [table 9 in Student Outline]*

Species	Storage conditions		Storage results	
	Temperature	Seed moisture	Time stored	Viability loss
	°C	*Percent*	*Years*	*Percent*
Citrus limon	-20	5	0.9	±5
Fagus sylvatica	-10	10	5.0	34
Gmelina arborea	-5	6-10	2.0	10
Populus deltoides	-20	6-10	6.0	21
Salix glauca	-10	6-10	1.2	0

Table *E.-Storage test results for temperate recalcitrant species (adapted from Bonner 1990) [table 10 in Student Outline]*

Species	Storage conditions		Storage results	
	Temperature	Seed moisture	Time stored	Viability loss
	°C	*Percent*	*Months*	*Percent*
Acer saccharinum	-3	50	18	8
Quercus falcata var. pagodaefolia	3	35	30	6
Q. robur	-1	4045	29	31-61
Q. rubra	−1 to -3	3845	17	18-46
Q. virginiana	2	.*	12	35

*Data not available.

3. Temperate recalcitrant

a. Temperate recalcitrant seeds are intolerant of desiccation (table 15) [table 10 in Student Outline].

(1) They cannot be dried below 20- to 30-percent moisture; thus, storage must generally be above freezing, although some *Quercus* species have been reported to survive storage at -2 °C.

(2) Metabolism and pregermination are common in storage.

(3) They cannot be stored in airtight containers; some gas exchange is necessary (table 12) [table 7 in Student Outline].

b. Examples include *Quercus* (high lipid) and *Aesculus* (high carbohydrate).

4. Tropical recalcitrant

a. Tropical recalcitrant seeds have the same moisture and gas exchange requirements as temperate recalcitrant seeds, but they are sensitive to low temperature. Chilling damage and death will occur below 12 to 20 °C, depending on the species (table 16) [table 11 in Student Outline].

b. They are the most difficult group to store, even for short periods.

c. Examples include species of *Shorea,*

Table *16.-Storage test results for tropical recalcitrant species (adapted from Bonner 1990) [table 11 in Student Outline]*

Species	Storage conditions		Storage results	
	Temperature	Seed moisture	Time stored	Viability loss
	°C	*Percent*	*Days*	*Percent*
Araucaria hunsteinii	19.0	25-30	54	±30
A. hunsteinii	2.0	30	365	82
Azadirachta indica	26.0	10–18	56	65
Hopea helferi	15.0	47	37	2
Shorea robusta	13.5	40-50	30	60
S. roxburghii	16.0	40	270	±30

Table 17. -Storage *test results for cryogenic trials of forest tree seeds (adapted from Bonner 1990) [Table 12 in Student Outline]*

Species	Seed moisture	Time stored	Viability loss
	Percent	*Days*	*Percent*
Abies alba	. *	6	5
A. concolor	<13	180	0
Fagus sylvatica	...	6	100
Larix decidua	...	6	5
Picea abies		6	1
Pinus echinata	.	112	0
P. ponderosa	<13	180	0
l? sylvestris	,,,	6	0
Populus tremula × *tremuloides*	,	6	1
Ulmus pumila	...	112	0

'Data not available.

Hopea, Dipterocarpus, and even some legumes; e.g., in Costa Rica, *Pithocellobium*.

E. Cryogenic Storage

Cryogenic storage is a method for very long-term storage for germplasm conservation (table 17) [table 12 in Student Outline].

1. **Techniques**
   a. Packages of seeds are immersed in liquid nitrogen (– 196 °C) or suspended above it in the vapor.
   b. The potential for germplasm conservation is good for small quantities but not for bulk lots.
   c. The upper (maximum) time limits of viability retention are not known. Only a few tests have been made on tree seeds.
2. **Costs-Comparable** with conventional storage in some cases of small seeds: $0.30 to $1.41 per year per sample of 1,000 seeds.

F. Physical Facilities

The optimum storage environment requires cold storage units, proper containers, and moisture management.

1. **Cold storage units**
   a. Cold storage units require a reliable power source, should not be used where floods or earthquakes may occur, should be located near other seed activities, should be rodent proof, and should be on high elevations when possible because ambient temperatures will be cooler.
   b. They should be built to hold a 5-year supply (or whatever the operational time).
   c. For germplasm conservation, 1 liter is needed of each sample (3,000 to 12,000 seeds) for medium-sized seeds (wheat and rice); e.g., 85 m^3 should hold 22,800 samples.
   d. Humidity control is not recommended in the Tropics; the seeds are dried and sealed in containers. If the power fails, seeds stay dry; this method is less expensive than humidity control.
   e. Direct or indirect vapor-compression refrigeration is best for the Tropics; it is usually available and reliable.
   f. Standby generators and safety alarms are recommended.
   g. When the power goes off and cooling stops, thermal time constants of 4 to 5 days should apply in large coolers, but if orthodox seeds are dry, serious damage may not occur for 2 weeks.
   h. Modular panel units are effective.
   i. Insulation depends on ambient conditions. A reliable local refrigeration specialist should be employed; an R value of 35 (heat transfer coefficient of 0.029 or less) is recommended.
2. **Containers**
   a. Fiber drums are very effective. Common capacities are 0.45 and 0.90 hL (25 and *50* kg for *Pinus taeda).*
   b. Generally, plastic is better than glass; plastic bags can be inserted inside glass containers.
   c. Rectangular containers utilize space better than round ones, but air spaces are still needed between containers. Round containers assure air spaces.
   d. Plastic bags should be 0.1 to 0.2 mm thick in humid atmospheres.
3. **Moisture management**
   a. Seeds will reach an equilibrium moisture content when exposed to the storage atmosphere.

b. If the storage unit has humidity control (50 to 60 percent relative humidity), orthodox seeds need not be sealed. Recalcitrant seeds cannot be sealed, so they cannot be stored in such a unit; the low humidity would desiccate the seeds.

c. Without humidity control, relative humidity will be 95 percent or more, which is fine for recalcitrant seeds. Orthodox seeds must be dried and stored in sealed containers in such a unit.

d. Humidity control is not recommended for the Tropics because both orthodox and recalcitrant seeds will be stored in the same facility.

e. Frost-free refrigerators are an alternative for humidity control because these systems remove the moisture from the unit.

G. Genetic Damage in Long-term Storage

1. Long-term storage could be devastating to germplasm conservation, but there is no strong evidence to date of lasting damage. Point aberrations occur on chromosomes, but they are not passed along to the next generation (fig. 32) [no equivalent figure in Student Outlinel.

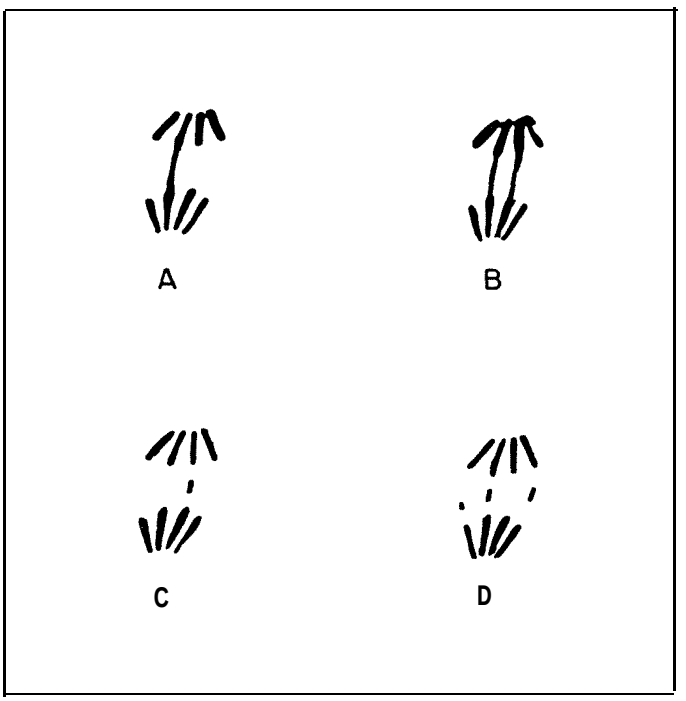


Figure 32.—*Chromosome aberrations found in germinated fresh and 50-year-old* Pinus echinata: *(A) single bridge, (B) double bridge, (C) single fragment, (D) multiple fragments (Burnett and Vozzo 1985) [no equivalent figure in Student Outline].*

2. Population changes are almost certain in heterogenous seedlots; some parts of the population die sooner in storage. The impact of this effect is still unknown.

H. Retesting in Long-term Storage

1. **Test procedures--ETA (1985)** recommends 4 replications of 100. Subsequent tests can be 2 replications of 100; if viability has fallen 5 percent, the test is repeated with another 2 replications of 100.

2. **Test interval for orthodox seeds**– Should be initial year, third year, and every fifth year thereafter.

3. **Nondestructive testing-** Leachate conductivity could be used.

4. **Regeneration-In** annual plants, regeneration is done when viability falls to 50 percent. There may be a better plan for trees.

I. Viability Constants in Storage

The use of viability constants in storage is another technique introduced by Roberts (1973).

1. **Theory-Viability** retention will,be the same for a given species under a given set of storage conditions. This property can be expressed as a "viability constant" for each species.

2. **Practice**

a. Good results have been obtained with agricultural seeds.

b. Critics say varieties of a single species will differ.

c. One must start with very good seeds.

d. There are few data for tree seeds. Tompsett (1986) has developed constants for several tree species, and other data are available for two pines, *Liquidambar styraciflua,* and *Platanus occidentalis.* The two hardwoods fit the expected pattern, but the pines have shown damage repair at high moisture levels after 8 years.

e. The above species are all orthodox. A different technique is needed to determine viability constants for short-lived recalcitrantspecies.

3. **Viability constants** for forest species could be useful in long-term storage for germplasm conservation or when good storage conditions are not available.

[2]Bonner, Franklin T. [n.d.] Unpublished research notes. On file with: U.S. Department of Agriculture, Forest Service, Southern Forest Experiment Station, Starkville, MS 39759.

4. Procedures

a. Store samples in many combinations of temperatures and moisture contents, and test frequently.

b. Plot seed death over time for each condition, using probit transformations.

c. Calculate coefficients for time, temperature, and seed moisture content.

d. See table 18 [no equivalent table in Student Outline].

J. Summary

Review table 12 [table 7 in Student Outline].

K. Sources

For additional information, see Bonner 1990, Bonner and Vozzo 1990, Chin and Roberts 1980, Harrington 1972, International Board for Plant Genetic Resources 1976, Justice and Bass 1978, Roberts 1973, Tang and Tamari 1973, Willan 1985.

Table 18. -*Viability equation' coefficients for seeds of* Ulmus carpinifolia *and* Terminalia brassii *(Tornpsett 1986) and four United States species [no equivalent table in Student Outline]*

Species	K_E	C_W	C_H	C_q
Liquidambar styraciflua	5.3435	1.7616	0.0307	0.000869
Pinus elliottii	5.2463	0.9832	0.0508	0.000571
P. taeda	3.2783	0.7300	0.0348	0.000328
Platanus occidentalis	5.1013	1.6742	0.0354	0.000838
Terminalia brassii	4.9990	2.1490	0.0350	0.000410
Ulmus carpinifolia	5.7150	2.9660	0.0340	0.000408

$$V = Ki \cdot p/_{10}K_E \cdot C_W \log_{10} m \cdot C_H t \cdot C_q t^2,$$

where

V = probit percent viability,

Ki = initial viability,

p = storage time (days),

K_E = species constant,

m = percent moisture,

t = temperature (°C), and

C_W, C_H, C_q are coefficients.

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].

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	2530	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	a12
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
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

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. Forexample:

   Number of drums in the lot
   12 3 4 5 6 7 10 23 50
   Number of drums to sample
   1 2 3 4 5 6 6 6 7 1 0
   Total number of primary samples
   5 5 5 5 5 6 6 6 7 1 0

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:

1. 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 (ZSTA 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: SO 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 live 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, l? echinata, P. 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 35.1).
   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) = $\sqrt{\text{variance}}$
Coefficient of variation (CV)
$= \left(\frac{\sigma}{\bar{x}}\right) 100$ where $\bar{x}$ = 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 n = 8
3.12 Σx = 22.64
3.00 $\bar{x}$ = 2.83
2.78 Σx^2 = 64.52
2.97 σ = 0.2530
2.42 CV = $\left(\frac{0.2530}{2.83}\right)$ 100 = 8.9%
3.02
2.83

Weight determination example 2

Lot 2 where:

2.80 *n* = *8*
2.78 Σx = 23.28
3.00 $\bar{x}$ = 2.91
2.94 Σx^2 = 67.80
2.97 σ = 0.0866
3.01 cv = $\frac{0.0866}{2.91}$ 100 = 3.0%
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. l,OOO-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.

$$\text{Number per gram} = \frac{1{,}000}{\text{weight of 1,000 seeds in grams}}$$

$$\text{Number per pound} = \frac{453{,}600}{\text{weight of 1,000 seeds in grams}}$$

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 [foot-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 lo-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
$x_1 = 85$	$\bar{x}_2 = 88$	$\bar{x}_3 = 86$
$R_1 = 10$	$R_2 = 20$	$\bar{x}$ 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
$x_1 = 85$	$\bar{x}_2 = 73$	$\bar{x}_3 = 64$
$R_1 = 10$	$R_2 = 51$ drop 40,	$R_3 = 75$ drop 15,
	$x_2 = 84$	$\bar{x}_3 = 81$
	$R = 13$	$R = 20$; 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, lo-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 l-l-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
	$\bar{x}$+	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*		GV†	
	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

*Peak value.
†Germination value.

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

Species	Suggested pretreatment	Medium+	Temperature	Duration	Comments
			°C	Days	
Acacia nilotica	Sc: M, A, HW; CW 24 hr	S, B	20/30‡	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 camaldulensis	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	B	20/30	14	§

*Pretreatment codes: A = acid scarification; CW = cold water soak; HW = hot water treatment; M = mechanical scarification; Pc = prechill; Sc = scarify.
†Medium 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	PV	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 much dead; abnormal total suggests damage; recondition to remove dead seeds if possible.

[1]Peak 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 seed-lot 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 l-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-

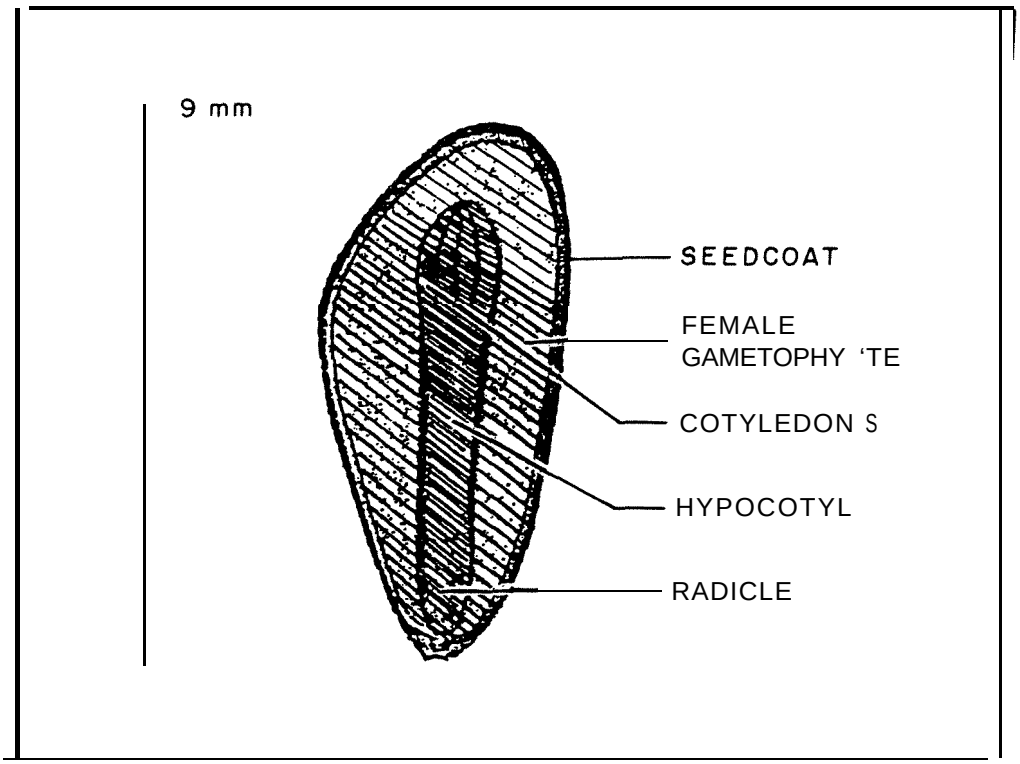


Figure 37. – Pinus: *Good stain (lined* area); *good seed (adapted from Krugman and Jenkinson 1974) [no equivalent figure in Student Outlinel.*

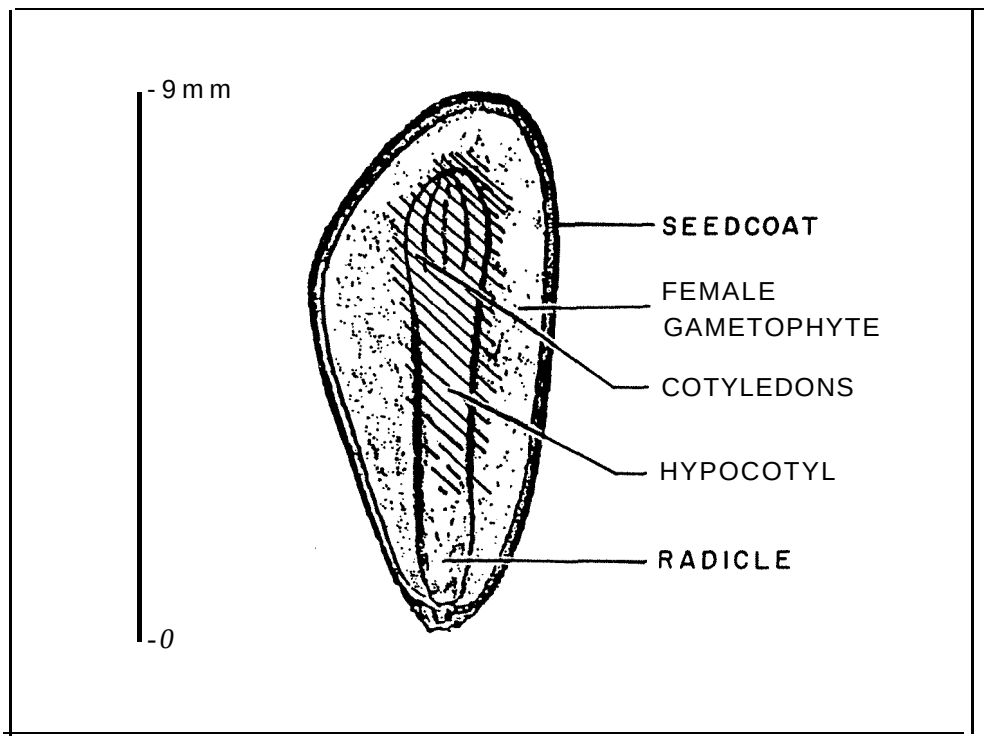


Figure *38.* – **Pinus:** *Cotyledons weakly stained (lined area); almost no radicle stain; probably nongerminable or perhaps poor TZ penetration (adapted from Krugman and Jenkinson 1974) [no equivalent figure in Student Outline].*

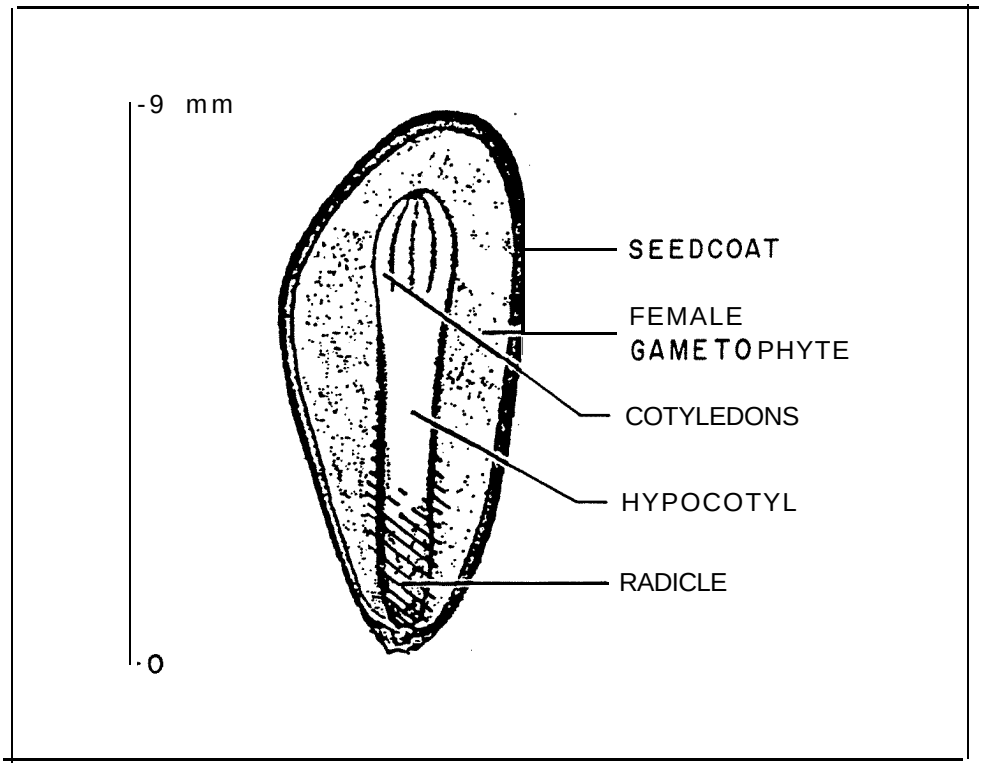


Figure 39.—Pinus: *Radicle stain only (lined area); nongerminable (adapted from Krugman and Jenkinson 1974) [no equivalent figure in Student Outline].*

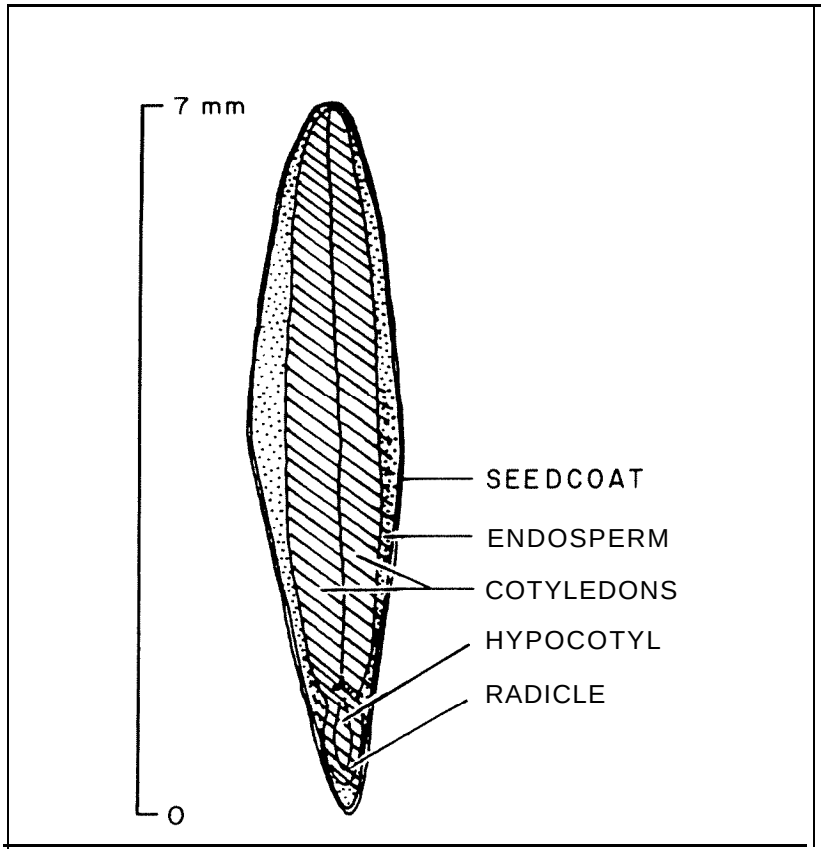


Figure 40. -Leucaena: *Complete stain (lined* area); *good seed (adapted from Whitesell 1974) [no equivalent* figure *in Student Outline].*

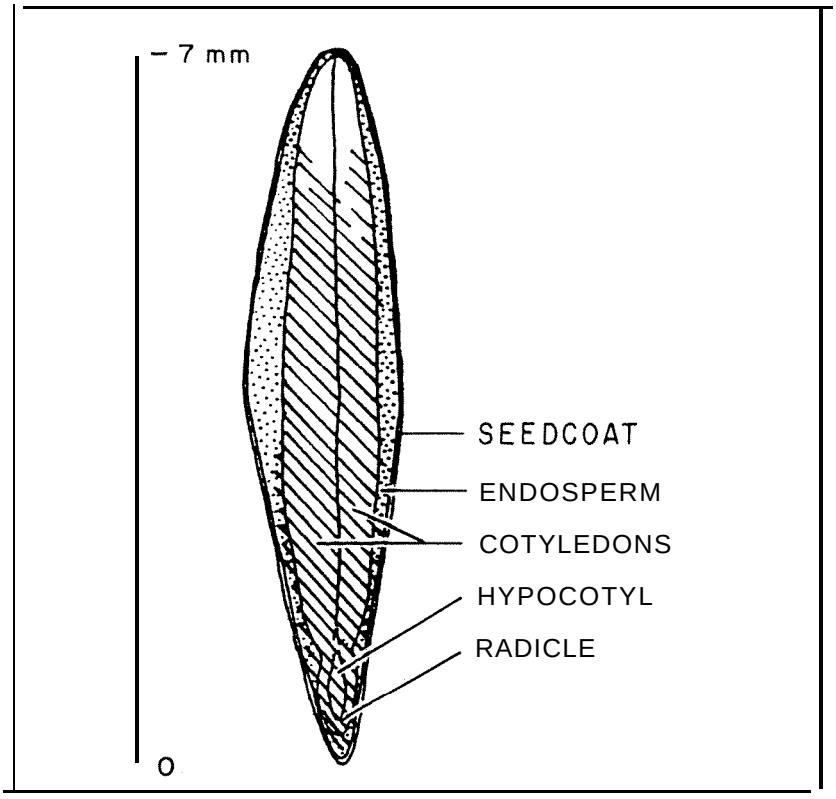


Figure 41.—Leucaena: *Unstained cotyledon tips (blank* area); *should germinate (adapted from Whitesell 1974) [no equivalent figure in Student* Outline].

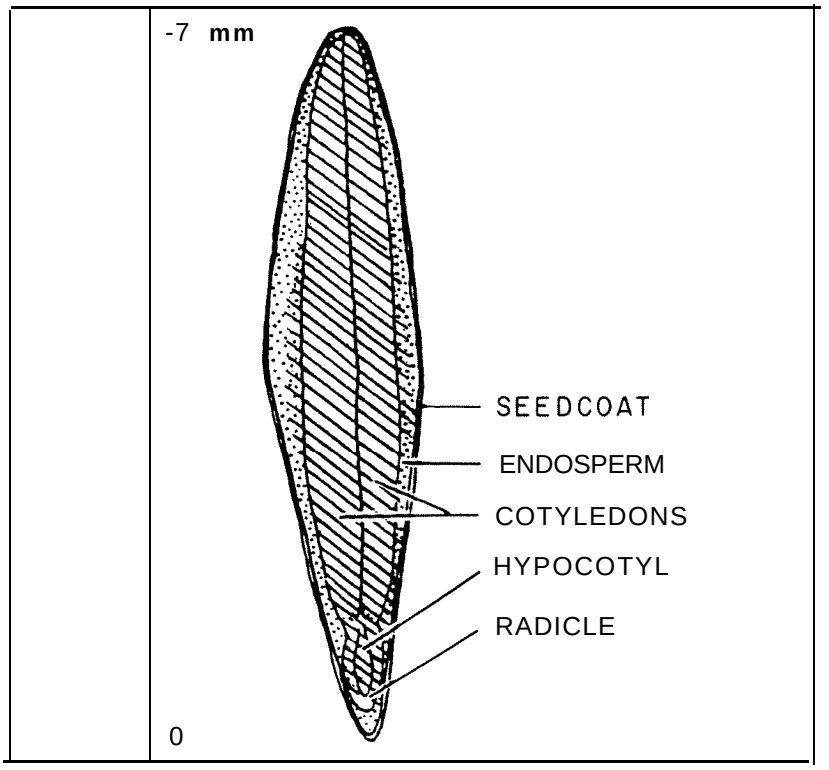


Figure 42. — Leucaena: *Less than one-third of the radicle tip is unstained (blank area); should germinate (adapted from Whitesell 1974) [no equivalent figure in Student Outline].*

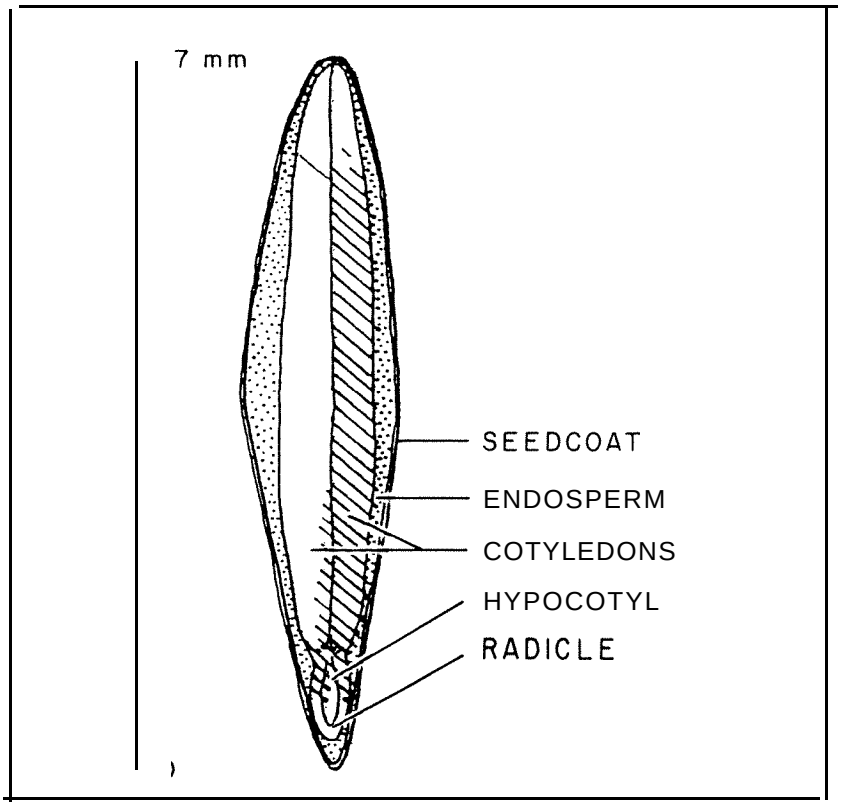


Figure 43. -Leucaena: *One cotyledon unstained and more than one-half of the radicle unstained (blank areas); nongerminable (adapted from Whitesell 1974) [no equivalent figure in Student Outline].*

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 agents are used

d. Disadvantages
   (1) Equipment is expensive
   (2) Extensive training is required
   (3) Interpretation is subjective

6. Leachate conductivity

a. 'Ibchnique: 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 to establish the relationship of conductivity to germination.
   (2) Still under development; some unknown factors causetrouble.

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) **Kilovoltage** (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.

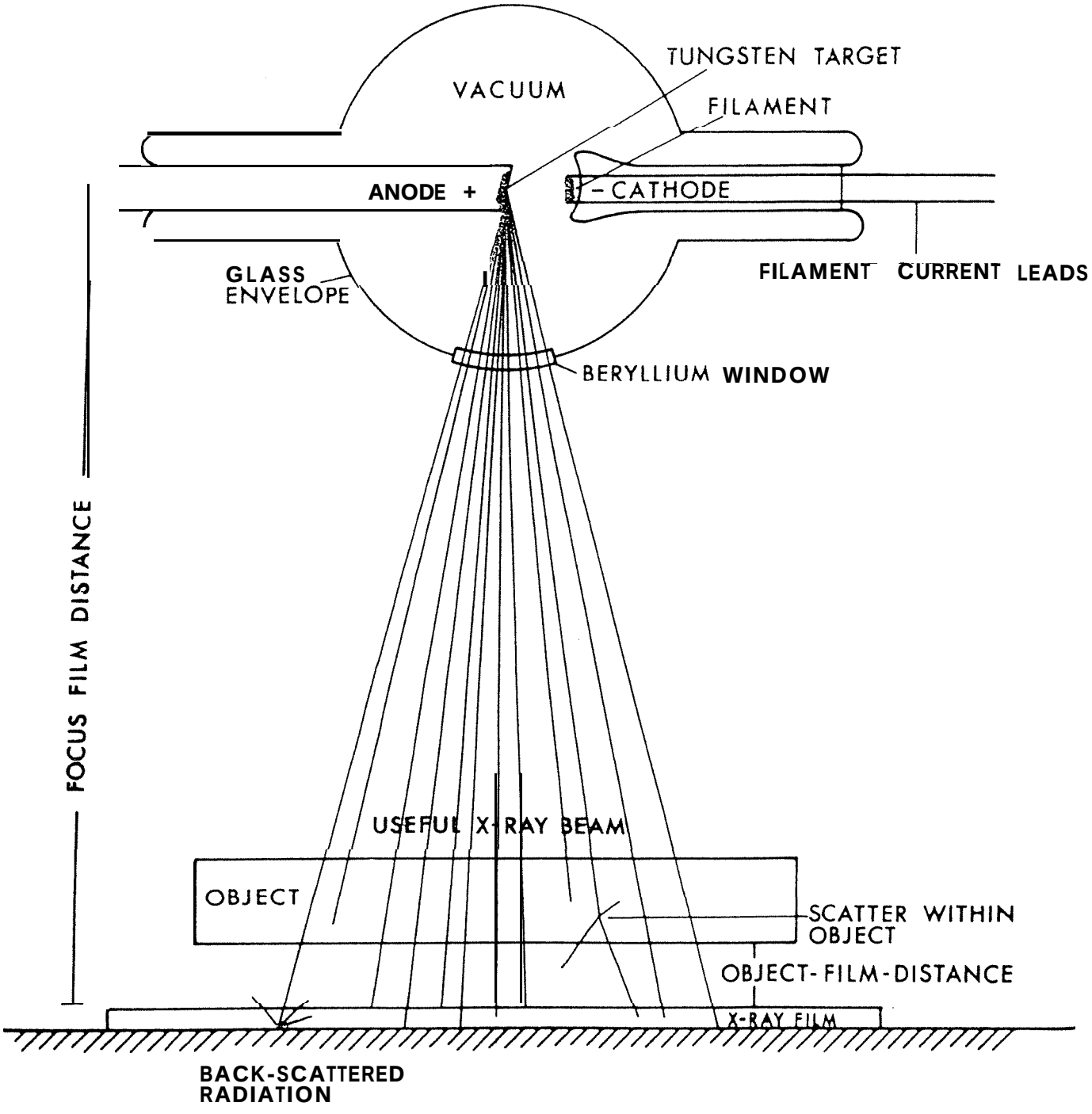


Figure 44.—*Diagramatic view of an x-ray apparatus (Simak 1980) [no equivalent figure in Student Outline].*

(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 areavailable.

(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 ($BaCl_2$) 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 ($CHCl_3$) (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

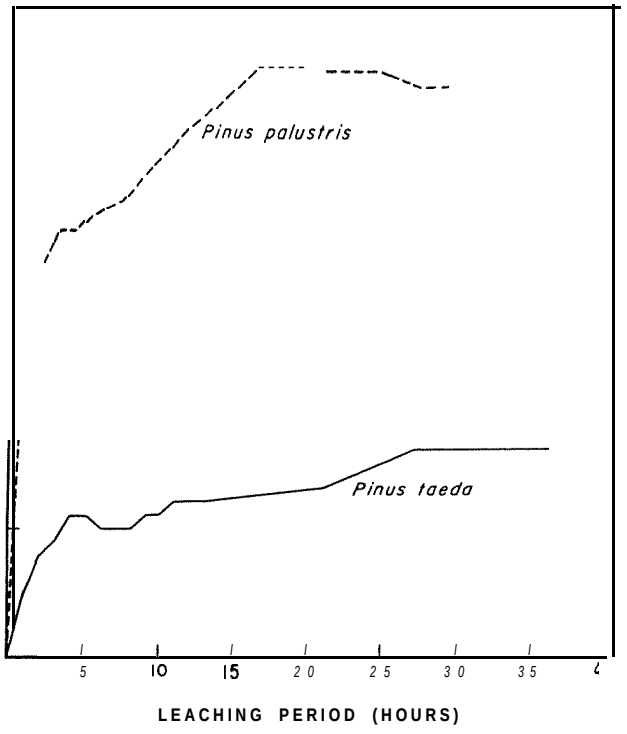


Figure *45.-Release of electrolytes over time from seeds of* Pinus palustris *and P.* taeda *(adapted from Bonner and Vozzo 1986) [no equivalent figure in Student Outline].*

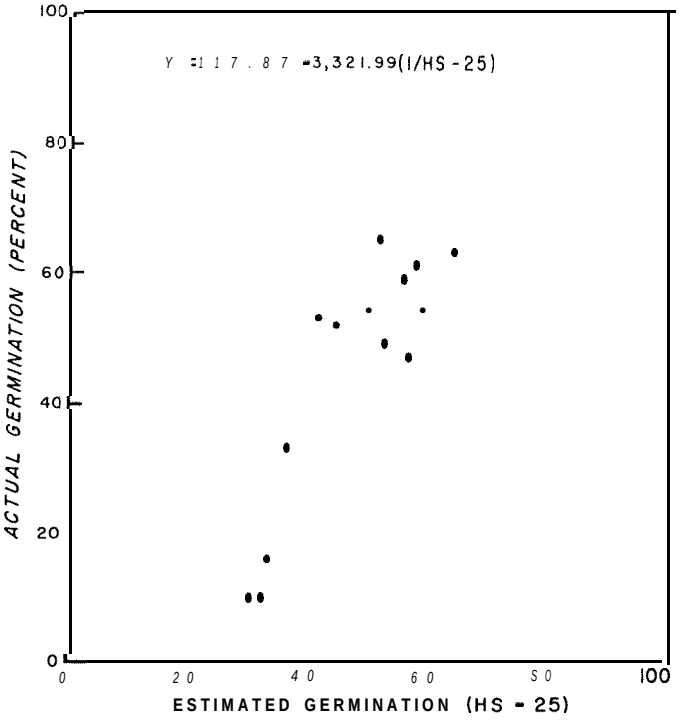


Figure *46. -Relationship of actual germination to estimated germination in* Pinus palustris *as determined with the ASAC-1000 Analyzer (adapted from Bonner and Vozzo 1986) [no equivalent figure in Student Outline].*

(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

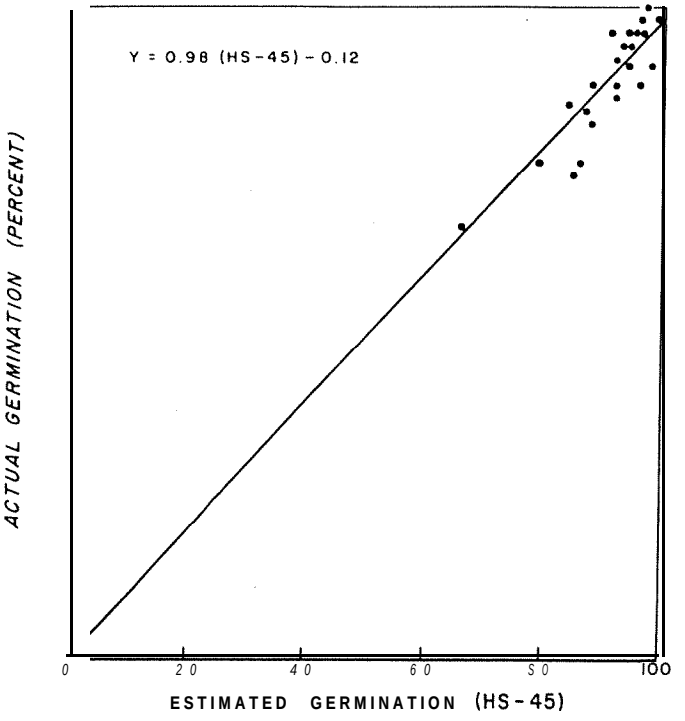


Figure 47. *-Relationship of actual germination to estimated germination in* Pinus taeda *as determined with the ASAC-1000 Analyzer (adapted from Bonner and Vozzo 1986) [no equivalent figure in Student Outline].*

(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
I? 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).

†No nursery tests with these species.

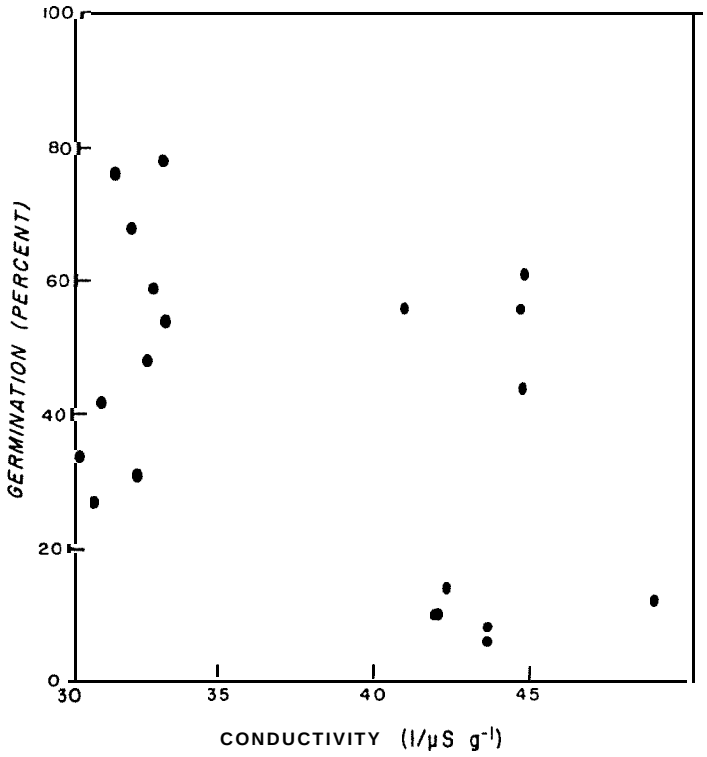


Figure *48. -Relationship of germination to leachate conductivity for* Eleagnus angustifolia *[no equivalent figure in Student Outline].*

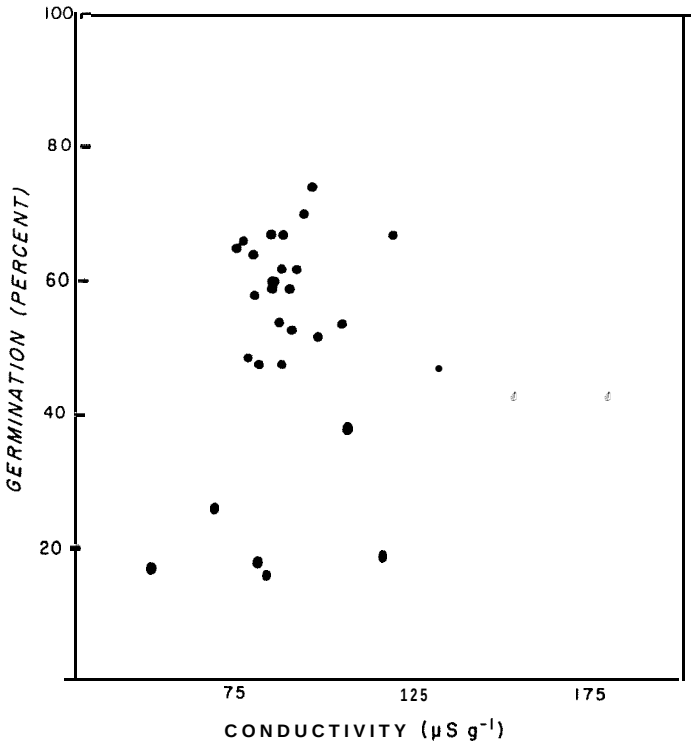


Figure *49. -Relationship of germination to leachate conductivity for* Picea glauca as *measured with a bulked sample (Bonner and Agmata-Paliwal 1992) [no equivalent figure in Student Outline].*

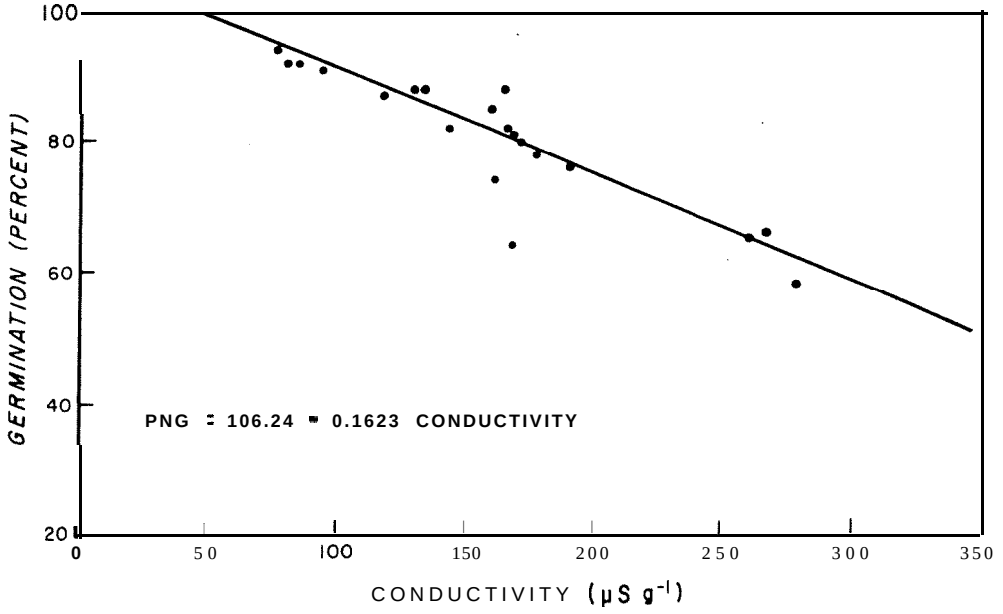


Figure 50. *-Relationship of germination to leachate conductivity for* Gleditsia triacanthos samples *that were given accelerated aging to produce varying levels of quality (Hooda and others, in press) [no equivalent figure in Student Outline].*

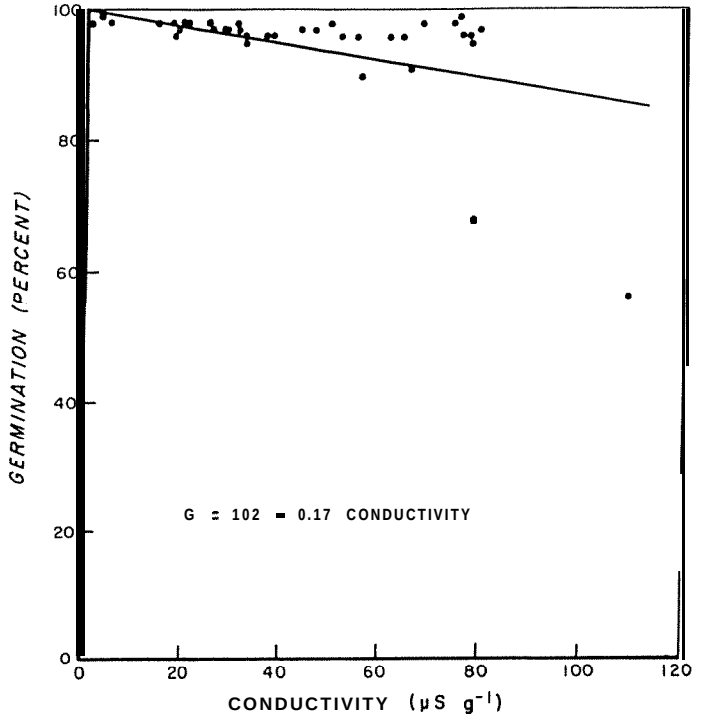


Figure 51 .-*Relationship of germination to leachate conductivity for* 38 *mixed seedlots of* Picea rubens *(Bonner and Agmata-Paliwal 1992) [no* equivalent *figure in Student Outline].*

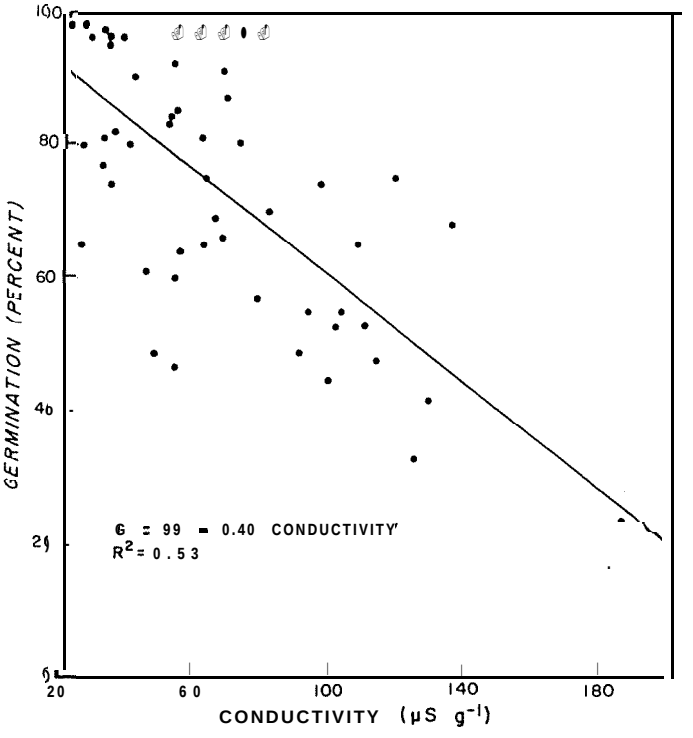


Figure *52. -Relationship of germination to leachate conductivity for 14 seedlots of* Picea rubens *that were given accelerated aging to produce varying levels of quality (Bonner and Agmata-Paliwal 1992) [no equivalent figure in Student Outline].*

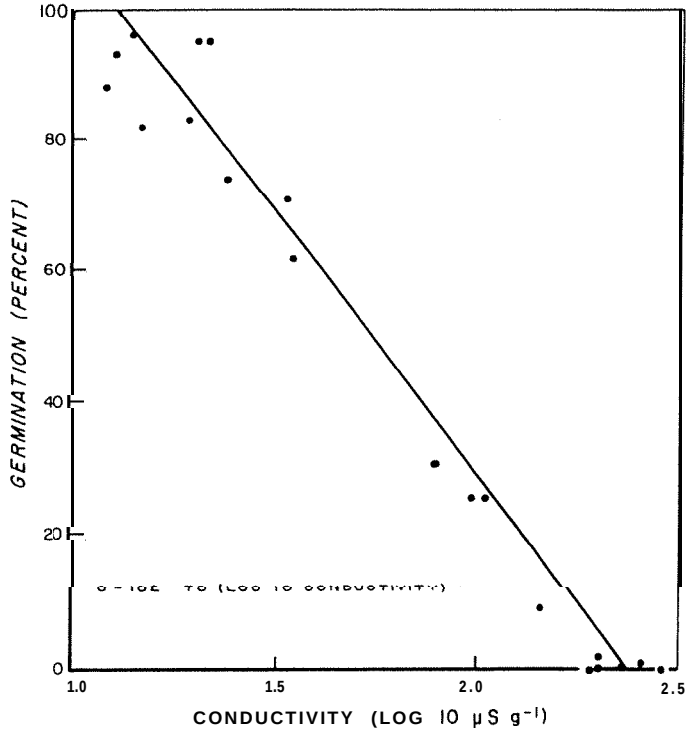


Figure *53. -Relationship of germination to leachate conductivity for seeds from a single mother tree of* Picea rubens, *which were given accelerated aging (Bonner and Agmata-Paliwal 1992) [no equivalent figure in Student Outline].*

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 °Cl) 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 *Hevea* 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.

Protection

I. Insects

A. Introduction

Insects are one of the greatest destroyers of tree fruits and seeds. They reduce both quality and quantity of seeds and affect angiosperms and gymnosperms equally. Damage is done through all reproductive stages, from developing buds to cleaned seeds in storage. Losses to seed insects are huge, and much is yet to be learned about their complete role in the reproductive cycle of woody plants.

B. Objectives

1. Learn the orders of insects that cause the most damage to tree seeds and the species they attack.
2. Recognize the types of injury that insects cause.
3. Learn some methods of insect control and management.

C. Key Points

The following points are essential in protecting seeds from insects:

1. Insects of the orders Hymenoptera, Diptera, Lepidoptera, Hemiptera, Coleoptera, Homoptera, and Thysanoptera do the most damage to flowers, fruits, and seeds of woody plants.
2. Damage ranges from causing reproductive structures to abort to causing loss of seeds in storage.
3. General types of damage include:
   a. Destroying the seeds only, Hymenoptera (wasps).
   b. Forming galls and mine scales, Diptera (flies).
   c. Free feeding, Lepidoptera (moths).
   d. Consuming endosperm, Hemiptera (true bugs).
   e. Mining cone axes, Coleoptera (beetles).
   f. Causing cone abortion, Homoptera (aphids and others) and Thysanoptera (thrips and others).
4. Control methods depend on identifying the insect's life cycle and the host-plant relationship.
5. Some methods for reducing damage are preventive measures, insecticides, natural biological control agents, and proper management techniques.

D. Damage

1. General concepts

a. Insects reduce seed production by infesting buds, flowers, cones, and seeds. Bud damage causes wilting, abnormal growth, and abortion of flowers and immature fruit. Damaged fruits may be deformed, worm infested, riddled with galleries, and susceptible to secondary infection by pathogens.

b. The most damaging insects to flowers, fruits, and seeds of most trees are largely restricted to six orders: Lepidoptera (moths and butterflies), Diptera (flies), Coleoptera (beetles), Hymenoptera (wasps), Hemiptera (true bugs), and Thysanoptera (thrips).

2. Specific concepts

a. Coleoptera (beetles) are the most damaging group in arid and semiarid zones.

(1) Bruchidae (bruchid beetles) are the most important by far for Leguminosae.

(a) ***Amblycorus, Bruchidius, Caryedon,*** and others feed on ***Prosopis, Tamarindus, Acacia, Parkinsonia, Gleditsia,*** and ***Cordia.***

(b) Eggs are deposited on developing fruits, and the larvae eat seed tissues. One group emerges at fruit maturity (trees and shrubs). **A** second group infests pods at later stages of development, emerges, **and** reinfests the same seeds in storage (e.g., peas and beans, Fabaceae).

(c) Bruchid beetles are serious seed predators of Acacia in arid zones.

(2) Curculionidae (weevils) lay their eggs on developing fruits.

(a) ***Conotrachelus*** are important cone worms in Mexican *Pinus* and other subtropical pines.

(b) *Curculio* and ***Conotrachelus are*** the common acorn weevils in ***Quercus.***

(c) ***Thysanocnemis*** are important seed weevils in ***Fraxinus.***

(d) ***Nanophyes*** can destroy 60 percent of the ***seed*** crop of *Terminatia ivorensis.*

(e) ***Apion ghanaense*** destroy many flowers and seeds of *Triplochiton.*

b. Lepidoptera (moths and butterflies) can damage stored seeds.

(1) Pyralidae attack many tropical legumes, primarily the developing pods and seeds. This family also contains the very damaging cone worms of *Pinus (Dioryctria).* Larvae destroy seeds, cone scales, and cone axes (60 percent loss).

(2) *Melissopus* and *Valentinia* are moths whose larvae destroy developing fruits of *Quercus.*

(3) Agathiphaga can destroy more than half the *seeds* in *Agathis* cones in the western Pacific area.

(4) Gelechiidae is a family of small moths that attack *Juniper-us* cones; they are very destructive.

c. Hemiptera (true bugs) feed on seeds with specialized sucking mouth parts. Saliva is injected into seed tissue to digest it.

(1) Coreidae attack *Erythrina* seeds in India and some *Acacia* species in Africa. The bugs reportedly move to trees when crops are sprayed. This family also includes *Leptoglossus,* the very damaging pine seedbug on tropical and subtropical *Pinus,* and *Leptocentrus,* which damages flowers and fruits on teak.

(2) Pentatomidae, the stink bug family, feed on seeds of *Pinus.*

d. Hymenoptera (wasps) includes many beneficial insects, but also some that feed on tree seeds.

(1) Torymidae includes a major conifer *seed* pest, *Megastigmus* spp. Its larvae feed on *Pinus, Abies,* and *Pseudotsuga.*

(2) Eurytomidae includes the genus *Bruchophagus,* whose larvae feed on *Acacia* seeds; most feed on smaller legumes than *Acacia* because Acacia pods are so tough.

e. Homoptera includes aphids, cicadas, and scales. Scales damage some Mexican pines, but they are not a major predator. Some damage to cones and seeds of *Pinus pinaster* is caused by aphids in South Africa; in one instance, seed yield was reduced by 72 percent.

f. Thysanoptera (thrips) cause damage, but little is known about the important predators. However, *Gnophothrips* damage or kill female buds and flowers of *Pinus* (Mexican and other subtropical species).

E. Controlling Insects

Control measures must be guided by the species and ecology of the insect to be controlled. The first step is to identify the problem and its source insect. Seeds with insects feeding internally are collected and placed in plastic bags to allow the insects to mature. The resulting information on the life cycle and insect-host relationship determines control measures.

1. **Prevention —** The insect may be prevented from reaching the seeds. For example, in The Gambia, long sleeves are put over the flowering branches to exclude pests in *Acacia* seed orchards. Such measures are feasible only in very valuable seed orchards.

2. **Chemical control** must be applied when the insect is at a vulnerable point in its life cycle. For example, if the insect spends most of its life inside of, and protected by, seed tissues, chemical sprays will not be effective during that period.

a. Foliar sprays: l-percent azinphosmethyl spray for cone worms and seedbugs was once common in Southern United States pine seed orchards, but environmental concerns reduced this practice. Sprays of nuvacron and endosulfan have controlled insect damage in teak seed orchards.

b. Systemic poisons: Carbofuran applied as granules in the soil (4.5 g per centimer of diameter at breast height [d.b.h.]) gives good control of cone worms, borers, and seedbugs, but it is not effective against seed chalcids.

c. Light traps: Can be used in seed orchards to reduce the population of egg-laying adults.

d. Chemical traps: Baited with sex attractants, chemical traps may reduce breeding populations.

e. Carbon dioxide: Treatment with carbon dioxide is a promising new measure to kill larvae in seeds. Shipping seeds in a carbon dioxide-rich atmosphere is also promising.

3. **Natural enemies-The** target insect's life cycle and history should reveal its natural enemies.

a. The bruchid beetles have natural parasitoids that attack the egg, larval, and pupal stages. The parasitoids are mostly from the insect order Hymenoptera. Mass-rearing the parasitoids to control the beetles may be possible if circumstances warrant.

b. In Hawaii, seed beetles have been successfully controlled biologically by introducing wasps to attack eggs, larvae, and pupae. The seed beetles lay their eggs on the outer surface of the pods and are, therefore, easily controlled by wasp predators. Larvae and pupae, however, are more difficult to control. Adult seed beetles have such short lives they are not susceptible to parasitoids.

c. A species of mite *(Pymotes)* will also feed on seed beetles. However, laboratory results do not corroborate field data, so this control method is in question.

4. Collection- Good seed collection is the first step in minimizing losses incurred in storage. Removing infested seeds during cleaning is the second step. Water flotation and density separation methods can be extremely effective. If good seeds were collected, they must be stored properly to control insects. Both low temperature and low moisture content will subdue insect infestations; however, it is much better to avoid transporting the insects to storage initially. Two weeks at −18 °C will kill most larvae. Drying seeds at 40 to 42 °C will kill insects. Heating seeds in warm water and fumigating with serafume, methyl bromide, or carbon bisulphide will also control insects. Chemical treatments should be avoided, however, because they can reduce viability if applied improperly. High moisture content makes recalcitrant seeds especially susceptible to damage.

F. Sources

For additional information, see Cibrian-Tovar and others 1986, Johnson 1983, Schopmeyer 1974, Southgate 1983.

II. Pathogens

A. Introduction

Pathogenic organisms (fungi, bacteria, and viruses) cause great economic losses. Not only are seeds the victim of pathogens, but they also are passive carriers (vectors) of pathogens that may not directly affect the seeds but may endanger other organisms. This fact is the basis of plant quarantine regulations that include seeds in the import and export restrictions on plant material.

B. Objectives

1. Learn the major types of seed pathogens and the typical damage that they cause.
2. Identify steps to decrease losses to seed pathogens.
3. Review documented occurrence of micro-organisms associated with tree seeds.

C. Key Points

The following points are essential to preventing seed pathogens:

1. The major disease-causing organisms are fungi, bacteria, and viruses.
2. All tree seeds carry micro-organisms, primarily on the surface of their seedcoats.
3. All seed micro-organisms are not pathogenic; some may even be beneficial.
4. Pathology of tree seeds has not been studied extensively; much work remains to be done.

D. Types of Pathogens

1. **Viruses**

a. Viruses are known to cause seven kinds of seed damage:
(1) abortion
(2) flower sterility
(3) seedcoat wrinkling
(4) shriveling
(5) chalky endosperm
(6) staining
(7) necrosis

b. In legumes, embryo-borne viruses reduce seed viability. Seedcoats shrivel and crack, shrinking the seeds. The seeds usually survive, but they remain small and, most significantly, transmit the virus to other seeds. In addition, the weakened state of the seeds allows secondary infection from other pathogens to reduce viability.

c. In agricultural legumes, a higher incidence of triploidy is associated with seed-borne viral infections. These triploid seeds germinate slower with less overall vigor than diploid seeds, and their seedlings usually do not survive.

d. There are economic complications in viral infections of agricultural seeds not important to tree seeds. For example, many virus infections discolor the seedcoat. Discoloration does not always mean poor seed viability, but it can influence the market price of seeds.

e. Although viruses live in seeds for very long periods (up to 30 years in bean seeds), it is not known how long viral-infected seeds will retain viability. The virus will usually outlive the seed.

2. **Bacteria-Bacterial** infections account for four kinds of seed damage:

a. Seed abortion includes problems ranging from shriveled seeds to size reduction to seed formation interruption; in each case, it will significantly reduce seed yields. The primary cause is *Xanthomonas*.

b. Seed rot usually begins in water-soaked lesions on the seedcoat. In young seeds, there is a general, overall rotting of tissue that then decomposes into bacterial slime. In mature seeds, the infection is usually localized in the seedcoat.

Latent symptoms are rapidly rotting seedlings. Infested seeds have discolored bacterial pendants attached. Seeds are sometimes so infested that cotyledons of surviving seedlings are covered by black lesions. *Xanthomonas* is also responsible for seed rot and is sometimes accompanied by rot fungi, such as *Colletotrichum*.

c. Seed discoloration is caused by pathogenic bacteria invading seedcoat lesions. The lesions usually begin as slight depressions caused by *Xanthomonas* or *Pseudomonas*. Common colors are usually reddish brown or yellow. In extreme cases, the seed eventually rots.

d. Slime disease (known also as tundu disease or tannau disease in Asia) is widespread (from Denmark to New Zealand) and of prime significance in agricultural seeds. It causes initial rotting, then a massive slime coat when wet, which dries to cover the seed with a varnishlike substance. Some symptoms also begin as slime and result in seed abortion. All causative agents are not clearly identified, but include several *Corynebacterium* species.

3. **Fungi** are a serious threat to seed health simply because of the great numbers of species known as seed pathogens. In addition to being lethal infections, some fungi drastically reduce the quality of seeds even though they do not kill them. There are eight kinds of fungal diseases, and two or more commonly combine to attack seeds.

a. Seed abortion is the result of infection by smut fungi that infest host flower parts and replace them with fungal fruiting parts. Flowers and young seed structures are particularly susceptible to Fungi Imperfecti, but as the host matures, it can often withstand the pathogen. Other species infect but do not damage the seeds.

b. Shrunken seeds typically have shriveled seedcoats. Fungal infections of stems and leaves may cause seed shrinkage, thus reducing seed yield. Rust fungi are an example of this problem. Certain other fungi reduce the oil content in sunflowers.

c. Seed rot during germination is commonly caused by *Fusarium*, which produces dry rot. A large genus causing severe problems for many tree hosts is *Botrytis*. Also of significance are *Ciboria, Sclerotinia, Phomopsis, Valsa,* and *Gloeosporium*. Cones of many conifers are particulary susceptible to *Schizophyllum*. It is also important to control rot in seed storage. *Mucor* usually comes from the soil and then penetrates seedcoat cracks.

d. Sclerotization and stromatization are defined as the transforming of host flowers and seeds into sclerotia or stromata of invading fungi. *Ciboria* and *Phomopsis* cause a commonly known "popcorn" disease in many forest tree seeds.

e. Seed necrosis refers to dead tissue accumulation, usually attributed to seed-rot fungi. However, the penetration of the fungi is usually superficial, extending only into the epidermal tissue. In legumes, however, necrosis will extend into lesions of the cotyledons.

f. Seed discoloration is not the problem for forest tree seeds that it is for agricultural seeds. Tree seeds are usually not subject to standards of form and color that many edible seeds must meet. Still, tree seeds are discolored by lesions, fungal coatings, and pigmentation. Not all fungi identified as seed-discoloring fungi will produce pigmentation on seeds, even though the fungi have infected the seeds.

g. Lowered germination capacity is a broad category, and perhaps any pathogen could he placed here. However, some species seem to affect germination specifically. *Ustilago* may be present in the embryo but remains dormant until the seed itself germinates. Some reports attribute the cause to fungal-produced toxins.

h. The metabolites of *some* infecting fungi have adverse physiological effects in the seed. These metabolites are sometimes not harmful to the seeds but are harmful to the animals that eat the seeds.

E. Control Mechanisms

Seed pathogens can be controlled by reducing infection and treating seeds in laboratories, storage, and nurseries.

1. **Infection reduction** – Infections in orchards can be reduced by:

a. Locating seed orchards in areas of low infection risk.

b. Removing alternate host plants from seed production areas or orchards.

c. Practicing good sanitation in orchards

and destroying infected trees, old fruits and cones, etc.

d. Applying fungicides (cone rust in *Pinus*).

e. Using good cone- and fruit-handling procedures; e.g.; reducing cone storage time and removing old cones. (Enormous amounts of infection can occur during extraction.)

2. Seed treatment in laboratories

a. Surface sterilization with hydrogen peroxide (H_2O_2) (30 percent for 20 minutes), sodium hypochlorite (NaOCl) (10-percent solution of commerical bleach), or ethanol (C_2H_5OH) (75 percent is somewhat effective).

b. Fungicides come in numerous types and concentrations; length of treatment and concentration must be tested before large lots are treated.

c. A hot water soak such as 50 °C for 15 minutes is used on rice to kill both external and some internal pathogens. This method has not been tested for tree seeds, but longer and hotter treatments might be effective on hard-seeded species.

3. Seed treatment in storage is common for some agricultural seeds but not for forestry seeds. Many fumigants or liquid, organic fungicides are likely to decrease seed viability in storage. Dry treatments with fungicidal dusts are recommended for tree seeds if a fungicide is needed.

4. Seed treatment in nurseries

a. Damping-off requires treatment.

(1) Soil is fumigated before planting.

(2) Seeds are treated with fungicide. Thiram is used in pines of the Southern United States.

(3) Soil is drenched with fungicide in container operations.

b. Seedling diseases can be controlled by treating seeds with the systemic fungicide Bayleton (800 mg/liter).

F. Micro-organisms Found on Tree Seeds

See Anderson (1986a) to identify those organisms that have been documented on the species of interest.

G. Sources

For additional information, see Anderson 1986a, International Seed Testing Association 1966, Neergard 1977, Sutherland and others 1987.

Basics for Nurseries

I. Production Systems

A. Introduction

This course is not intended to cover all aspects of nursery establishment and management. However, a few nursery problems involve seeds and seed management practices. The type of nursery system, size of the nursery, and location are important for seeds. It was once believed that all seedling production and planting in the Tropics had to be done in containers. This is not true; in general, however, bare-root production systems predominate in the Temperate Zones and container production systems predominate in the Tropics.

B. Objectives

1. Recognize different nursery systems and the conditions most favorable for each.
2. Learn the relationship of nursery systems to national seed program management.
3. Review basic seed technology for sowing in each system.

C. Key Points

The following points are essential in understanding seed basics for nurseries:

1. Bare-root systems are more common in Temperate Zones; container systems are more common in the Tropics.
2. Bare-root production is possible in the Tropics with some pines and for stump production of selected species.
3. In container systems, large seeds are usually sown directly in containers, whereas small seeds are sown in germination beds or trays and transplanted (pricked out).
4. Tray mobility is an advantage in caring for and protecting young seedlings.
5. In small nurseries, seed treatments for germination are usually done by hand.

D. Core Material

1. Type of Nursery
   a. **Bare-root systems**
      (1) Are suitable in countries with large-scale planting programs.
      (2) Can produce seedlings or stumps.
      (3) Require same-day planting in tropical environments when seedlings are lifted; thus, distribution of seedlings to many planting locations may not be feasible.
      (4) Used with *Pinus caribaea* in Venezuela and stump plantings of *Gmelina, Dalbergia sissoo,* and Cassia *siamea.*
   b. **Container production systems**
      (1) Are usually preferred in most tropical locations because
         (a) They can be small, labor-intensive operations.
         (b) Containerized seedlings can stand the harsh environments during transport to the planting sites.
      (2) System options are:
         (a) A large centralized nursery with 0.5 to 1.0 million seedling production
         (b) Numerous small nurseries with 10,000 to 100,000 seedlings each
      (3) Containers can also be used for "wildings," natural seedlings that are dug up and transplanted. Wildings are useful for species with recalcitrant seeds that cannot be collected and delivered alive to nurseries for planting (e.g., *Shorea* spp. in Asia).
      (4) Movement of a large number of seedlings at a time is difficult because of container bulk, but the system is suitable for many small nurseries in dispersed locations.
   c. **Seed program considerations**
      (1) If most production is in a large, centrally located nursery, seed cleaning and storage should be located nearby.
      (2) If most production is in small, dispersed nurseries, cleaning and short-term storage should be in a regional center. Long-term storage should still be at a "national" seed center.
      (3) In small nurseries, much seed collecting, extracting, and cleaning are done locally. This centralization decreases the need for mechanical operations and favors the use of labor-intensive manual methods.
      (4) Localized collection forces the use of local seed sources.
      (5) When seedlings of tropical recalcitrants are grown, small local nurseries must be used to avoid losing seed viability during long transport.
      (6) In most successful reforestation programs, a combination of approaches will probably evolve, depending on species, transportation systems, personnel, and politics.
2. Bare-Root Production
   a. **Small seeds-For** small seeds, such as

those of *Pinus,* mechanized sowing and culture are typical.

b. **Large seeds-For** large seeds, such as those of *Quercus, Juglans,* and *Melia,* sowing by hand in furrows across the beds is typical.

c. Covering

(1) Small seeds-Press into the soil surface and cover with a light mulch (2 to 3 mm>.

(2) Large seeds -Place on their sides, press into the soil, and cover with 5 mm of soil (or not more than three times the seed diameter). Add mulch if moisture conditions require it. Some species, such as *Swietenia macrophylla,* should not be pressed into the soil because pressing can cause J-root formation.

3. Container Production

Seeds can be sown directly into containers or sown into seedbeds or seed trays and transplanted later (pricking out).

a. **Sowing into containers**

(1) Sowing into containers is good for the root systems because damage or distortion from transplanting is avoided.

(2) Sowing directly into containers is used for:

(a) Large seeds that can be handled individually

(b) Seedlots with expected high germination

(3) Suggested sowing rates are shown in table 28 [table 16 in Student Outline].

(4) "Doubles" are pricked out to fill in blanks.

(5) Only one seedling should be grown per container.

(6) Seed needs are calculated as follows. If the following conditions exist: the goal is 4,000 seedlings, seeds per kilogram are 3,500, germination is 70 percent, plant survival is 85 percent, and plantable seedlings are 80 percent, then 5,882 containers are needed. (Four thousand seedlings divided by 0.8 times 0.85 equals 5,882 containers). With 70-percent germination, 2 seeds per container are sown (5,882 containers times 2 seeds per container divided by 3,500 kg equals 3.4 kg of seeds needed).

b. **Sowing into seedbeds or seed trays**

(1) Sowing into seedbeds or seed trays concentrates germination in small areas, allowing better protection and care.

(2) This method is used for:

(a) Seedlots with expected germination of less than 40 percent

(b) Seedlots with slow germination

(c) Species that produce several seedlings from one seed unit

(d) Very small seeds that are hard to handle

(e) Scarce or expensive seedlots

(3) Seed tray mobility can be an advantage because it allows trays to be moved in and out of shade or protected from heavy rains. Trays can be made cheaply from local materials.

(4) The steps in sowing into seedbeds or seed trays are:

(a) Use a sand:topsoil mix of 1: 1 for slow germinators and growers. They need nutrients from the soil for 4 to 8 weeks.

(b) Use pure sand for *Pinus, Eucalyptus,* etc., that germinate quickly and are pricked out after only a few days.

(c) Press seeds into the soil and barely cover with washed sand; then mulch lightly with pine needles, rice husks, and water. (Washed sand will not crust when it dries.)

(d) Monitor closely to maintain proper moisture level.

(5) There are other considerations when using seedbeds.

(a) Seedbeds must be well drained, and the soil mix should be at least half sand.

(b) Small seeds are broadcast, pressed lightly into the soil, and

Table *28. -Suggested sowing rates for seedling production in containers (Napier and Robbins 1989) [table 16 in Student Outline]*

Expected germination	Seeds per container
Percent	*Number*
80	1 or 2'
60-79	2
40-59	3
<40	use seedbeds

'Sow half the containers with one seed and half the containers with two seeds.

covered lightly with sand and mulch.

(c) If rodents are a problem, the beds are covered with wire mesh.

(d) For very small seeds (e.g., *Eucalyptus* and *Anthocephalus*), the seeds are mixed with fine sand and shaken from a salt shaker to distribute them evenly in the beds.

(6) Rates for sowing in seedbeds:

(a) Desired densities are 2,000 seedlings per square meter for seedbeds and 4,000 seedlings per square meter for seed trays.

(b) If there are 100,000 seeds per kilogram and if the expected germination is 40 percent, then 1 g of seeds should produce 40 seedlings. In seedbeds, 2,000 divided by 40 equals 50 g of seeds to be sown per square meter of seedbed. This rate is doubled (100 g) for sowing in seedtrays.

D. Sources

For additional information, see Lantz 1985, Liege1 and Venator 1987, Napier and Robbins 1989, Willan 1985.

Seed Programs

I. National Programs

A. Introduction

National seed programs are necessary to support national reforestation and afforestation efforts by ensuring an adequate supply of high-quality seeds of suitable species and sources. Countries of the Association of Southeast Asian Nations are losing over 1.2 million hectares of forest lands annually to other uses. Deforestation of "officially" designated forest lands in India has not been excessive since the 1950's (about 3 percent of the lands under the Forest Department), but more than 10 times this area of wastelands, small groves, etc., has been denuded. Reforestation efforts on a national scale are needed. Within the framework of national programs, State or Provincial seed programs may also be needed. A national forest-seed program can serve many functions.

B. Objectives

1. Learn the general functions of a national forest-seed program.
2. Examine possible administrative structures of a national program.
3. Examine an existing national program as a casestudy.

C. Key Points

The following points are important in national seed programs:

1. The primary function of a national forest-seed program is to ensure an adequate supply of suitable tree seeds.
2. National programs can serve many other important functions.
3. National programs should serve the needs of all tree planting: industrial wood plantations, watershed protection, social forestry plantings, agroforestry, etc.

D. Tree-Planting Activities

Many purposes are served by a national forest-seed program.

1. **Industrial wood products,** such as lumber, pulp, veneer, and speciality products, are made from softwoods or fast-growing hardwoods.
2. **F'uelwood and charcoal are** made from short-rotation hardwoods (e.g., *Acacia, Eucalyptus,* and *Prosopis).* These are good, multipurpose species for:
   a. Village forests
   b. Individual landowners
   c. Commercial production in small-scale plantations on nonarable land
3. **Watershed protection,** especially around reservoirs, mine spoils, and dune stabilization areas, is essential.
4. **Windbreaks or shelterbelts**
5. **Urban planting** can be primarily ornamental, but can include planting for climate modification.
6. **Wildlife habitat and food plantings** occur in parks and game preserves.
7. **Agroforestry planting** is done for fodder production, nitrogen fixation, and fuel-wood.
8. **Social forestry** includes planting on roadsides, canal banks, and other common publicareas.
9. **Conservation of genetic resources can** be achieved by ex situ planting in clone banks or "gene archives."

E. Scope Of The Program

1. The population distribution must be known.
2. Physiographic characteristics of the country or State must be identified.
3. Available land area and ownership are important.
4. Annual goals must be realistic; the first year's may be modest to allow controlled growth and expansion into a comprehensive program.
5. Seed storage goals may include 2-, 3-, or 5-year supplies. Regional seed banks may be needed. There may be sales to other countries.
6. Distribution of indigenous species and their potential seed collection zones must be mapped.

F. Species Choices

Choice of species to be included in the program is a critical step.

1. Indigenous species and land races may be best and are usually favored.
2. Exotics are often discouraged, and caution should be used with them; however, there is a definite place for good exotics.
3. Seed source choices are extremely important. If possible, these decisions should be based on results of provenance tests.
4. Plantings should follow the natural plant succession where possible. Pioneer species should be planted first, with mycorrhizae or nitrogen-fixing bacteria innoculation, site preparation, fire control, and protection from livestock. These plants will form nurse trees for the second group of shade-tolerant species. The type of planting will dictate the approach.

G. Administrative Structure

Many government agencies or ministries may be involved because the same seeds may be used for several purposes (agroforestry, watershed protection, etc.). In the United States, the U.S.

Department of Agriculture, Forest Service and Soil Conservation Service; the US. Department of the Interior, National Park Service and Bureau of Land Management; and State and local counterparts are involved.

1. **Forestry ministry** levels — The administrative structure can be:
   a. National
   b. Provincial or State
   c. Village or other local structures
2. **Comprehensive natural resource agencies** may combine forestry and wildlife.
3. **Agricultural agencies** -Indian Council of Agricultural Research (ICAR) in India is an example for agroforestry.
4. **Military departments-in** the United States, military bases manage their own forests.
5. **Division of responsibilities** can be made for the various functions that are required:
   a. Overall planning must consider total production, seed collection zones, potential seed crops, collection vs. purchase, source of funds, etc.
   b. Seed acquisition and distribution can be concentrated at one central location or dispersed to regional centers. One strong national seed center for research, testing, and long-term storage is strongly recommended. Dispersed secondary centers are usually needed to collect and clean seeds. In large countries, such as India or Brazil, regional centers as well as subregional centers are necessary. Important elements of this function are:
      (1) Collecting and cleaning of seeds. Seed production areas and seed orchards may influence the location of this work.
      (2) Testing should be required on all lots.
      (3) Storage characteristics must be recognized. Workers must know which species can or cannot be stored and thus plan accordingly.
      (4) Certification is usually not feasible early in a program, but it may come later if needed. It should be well planned in advance.
      (5) Record keeping is an important function that should be centralized.
      (6) If there will be sales to other countries, national needs must be satisfied first.
      (7) If sales are within countries, sales policies must be established. Villages or individuals may or may not be charged for seeds and seedlings; seeds could be supplied at cost.
   c. Seedling production can be a part of national seed programs. It usually is not, but much depends on the government organization and the size of the country. There are several options:
      (1) National or State nurseries
      (2) Village nurseries
      (3) Private nurseries owned by individuals. (In some countries, farmers can grow tree seedlings as a cash crop.)
      (4) Commercial nurseries. (Government nurseries must not conflict with free enterprise.)
   d. Plantation care is not usually a function of the seed program unless seed stands are involved.
      (1) Protection, primarily from animals, fire, and people is usually needed.
      (2) Survival and early growth should be documented because early plantations can be used for seed source evaluation and then be turned into seedling seed orchards.
   e. Research may or may not be within the structure of the national program, but the program must help set research priorities.
      (1) Some common seed problems include maturity indices, pretreatment, storage, and testing. One ultimate goal is a seed manual for each country, either a comprehensive book like the USDA manual (Schopmeyer 1974) or a smaller manual that gives only basic seed handling and treatment information (tables 29 and 30) [no equivalent tables in Student Outline].
      (2) Species, site, and seed source evaluations are critical, but they usually fall under the tree improvement program.

H. Critical Steps and Decisions

1. Planting goals must establish what, where, and how much to plant.
2. Availability of seed supply
   a. Is the crop potential for indigenous species sufficient?
   b. Are there suitable commercial sources? Many commercial operations deal pri-

Table *29. -Cone and seed production characteristics of conifers in British Columbia (adapted from Dobbs and others 1976) [no equivalent table in Student Outline]*

Species	Cone bearing age begins	Cone length	Period between collectible crops		Cone yield per mature tree	Cones per hectoliter	Minimum filled seed count for collection		Collection period
			Avg.	Range			0.5 sect. per cone		
	Years	*cm*	*--- Years ---*		*hL.*	*---------- Number ----------*			
Abies amabilis	20	9-13	2-3	...*	...	700	.	...	Late Aug. to mid-Sept.
A. grandis	20	5-12	5-6	3-8	..	700	...	...	Late Aug. to mid-Sept.
A. lasiocarpa	20	6-12	3	2-5	...	850	...	...	Mid-Sept. to mid-Oct.
Chamaecyparis nootkatensis	15	0.5–1.5	2-4	...	...	130,000	.	2	Aug. to Oct.
Larix laricina	40	1.0-1.5	5-6	...		...	...	...	Aug. to Sept.
L. lyalli	30	4-5	...	...	...	...	...	...	Aug. to Sept.
L. occidentalis	25	3-4	5-6	...	...	11,000	6-8	40	Aug. to Sept.
Picea engelmanni	15	3-8	5	2-10	...	8,300	7-10	...	Mid-Aug. to Sept.
P. glauca	20	3-6	6	2-12	...	11,000	7-10	...	Mid-Aug. to Sept.
P. mariana	10	1-4	4–5	2-6	...	...	...		Sept.
P. sitchensis	20	6-10	3-4	2-5	0.5–1.0	4,700	7-10	:::	Sept.
Pinus albicaulis	20	4-8	...	3-5	...	...	...	..	Aug. to Sept.
P. contorta	10	3-5	3	2-4	0.5-1.0	8,300	...	20	Oct. to Mar.
P. flexilis	20	a 2 0	3	2-4	...	...	...	...	Late July, Aug. to Sept.
P. monticola	10	10–25	4	3-7	0.2-0.5	280	...	90	Late Aug. to early Sept.
P. ponderosa	15	8–15	3	2-5	1.0-1.5	700	...	75	Late Aug. to early Sept.
Pseudotsuga menziesii	15	5-10	5	2-10	0.5–1.0	2,800	5-7	...	Mid-Aug. to early Sept.
Thuja plicata	15	1-2	2-3	1-4	l..	110,000	...		Aug. to Sept.
Tsuga heterophylla	20	2-3	3-4	2-8	...	83,000	3-4	:::	Sept. to Oct.
T. mertensiana	20	2–8	...	1-5	...	...	...	...	Sept. to Oct.

'No data given

Table *30.-Selected data on seed quality of some Colombian tree species (adapted from Trujillo 1986) [no equivalent table in Student Outline]*

Species	Average purity	Pure seeds/kg	Average viable seeds/kg	Average germination	Range of germination	
					start	End
	Percent		*-- Number*		*--- Days ----*	
Cordia allidora	92.1	35.0	***27,098***	63.3	9	22
C. gerascanthus	89.2	64.0	***32,459***	65.5	6	16
Cupressus lusitanica	97.7	143.0	24,778	13.8	10	23
Delonix regia	98.9	2.3	1,394	58.1	5	16
Didimopanax monototonii	95.9	2.9	11,305	35.3	69	86
Erythina glauca	100.0	2.2	1,374	63.5	5	21
Hymenaea courbaril	99.5	2.6	142	54.5	22	32
Jacaranda copaia	84.5	65.5	12,097	20.0	11	25
Ochroma lagopus	98.7	137.0	61,423	69.0	10	40
Samanea saman	96.0	5.3	4,485	94.5	4	14
Tecoma spectabilis	95.8	120.0	97,239	77.6	5	1 2

marily with exotics (See table 31 for a list of commercial seed dealers) [no equivalent table in Student Outline].

3. Collection crews

a. Equipment and transportation must be on hand.

b. Training of all crews will be required.

c. Legal assistance, such as permission from landowners, government agencies, or tribal administrators will be needed.

4. Nursery administration requires:

a. A suitable site

b. Trained personnel

c. Adequate equipment for all operations

5. Collection goals are related to planting goals, potential seed crops, and storage potential of the seeds. (Will more than 1 year's supply be collected?)

6. Seed centers-A national seed center and/or State or regional seed centers should be established.

Table *31. -International seed dealers [no equivalent table in Student Outline*

Aggarwal Nursery and Seed Stores
Panditwari
P.O. Prem Nagar
Dahra Dun 248007, U.P.
India

Roger Anderson, Ltd.
P.O. Box 51-325 Pakuranga
Auckland
New Zealand
Telex: NZ 21721 (Roger A.)

Asmer Seeds
Asmer House
Ash Street
Leicester
United Kingdom
Telephone: (0533) 26733

Barilli and Biagi
l-40.100
Bologna
Italy

EFG (Nurseries), Ltd.
Maelor Nursery, Conery Lane
Bronington nr Whitchurch
Shropshire
United Kingdom
Telephone: 094873 301

Florsilva Ansaloni
C.P. 2100
40100 Bologna
Italy
Telephone: 51-625-5218
FAX 51-6525-6857

The Forest Research Centre
P.O. Box HG595
Highlands
Harare
Zimbabwe

A.J. Frost
7080 Borkop
Denmark

The Inland and Foreign Trading Co.
(PTE) Ltd.
P.O. Box No. 2098
Maxwell Road Post Office
Singapore 9040
Telephones: 27222711, 2721801, 2782193
Telex: RS25254 IFTCO

International Forest Seed Co.
P.O. Box 290
Odenville, AL 35120
U.S.A.
Telephone: 205/629-5749

International Paper Co.
Seed Center
Nacogodches, TX 75962
U.S.A.
Telephone: 409/569-1069

Lawyer Nursery, Inc.
950 Hwy 200 West
Plains, Montana 59859
U.S.A.
Telephone: 406/826-3881
Telex: 31-9547

Soren Levinsen
Post Box 86
Kollerod
Denmark

Louisiana Forest Seed Co., Inc.
Rt. 2, Box 123
Lecompte, LA 71346
U.S.A.
Telephone: 318/443-5026

Nova Tree Seed Co., Inc.
Rt. 2, Middle Musquodoboit
Nova Scotia BON 1X0
Canada

The Old Farm Nurseries
H.den Ouden and Son b.v.
P.O. Box 1
2770 AA Boskoop
Netherlands
Telex: 39810

D. Oriell-Seed Exporters, Unit 11
10 Golfview St.
Mt. Yokine
6060 W. Australia

Pal Seed Traders
7-Araghar
Dehra Dun, U.P.
India

Reid, Collins, & Assoc.
Vancouver, BC
Canada

Renz Nachf GmbH and Co.
D-7270 Nagold-Emmingen
Germany

F.W. Schumacher Co.
36 Spring Hill Road
Sandwich, MA 02563
U.S.A.
Telephone: 617/888-0659

Table 31. -*International seed dealers [no equivalent table in Student Outline] (Continued)*

Seed Branch
Forestry Commission Research Station
Alice Holt Lodge
Wrecclesham
Farnham, Surrey
United Kingdom
Telephone: 0420 22255

Seed Export Company
7 Bellata St.
The Gap
Queensland 4061
Australia
Telex: AA 42027

Setropa Ltd.
P.O. Box 203
1400 AE Bussum
Netherlands

Sheffield's Seed Co., Inc.
273-Auburn Rd., Rt. 34
Locke, NY 13092
U.S.A.
Telephone: 315/497-1058

Silvaseed Company
P.O. Box 118
Roy, WA 98580
U.S.A.

Southern Seed Company, Inc.
Baldwin, GA 30511
U.S.A.
Telephone: 404/778-4852
Telex: 4611041

Southpine, Inc.
P.O. Box 7404
Birmingham, AL 35253
U.S.A.
Telephone: 205/879-1099
Telex: 703040

G.J. Steingaesser and Company
Postf. 1765
8760 Miltenburg
Germany

Timmers and Leyer
P.O. Box 17
2100AA Heemstede
Netherlands
Telex: 41754 flori nl

Tree Seeds
The Boat House, Potters Lane
Samlesbury, Preston
Lancashire
United Kingdom
Telephone: 077477 213

University of Hawaii at Manoa
Dept. of Agronomy & Soil Science
190 East-West Rd.
Honolulu, HI 96822
U.S.A.
Telephone: 808/948-7530

Van Dijk and Company
Einhuizen
Netherlands

Versepuy
43000 Le Puy
France

Vilmorin-Andrieux
La Menitre
49250 Beaufort-en-Vallee
France

Yamato-Ya, Ltd.
16-6, 1-Chome
Ginza, Chuo-ku
Tokyo
Japan

I. Other Considerations
   1. **Continuity of operations-Well-trained** people should be kept at the same jobs for 3 to 5 years, preferably longer.
   2. **Training** of all personnel is a key to success.
   3. **Multiple functions** sometimes occur.
      a. In Morocco, government foresters also grow and distribute fruit trees to the farmers.
      b. Some countries have only one seed laboratory so the same people must test agricultural and tree seeds in the same laboratory.
   4. **International organizations-The** following organizations can help seed programs.
      a. ISTA – ISTA Secretariat
         Reckenholz, P. 0. Box 412
         CH-8046 Zurich
         Switzerland
      b. IUFRO-IUFRO Secretariat
         Schonbrunn
         A-1131 Vienna
         Austria
      c. FAO-Forest Resources Development Branch
         Forest Resources Division
         Forestry Dept., FAO
         Via delle Terme di Caracalla
         I-00100 Rome, Italy

d. ICRAF-International Council for Research in Agroforestry
P.O. Box 30677
Nairobi, Kenya

5. Agricultural seeds- The relationship of agricultural seeds to tree seeds must be kept in perspective. Except for conifer seed extraction, dewinging, and x rays, all the tree seed technology is borrowed from agriculture: cleaning, storing, testing, etc. Seed maturation and general biochemistry are similar, and much can be learned from agricultural seed technologists.

J. Case Study

See Robbins and Shrestha (in press) for an example from Nepal.

K. Summary

The functions of a national seed center are to:

1. Further develop taxonomy and aids to species identification.
2. Collect and disseminate data on the ecology of individual species, thus enhancing understanding of the performance of species.
3. Promote measures, as necessary, to conserve the genetic resources of important species.
4. Develop optimum seed collection strategies based on knowledge of breeding systems.
5. Maintain existing seed collections and ensure their future development as programs evolve to use promising species and provenances.
6. Assist collectors from other countries within the framework of national policy; some countries (e.g., Brazil and Indonesia) restrict collections by foreign nationals.
7. Provide information on the physical and physiological characteristics of seeds, and any diseases that might be borne by seeds.
8. Encourage quarantine practices that minimize the chances of domestic insects becoming established in other countries.
9. Disseminate information by providing appropriate training, symposia, and publications.
10. Disseminate seed samples for research or species trials to other institutions or countries on a cost or exchange basis.

L. Sources

For additional information see Gregg 1983, Hellum (in press), Robbins and Shrestha (in press), Rudolf 1974.

II. Seed Centers

A. Introduction

National forest-seed programs require some sort of national tree-seed center, institute, or laboratory. Dedicated facilities and some centralized authority are suggested for tree-seed centers. Their level of technology may vary with the country's needs, but these centers should serve as the focal point or hub of seed activities.

B. Objectives

1. Learn the general functions of national tree-seed centers and how they support national seed programs.
2. Examine several options for center design.

C. Key Points

The following points are essential to designing and operating successful seed centers:

1. The primary function of a seed center is to support the national forest-seed program.
2. Seed centers provide seed services, research on seed problems, training of seed workers, and extension activities for seed users.
3. Many countries will require regional or subcenters for efficient operation.

D. Functions

The functions of seed centers include seed services, seed research, and training and extension programs.

1. **Services** -A seed center:
   a. Coordinates seed collection by establishing seed zones, setting collection quotas, and training crews.
   b. Conditions seed collections by extracting, cleaning, and upgrading.
      (1) All operations may be centrally located.
      (2) Drying and extracting can be at regional centers, and seeds shipped to a national center for final cleaning and storage.
   c. Stores the seeds for the following purposes:
      (1) Operational storage for nearby users
      (2) Long-term storage of surplus stocks
      (3) Very long-term storage for germplasm conservation
   d. Tests the seeds of:
      (1) National seed program collections
      (2) Other in-country users (e.g., universities) that could generate income
      (3) Third parties (to settle legal disputes) that could also generate income
   e. Assists in certification-If a program is established, seed center staff must be involved, although primary responsibility rests with the "designated certification authority," not usually the seed center staff.

2. Seed research

a. Applied research on problems that hinder efficient and economical seedling production is vital; e.g., scarification techniques, maturity indices, and treatment of seed pathogens.

b. Basic research is better done in cooperation with universities, but a basic research group in the seed center would have advantages; e.g., studying critical drying rates for recalcitrant seeds, models of storage potential, and fatty acid metabolism during storage.

3. Training and extension programs

a. Centers should train seed collectors, analysts, and other personnel for specific programs.

b. Extension programs for nursery workers and farmers to teach efficient seed utilization are vital to national seed programs and should be staffed by trained extension workers. The training of such people is beyond the scope of this course.

E. National or Regional Centers

The decision to establish a national vs. a regional center depends on the following considerations:

1. **National centers** can be more responsive to political realities and capitalize on national pride.

2. Regional centers can expand scope and function by pooling resources, an attractive feature to donors. Regional centers may be necessary because of the high cost of transporting large collections or because of the use of short-lived recalcitrant seeds that would not survive long trips in stressful environments.

3. **Compromise-National** centers can be used for storage, testing, and research; regional centers can be used for collecting and cleaning.

F. Location Concerns

1. **Proximity to** seeds-The centers should be located near major seed production zones.

2. Transportation – Good transportation links (roads, rail, and water) are necessary.

3. **Isolation** – Complete isolation from population centers makes it hard to recruit and retain good people.

4. **Technical help-Because** university affiliation helps in many ways, location near a campus is desirable.

5. **Disaster potential-Flood** or earthquake zones should be avoided for the safety of the facility and the workers.

G. Center Design

Seed center designs depend on the following factors.

1. **Activity zones** include the following areas:
   a. Loading dock
   b. Drying area
   c. Extraction equipment (There may be problems with dust and trash.)
   d. Cleaning equipment
   e. Conditioning equipment
   f. Seed storage
   g. Testing laboratory
   h. Offices for records and supervision
   i. Supply storeroom

2. **Building design – See** figures 54 and 55 [no equivalent figures in Student Outline] for the floor plans of Seedlabs 2000 and 5000 by ISTA.

3. Equipment

a. Commercial sources are best, but much can he made locally.

b. Spare part sources are crucial for commercial equipment.

c. Maintenance must be available, either from equipment suppliers or from local people. If local people are used, then they must be trained.

d. Electrical supply must be dependable.

e. Seed centers should get the best equipment because the size of the operation will demand it. Subcenters and village operations can get by with simple equipment because of their small size.

4. Staffing- Supervisors should have defined areas of work; see figure 56 [no equivalent figure in Student Outline]. The staff should be made up of the following:

a. The director coordinates seed supply needs and distribution.

b. The collection supervisor directs collection teams and coordinates State/Province collections.

c. An extraction/cleaning supervisor and two to five technicians.

d. A testing supervisor and two analysts (one for purity and one for germination). Seasonal workers will also be needed.

e. A storage/shipping supervisor, one technician, and one clerk.

5. Training-All staff members should be trained in their specialties by university staff, special short courses, or on-the-job training at an established center. If personnel change jobs, the new people must be trained immediately. The skills of long-time staff should be updated as new methods are developed.

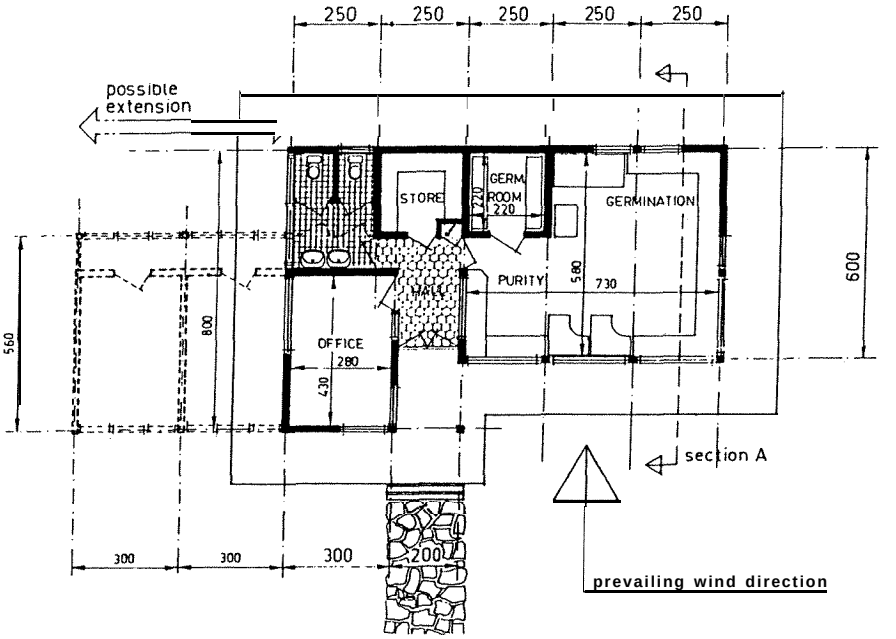


Figure 54. -*Suggested floor plan for a small testing laboratory (adapted from van der Burg and others 1983). Dimensions are in centimeters [no equivalent figure in Student Outline].*

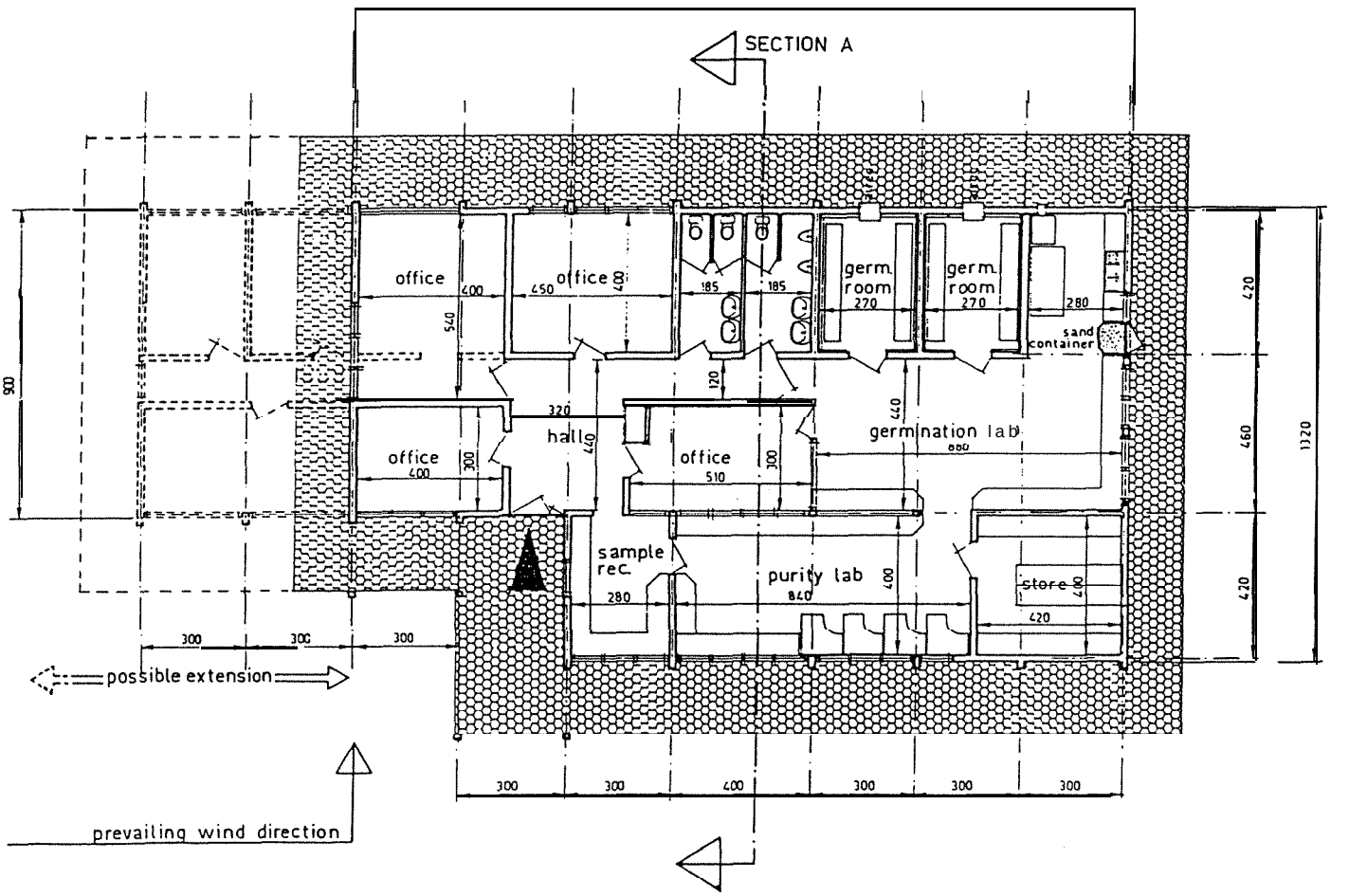


Figure 55. -*Suggested floor plan for a large testing laboratory* (adapted *from van der Burg and others 1983). Dimensions are in centimeters [no equivalent figure in Student Outline].*

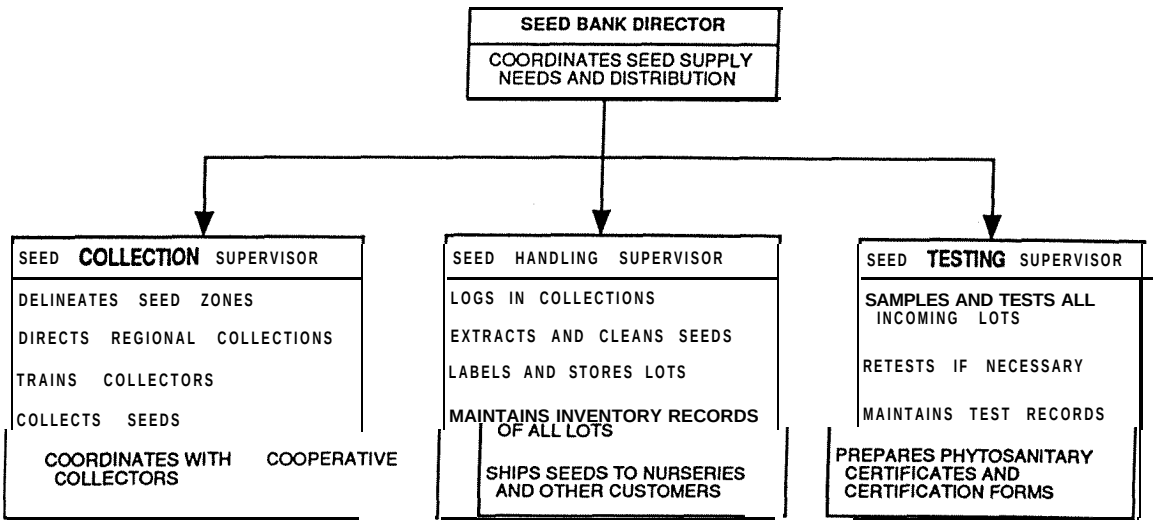


Figure *56.-A suggested staff organization for a small seed bank [no equivalent figure in Student Outline].*

H. Sources
For additional information, see van der Burg and others 1983.

III. Labeling and Certification

A. Introduction
When forest reproductive materials (seeds, seedlings, and vegetative propagules) are not collected or grown by the user, that user should have reasonable assurance of the identity and quality of the material he is buying. Many seed-labeling laws require detailed labeling to assure the buyer of the seeds' identity, purity, viability, and freedom from pests; i.e., the physiological quality of the seedlot. Certification is more than labeling required by seed laws; it is a statement about the genetic quality and identity of the seedlot.

B. Objectives
1. Understand the purpose of certification.
2. Identify the general elements of a certification program.
3. Describe the four certification categories used in the Organization for Economic Cooperation and Development (OECD) standards for international trade.

C. Key Points
The following points are essential to understanding labeling and certification of forest reproductive materials:
1. Certification is the guarantee by an officially recognized organization that forest reproductive materials of identified varieties have been grown, collected, processed, and distributed in a manner to maintain high quality and genetic identity.
2. A certification program requires a certification agency, a producer who wishes to sell certified material, records of the breeding program, certification standards, independent inspections, and certification labels.
3. The four certification categories used by OECD are:
   a. source-identified (yellow tag)
   b. selected (green tag)
   c. untested seed orchards (pink tag)
   d. tested reproduction material (blue tag)
4. Certification usually requires inspections of the production unit prior to pollination, a crop inspection before harvest, inspections during the collection-to-storage phases, and inspections at the time of packaging materials for sale.

D. Certification
1. **Definition-** the guarantee of character and quality of reproductive materials by an officially recognized organization, usually evidenced by a color-coded tag and a certificate containing such information as certification category, genuineness of species and variety, year of collection, origin, purity, soundness, and germinative capacity.
2. Purpose-Certification is more than just labeling. The purpose of certification is to maintain and make available to the public high-quality seeds and propagating materials of superior crop plant varieties. For agricultural and woody plant material, the word "certified" implies genetic improvement.

3. International aspect -An international scheme for certifying forest reproductive material was developed by the Organization of Economic Cooperation and Development (OECD). Its features are now being incorporated into forestry certification schemes in North America.

E. Definition of Terms

The following definitions are for terms used in the OECD Scheme (Organization for Economic Cooperation and Development 1974):

1. **Forest reproductive material**
   a. Seeds: cones, fruits, and seeds intended for the production of plants
   b. **Parts of plants:** stem, leaf, and root cuttings, scions and layers intended for the reproduction of plants
   c. **Plants:** plants raised by means of seeds or parts of plants; also includes natural regeneration
2. **Clone-a** genetically uniform assemblage of individuals derived originally from a single individual by vegetative propagation, such as by cuttings, divisions, grafts, layers, or apomixis
3. **Cultivar** – an assemblage of cultivated individuals, which is distinguished by any characters (morphological, physiological, cytological, chemical, or others) significant for the purposes of agriculture, forestry, or horticulture and which, when reproduced (sexually or asexually), retains its distinguishing features
4. **Provenance-** the place in which any stand of trees is growing; the stand may be indigenous or nonindigenous. (This is the location of the seed source.)
5. **Origin-** for indigenous stands of trees, the origin is the place in which trees are growing; for nonindigenous stands, the origin is the place from which the seeds or plants were originally introduced
6. **Designated authority-** an organization or institution designated by and responsible to the government of a country participating in the OECD scheme for the purpose of implementing the rules of the scheme on its behalf

F. General Elements of a Certification Program

1. The designated authority sets standards for certification and provides inspectors and certification tags and must have legal standing.
2. A producer wishing to sell certified material applies to the designated authority for qualification.
3. The history of the material (provenance, seed source, and breeding program) must be supplied to the designated authority who determines if the material meets certification standards for genetic quality and identity.
4. If the reproductive material is eligible for certification, production of the material for sale is supervised by the designated authority through independent inspections at intervals during the growing, collecting, and processing of the material.
5. The material to be sold must meet certification standards for viability, purity, and, in some programs, freedom from pests.
6. Certification labels are placed on each container of reproductive material at the time of packaging, and certificates of variety identity are given to the buyer.
7. The producer or seller is justified in charging more for certified material because certification ensures known genetic quality, provenance identity, viability, purity, and freedom from pests.
8. In summary, certification requires supervision, independent inspection, labeling, and record keeping while allowing the seller to sell at a higher price or at a competitive advantage.

G. Standards for Certification

1. **Certification classes** for forest reproductive material differ from classes for agricultural seeds. Forestry programs typically use the following OECD standards for forest reproductive material that includes "tested reproductive material," which is the equivalent of the "certified" category for agricultural seeds, and three additional classes of less rigid genetic control. The four classes and their requirements are:
   a. Source-identified reproductive material (yellow tag) comes from stands within an identified seed collection zone. It is required that:
      (1) Seed source and/or provenance must be defined and registered with the designated authority. (See "seed collection zones" below.)
      (2) Seeds must be collected, processed, and stored under inspection by the designated authority.
   b. Selected reproductive material (green tag) comes from phenotypically selected stands and cultivars. These stands and cultivars have not been tested for genetic quality, but they must:
      (1) Be isolated by distance from poor stands.

(2) Show normal variation among trees within a stand.
(3) Be large enough for adequate cross-pollination.
(4) Be old enough and developed enough to allow evaluation of phenotypes.
(5) Exhibit phenotypic superiority in some desirable quality, such as volume, wood quality, form or growth habit, resistance to disease, fodder production, or fruit production.

c. Reproductive material from untested seed orchards (pink tag) comes from phenotypically selected parent trees in a seed orchard or from the progenies of such trees.

d. Tested reproductive material (blue tag) must come from seed orchards, stands, or cultivars whose genetic superiority in at least one desirable quality has been proven in tests approved by the designated authority. Superiority can only be certified in terms of the environment and the age of the test.

e. Forestry certification classes can be applied to parts of plants (cuttings) as well as seeds.

f. Agricultural seed classes are different: breeder seeds and foundation seeds have white tags; registered seeds have purple tags; and certified seeds have blue tags.

2. Seed collection zones

a. Seed collection zones, or regions of provenance, should be delimited by administrative and geographic boundaries and, where applicable, by elevation and other appropriate boundaries.

b. Maps showing boundaries and reference numbers of seed collection zones should be established and published by the designated authority.

c. Seed collection zones are necessary for "source-identified reproductive material." The specific location and site characteristics of seed sources should be used for the other three subclasses.

3. Other requirements of certification

a. Basic information-The originator, developer, owner, agent, or producer must request certification and must provide the following information:
(1) Name of the variety
(2) Statement of the variety's origin and the breeding procedure used in its development
(3) Detailed description of any morphological, physiological, and other important characteristics that distinguish the variety
(4) Evidence of performance, including comparative yield data, insect and disease resistance, and other factors supporting the identity of the variety (not necessary for source-identified reproductive material)
(5) Statement on the suggested area of adaptation and the purpose for which the variety will be used, including a description of the regions where the variety has been tested and is recommended to be grown

b. Inspections may include:
(1) Initial field inspections when certification is first requested are conducted before pollination to check pollen-dilution zone and to remove phenotypically or genetically poor parent material.
(2) Crop inspections come just before harvest to estimate the amount of material that will be certified.
(3) Inspections during collection, conditioning, and storage of material determine that the genetic identities and viability are being maintained.
(4) Inspection at the time of packaging for sale to check for "off-types," disease, insects, viability, purity, and germinative capacity are conducted before tags are attached.

c. Fees-In the United States, certification is financed by fees producers pay to the designated authority.

H. Other Documentation

1. **Labels-Some** countries or other political entities require labels for commercial sales with identity (species), purity, germination, etc., on the labels. No certification is implied.

2. **Phytosanitary certificate-** Phytosanitary certification is required by most countries to stop the spread of insects and pathogens. It certifies that the seeds have been inspected and/or treated (fig. 57) [no equivalent figure in Student Outline].

I. Sources

For additional information, see Bonner 1981a, Organization for Economic Cooperation and Development 1974, Rudolf 1974.

THIS IS A SAMPLE

NO phytosanitary certificate can be issued until an application is completed (7 CFR 353) See reverse for additional OMB information

FORM APPROVED
OMB NO. 0579-0052

UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
PLANT PROTECTION AND QUARANTINE

PHYTOSANITARY CERTIFICATE

TO: THE PLANT PROTECTION ORGANIZATION(S) OF

FOR OFFICIAL USE ONLY

PLACE OF ISSUE
NEW ORLEANS, LOUISIANA

NO.: FPC 134236

DATE INSPECTED

CERTIFICATION

This is to certify that the plants **or plant products described below have been inspected according to appropriate procedures and are considered to be free from quarantine pests, and practically free from other injurious pests; and that they are considered to conform with the current phytosanitary regulations of the importing country.**

DISINFESTATION AND/OR DISINFECTION TREATMENT

1. DATE	2. TREATMENT
3. CHEMICAL *(active ingredient)*	4. DURATION AND TEMPERATURE
5. CONCENTRATION	6. ADDITIONAL INFORMATION

DESCRIPTION OF THE CONSIGNMENT

7. NAME AND ADDRESS OF THE EXPORTER	8. DECLARED NAME AND ADDRESS OF THE CONSIGNEE

9. NAME OF PRODUCE AND QUANTITY DECLARED

10. BOTANICAL NAME OF PLANTS	11. NUMBER AND DESCRIPTION OF PACKAGES	12. DISTINGUISHING MARKS
13. PLACE OF ORIGIN	14. DECLARED MEANS OF CONVEYANCE	15. DECLARED POINT OF ENTRY

Any intentional false statement in this phytosanltary certificate or misrepresentation relative to this phytosanitary certificate Is a violation of law, punishable by a fine of not more than $10,000, or Imprisonment of not more than 5 years, or both. (18 U.S.C. s1001)

ADDITIONAL DECLARATION

16. DATE ISSUED	17. NAME OF AUTHORIZED OFFICER *(Type or Print)*	18. SIGNATURE OF AUTHORIZED OFFICER

No liability shall attach to the United States Department of Agriculture or to any officer or representative of the Department with respect to this certificate.

PPQ FORM 577 (SEP 92) *Previous editions may be used.* PART 1 - SHIPPER'S ORIGINAL

Figure 57.-A *phytosanitary certificate* used *in the United States [no* equivalent *figure in Student Outline].*

IV. Germplasm Conservation

A. Introduction

Loss of forests around the world is widely deplored for many reasons, some valid and some not so valid. One consequence of deforestation is the loss of valuable germplasm that could be used in artificial regeneration and future breeding programs. The Food and Agriculture Organization of the United Nations (FAO) lists more than 300 tree species or provenances as endangered. Fortunately, there are steps that can be taken to conserve this germplasm.

B. Objectives

1. Recognize the consequences of excessive loss of germplasm of forest trees.
2. Learn the strategies available to conserve germplasm.

C. Key Points

The following points are essential to understanding germplasm conservation:

1. The ideal practice would be extensive in situ preservation.
2. Ex situ conservation is widely practiced already, but "passport data" on planted material need to be maintained.
3. Seed storage can play a critical role in germplasm conservation.
4. National programs of conservation should be carefully planned and established.

D. Importance of the Problem

1. The deforestation to replanting ratio is about 30: 1 worldwide, although some of the deforested areas regenerate naturally.
2. Insects and diseases can eliminate certain gene pools or sometimes entire species. For example, ***Castanea dentata, Ulmus americana,*** and ***Abies fraseri*** are almost gone in North America.
3. Global climate changes could eventually change distribution of species through changing selection pressures. Some gene pools could be lost, or at least, gene frequencies would change.
4. According to FAO statistics, more than 300 species or special provenances are endangered. Complete species loss is rare, but some provenances could be easily lost.

E. Available Technologies for Conservation

The following strategies are options for germplasm conservation:

1. **In situ conservation** is good for tropical hardwoods; 100 species can be found in 0.4 ha. Land use pressures and economics make this option increasingly difficult, however.
2. **Ex situ conservation** is widely used for fast-growing plantation species. "It is well suited to international cooperative efforts because seeds can be shipped to available sites. Blocks of 10 ha on several sites are recommended. Current recommendations are to save 50 to 400 individuals per geographic race or provenance.
3. **Conventional seed storage** plays a supporting role for tree germplasm because regeneration of seed supplies requires long periods of time. Storage is becoming more popular because of its low cost. Genetic damage during storage is possible, but this is largely unproven in tree seeds.
   a. True orthodox seeds can be stored for long periods (longer than one rotation) at subfreezing temperatures.
   b. Suborthodox seeds can be stored the same as true orthodox seeds but for shorter periods because of high lipid content (e.g., *Carya*) or thin seedcoats (*e.g., Populus*).
   c. Temperate recalcitrant seeds can be stored up to 3 years at a high moisture content and temperatures just above freezing.
   d. Tropical recalcitrant seeds can be stored the same as temperate recalcitrant seeds, but chilling damage occurs below 15 to 20 "C, and seeds live for only a few months.
4. **Cryogenic storage** is storage in liquid nitrogen at -196 °C. This method is potentially very useful, but more research is needed. Limits for true orthodox seeds are unknown. Cost is about the same as for conventional storage for small seeds (e.g., ***Eucalyptus*** and some *Pinus* spp.), but too high for larger seeds.
5. **Storage of pollen** has little potential, except as micropropagation tissues. Haploid tissue, which may be useful in breeding, could be stored this way. Storage life is shorter than for seeds.
6. **Micropropagation tissues** have potential for long-term storage of suspension cultures. This concept is intriguing, but research is in the early stages. It could help most with endangered, tropical, recalcitrant species. Technology will soon be available, but cost may be high.

F. Current Efforts

1. The FAO, Forest Resources Division, through the Panel of Experts on Forest Gene Resources, promotes national and international efforts.

2. Supported by FAO, the International Board for Plant Genetic Resources (IBPGR) is an autonomous scientific organization of 13 countries. The board has recently added forest trees to its agenda.
3. The Central America and Mexico Coniferous Resource Cooperative (CAMCORE) is directed from North Carolina State University in Raleigh. Members include forest industries and governments; the Cooperative includes hardwoods in its efforts.
4. The Oxford Forestry Institute (OFI) (formerly, the Commonwealth Forestry Institute) in the United Kingdom is active in seed source and tree improvement for tropical species.
5. Danish International Development Agency (DANIDA) operates the Forest Seed Centre in Humblaek, Denmark. This agency is active in Asia.
6. The Centre Technique Forestier Tropical (CTFT) is a French organization that is very active in Africa but less so in Asia.
7. Commonwealth Scientific and Industrial Research Organization (CSIRO), Division of Forest Research, an Australian organization, is active in Asia and eastern Africa.
8. Numerous countries' seed banks concentrate on indigenous species. Current efforts at forest tree germplasm conservation include seed storage at several international and national centers (table 32) [table 17 in Student Outline]. In addition, many countries have national seed storage facilities that serve their domestic needs. It is questionable how much of this seed storage is for long-term preservation of germplasm; most of it is designed for short-term storage to establish provenance trials and ex situ conservation stands.

G. Recommendations for Action
1. In situ conservation efforts should increase, especially in tropical forests, both moist and dry. The extent of genetic variation in natural stands must be determined.
2. Assistance in international efforts for more ex situ conservation planting, similar to that provided by FAO and OFI, is needed.
3. Research should continue on modeling conventional seed storage of true orthodox and

Table *32.-Some major international seed storage centers' [table 17 in Student* Outline]

Center	Country	Approximate size of collection: Species	Approximate size of collection: sources	Reference
		Number		
United States Forest Tree Seed Center	United States	67	197	Karrfalt (1985)†
National Seed Storage Laboratory	United States	18	41	Bass (1985)†
Petawawa National Forestry Institute	Canada	118	2,130	Janas (1984)
DANIDA Forest Seed Centre	Denmark	46	187	Anonymous (1985)
CSIRO Tree Seed Centre	Australia	900	4,000	Turnbull and Doran (in press)
OF1 Oxford, UK	United Kingdom	‡	‡	‡
Banco Latinoamericano de Semillas Forestales	Costa Rica	153	308	Anon. (1983)
Banco de Semillas COHDEFOR	Honduras	4	46	Gustavo (1985)†

'Bonner, F.T. 1986. Unpublished report. On file with: USAID Science and Technology Office, Washington, DC. [Number of pages unknown].

†Personal communication from center directors.

*Data not available.

suborthodox seeds to predict just how long they can be stored. Increased efforts with these species in cryogenic storage is also needed.

4. More research effort to conserve species with recalcitrant seeds is crucial. High-priority areas are reproductive biology, conventional seed storage, and micropropagation techniques.
5. More seed banks must be established now for forest species. Rangewide collections can be stored as seed reserves for ex situ plantings and as cellular reserves for micropropagation. These will also provide data on conventional seed storage.

V. Applied Research

A. Introduction

Many seed problems can be solved locally without sophisticated research equipment that is costly to acquire and operate. Some investigations furnish answers without statistical treatment; others need statistical work to demonstrate their reliability. Simple designs are usually satisfactory in seed work, including completely randomized treatments and factorials. The main requirements are curiosity and dedication.

B. Objectives

1. Learn a few principles of simple research studies.
2. Review case study examples of applied seed research.

C. Key Points

The following points are essential to applied seed research:

1. Problems can often, but not always, be solved with simple tests and experiments.
2. Standard procedures are always used when they are available; e.g., ISTA (1985) rules for germination testing.
3. Treatments are always replicated with several seed sources or in different seed years.
4. The limitations of the procedures in use must be recognized; e.g., electric seed moisture meters cannot be accurate to 0.1 percent.

D. General Considerations

1. **Replication-** The "standard" for seed work is usually 4 replicates of 100 seeds each (4 x 100), although in research, 50 (or even 25) are acceptable. If seeds are limited, the number in each replicate is decreased, but not the number of replicates. If the material is variable, five or six replicates would be satisfactory. If possible, more than one seed source should be used or more than one seed year from the same trees. Half-sib collections are also good for certain tests, such as determining maturity indices.
2. **Documentation** – Complete records are essential. Entries should be in ink in bound notebooks, if possible. Carbon or photocopies stored in a different place are also desirable. Computer files are extremely useful, but not essential. The key is to make a complete record of all treatment details and results.
3. **Statistics-Because** this is not a statistics course, experimental design will not be taught. However, it is important to:
   a. Design studies to allow statistical analysis.
   b. Use simple designs whenever possible, but be sure that they are valid.
   c. Temper statistics with common sense because statistical significance may not always equate with practical significance.
4. **Publication** – Publication of results is usually desirable because it disseminates knowledge to others who may face the same problems. However, it should never be the primary goal of research.

E. Case Studies

1. **Maturity indices of fruits or seeds**– The steps for this research are:
   a. Use a minimum of five trees.
   b. Sample over a reasonable period.
   c. Collect 10 to 15 fruits per tree.
   d. Take color photographs if possible.
   e. Take the following measurements:
      (1) Size (length and diameter)
      (2) Weight (wet and dry; dried at 103 °C for 15 to 24 hours)
      (3) Moisture content
      (4) Germination
      (5) Chemical analyses (optional)
      (6) Histochemistry (an alternative)
   f. Plot means on a time scale. (See the example in the "Seed Maturity" section.)
   g. Repeat at least twice to cover three seed crops.
2. **Extracting and cleaning methods**– The steps for this research are:
   a. Make pertinent comparisons
      (1) Sun drying vs. shade drying
      (2) Hand extraction vs. machine extraction
      (3) Any mechanical action vs. hand cleaning

(4) Seed size (size into three groups and test germination)
(5) Dewinging vs. sowing winged seeds

b. Replicate each treatment 5 times; test each replicate with 4 samples of 50 seeds each.

c. Retests unusual results.

d. Use the "t" tests for two treatments and complete randomization for more than two treatments.

3. **Pretreatment for germination** – The steps in pretreatment are:

a. Make pertinent comparisons (tests)
(1) Hand scarification vs. mechanical scarification
(2) Hot vs. cold water soak
(3) Stratification (time and temperature parameters)
(4) Chemical stimulation

b. Use the same general directions as for extracting and cleaning. Factorial designs will be more common for treatment combinations (e.g., time vs. temperature and soak time vs. chemical level).

4. **Storage conditions-The** steps for storage tests are:

a. Make pertinent comparisons (tests).
(1) Room temperature vs. refrigerated conditions
(2) Different refrigeration temperatures
(3) Seed moisture levels
(4) Type of storage containers

b. Use large enough replicates to allow sampling over time.

c. Test orthodox seeds at 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 years, and test recalcitrant seeds at 1, 2, 4, 8, 12, 18, and 24 months, then every 6 months thereafter.

d. Use at least four replicates.

5. **Testing for recalcitrance-The** steps to test for recalcitrance are:

a. Bring the seedlot to full imbibition.

b. Start drying with at least two rates (slow and fast), either indoors or outdoors, shade or sun.

c. Take periodic samples for moisture content and germination. Collect samples every 2 to 4 hours for the first 24 hours, then double that time in the next 24 hours, and double again in the next 48 hours.

d. Maintain the drying range from full imbibition to lo-percent moisture or until death of the seeds.

e. Designate seeds that cannot be dried below 20 percent as recalcitrant.

f. Repeat this test to confirm recalcitrance; never trust just one measurement cycle. Tests of additional seedlots are desirable.

g. Check chilling injury at 0 to 5 °C by exposing fully imbibed seeds to this temperature for 24 hours.

h. Keep statistics in perspective. Realize that they are not as important as common sense in interpretation of results.

Conclusion

I. Review Session

The purpose of the final review session is to cover items that have been poorly understood by the participants and need further explanation and to present material that for some reason has been omitted during the course. If the answers to the problems have not been reviewed, that material should also be covered in this session. For general review of the material, three approaches are suggested:

A. Questions from the participants to the instructors, chapter by chapter.
B. A "participants-only" session in which they discuss the topics among themselves and make up a list of questions for the instructors to answer later.
C. If report assignments have not been discussed, the participants can present summaries of their findings and thus present plenty of opportunity for review.

The first approach is the simplest, but many participants may be reluctant or embarrassed to ask some questions with the instructors present. The second approach works well in this case. Give the participants a minimum of 1 hour with an appointed recording secretary. The instructors can be called back for the answer session. The third approach can be used if the assignment reports have not been presented at another time. It is also a good way to review if time becomes limited.

II. Course Evaluation

If an evaluation of the course is desired, the general evaluation questionnaire of the USDA's Office of International Cooperation and Development (OICD), or a similar instrument, may be distributed. If a test on subject matter is desired, a separate test must be assembled. The appendix contains the OICD questionnaire.

III. Closing Ceremony

Closing ceremonies are a "must" in most countries, allowing for speeches and platitudes from local administrators and heads of institutions. Some sort of certificate for the participants is also necessary because many of them must show proof of attendance when they return home. Time must also be allowed on the program for a spokesperson from the participants to say a few words. The agenda and choice of speakers should be left up to the local administrator or person in charge of local arrangements.

Laboratory Exercises

Exercise 1- Seed Structure

Objective:

To learn basic seed structures and their function in important seed types.

Methods:

1. Presoak seed samples in tapwater at room temperature (or 27 "C) for 15 to 24 hours. The imbibition will soften tissues and facilitate dissection.
2. Using a knife, clippers, or a single-edged razor blade and depending on type of seeds, carefully cut the seeds in one of two different ways:
   a. cross section (transverse)
   b. lengthwise (longitudinal)
   Several cuts may be necessary to expose the embryo and other internal tissues.
3. Examine the tissues exposed by the cuts and label them on freehand sketches of the cut material. Determine which tissues are for embryo protection, storage of food reserves, etc. Look for abnormal structures, insect damage, etc.
4. On at least one seed of each species, try to remove the embryo without damage, sketch it, and label the parts.

Supplies:

Clippers (or knives), single-edged razor blades, dissecting needles, a small magnifying glass (or hand lens), pencil and paper, and seed samples of five tree species.

Exercise 2 — Seed Crop Estimation

Objective:

To predict seed crops in advance of collection by estimating the number of:
1. Good seeds per fruit
2. Fruits per tree

Methods:

1. Good seeds per fruit
   a. Choose a multiple-seed fruit and collect 15 fruits prior to maturity.
   b. Cut fruits in half lengthwise and count good seeds visible on each half
   c. Dry the fruit halves in an oven (40 to 50 "C) to extract seeds and to obtain actual counts of good seeds.
   d. Calculate regression equations to predict total seeds from fruit cross-section counts.
2. Fruits per tree-Visit nearby trees and estimate fruit crops by:
   a. Total count
   b. One-fourth crown count
   c. Sample branch count
   d. Any other known ways
3. Combine results of both methods to estimate size of the seed crop.

Supplies:

Cone cutters or sharp blades, an oven, drying containers, and binoculars.

Exercise 3a—Cone Drying and Seed Extraction (Central America)

Objective:

To learn how to calculate seed and fruit needs for a planting program.

Assumptions:

Area to plant-2,000 hectares (ha) at 1,700 trees per hectare
Species — *Pinus caribaea*

1. All moisture contents are percentage of wet weight.
2. There are 800 closed cones per hectoliter (hectoliter) (40 kg).
3. Moisture content of closed cones is 40 percent.
4. Cones double in size when open.
5. Yield averages 400 grams (g) of pure seeds per hectoliter of closed cones.
6. There are 68,200 seeds per kilogram (kg).
7. Laboratory germination is 80 percent; 50 percent of the germinated seeds produce plantable seedlings.
8. Cones are put into the kiln when they reach 25-percent moisture content.
9. Drying trays hold 0.5 hL of closed cones; each stack of eight drying trays holds 4.0 hL; eight stacks can fit in the kiln at once.
10. It takes 12 hours to dry a full charge to the lo-percent cone moisture needed for the cones to open fully.
11. It takes 700 kilocalories (Kcal) to heat 1 hL of cones for 1 hour.
12. Fuel value for wood of *Casuarina equisetifolia* is 4,950 Kcal per kilogram; for *I! caribaea* cones, 4,500.
13. Open *I? caribaea* cones weigh 104 g per liter (L).

Questions:

1. How many cones must be collected to meet the planting goal?
2. How much total moisture must be lost in predrying (prior to entering kiln)?
3. How many drying stacks will be needed to predry everything at once?
4. How many kiln charges will be needed?
5. How long will it take to open all cones?
6. How much fuel will be needed with *C. equisetifolia* wood? with *P. caribaea* cones?
7. Have enough cones been collected to heat the kiln?

Answers:

1. 312.5 hL

seeds required $= \frac{(1{,}700)}{(0.8)\ (0.5)} \left(2{,}000\right) = 8{,}500{,}000$; or

$\frac{8{,}500{,}000}{68{,}200} = 125$ kg

0.4 kg pure seed/hL: $\frac{125}{0.4} = 312.5$

2. 2,500 kg of moisture (312.5) (8)

1 hL = 40 kg; 16 kg H_2O and 24 kg dry matter

At 25%, $\frac{x}{24+x} = 0.25$

$x = 8$ kg

Since there were originally 16 kg, 8 kg must be lost.

3. **78+**

$\frac{312.5}{4} = 78.12$

4. **10**

$\frac{78.12 \text{ stacks}}{8 \text{ stacks/charge}} = 9.8$

5. **120 hr**

(12 hr) (10 charges) = 120

6. *Casuarina equisetifolia* = *540* **kg;**
Pinus caribaea = *600* **kg**

Casuarina equisetifolia:
(32 hL/charge) (12 hr/charge) = 384 hL-hr
(384) (700 Kcal) = 268,800 Kcal/charge
$\frac{268,800}{4,950}$ = (54 kg/charge) (10 charges) = 540 kg

Pinus caribaea:
$\frac{268,800}{4,500}$ = (60 kg/charge) (10 charges) = 600 kg

7. Yes
Open cones weigh 10.4 kg/hL
At 2 x expansion, we have (312.5) (2) = 625 hL of open cones
(625) (10.4) = 6,500 kg

Exercise 3b—Cone Drying and Seed Extraction (India/Pakistan)

Assumptions:

Area to plant-2,000 ha at 1,700 trees per hectare
Species — *Pinus roxburghii*

1. All moisture contents are expressed as a percentage of wet weight.
2. There are 400 closed cones per hectoliter (hL) (40 kg).
3. Moisture content of closed cones is 40 percent.
4. Cones double in size when open.
5. Yield- averages 1.2 kilograms (kg) pure seeds per hectoliter of closed cones.
6. There are 12,000 seeds per kilogram.
7. Laboratory germination is 80 percent; 50 percent of the germinated seeds produce plantable seedlings.
8. Cones are put into the kiln when they reach 25-percent moisture content.
9. Drying trays hold 0.5 hL of closed cones; each stack of eight drying trays holds 4.0 hL; eight stacks can fit in the kiln at once.
10. It takes 12 hours to dry a full charge to the lo-percent cone moisture needed for cones to open fully.
11. It takes 700 Kcal to heat 1 hL of cones for 1 hour.
12. Fuel value of wood of *Casuarina equisetifolia* is 4,950 Kcal per kilogram; for *P. roxburghii* cones, 4,500. Open *P. roxburghii* cones weigh 110 g per liter (L).

Questions:

1. How many cones must be collected to meet the planting goal?
2. How much total moisture must be lost in predrying (prior to entering kiln)?

3. How many drying stacks will be needed to predry everything at once?
4. How many kiln charges will be needed?
5. How long will it take to open all cones?
6. How much fuel will be needed with *C. equisetifolia* wood? With *P. roxburghii* cones?
7. Have enough cones been collected to heat the kiln?

Answers:

1. 590 hL

seeds required = $\frac{(1,700)}{(0.8)(0.5)}\left(2,000\right) = 8,500,000$; or

or $\frac{8,500,000}{12,000} = 708$ kg

1.2 kg pure seeds/hL: $\frac{708}{1.2} = 590$ hL

2. 4,720 kg of moisture (590) (8)

1 hL = 40 kg; 16 kg H_2O and 24 kg dry matter

At 25%, $\frac{x}{24+x} = 0.25$

$x = 8$ kg

Since there were originally 16 kg, 8 kg must be lost.

3. 147+

$\frac{590}{4} = 147.5$

4. 19

$\frac{147.5 \text{ stacks}}{8 \text{ stacks/charge}} = 18.4$

5. 228 hr

(12 hr) (19 charges) = 228

6. Casuarina equisetifolia = **1,026 kg;**
Pinus roxburghii = **1,140 kg**

Casuarina equisetifolia:

(32 hL/charge) (12 hr/charge) = 384 hL-hr

(384) (700 Kcal) = 268,800 Kcal/charge

$\frac{268,800}{4,950}$ = (54 kg/charge) (19 charges) = 1,026 kg

Pinus roxburghii:

$\frac{268,800}{4,500}$ = (60 kg/charge) (19 charges) = 1,140 kg

7. Yes

Open cones weigh 11.0 kg/hL

At 2 x expansion, we have (590) (2) = 1,180 hL of open cones

(1,180) (11.0) = 12,980 kg

Exercise 4 — Storage Space Requirements

Objectives:

Once annual seed requirements are known, space requirements for cold storage must be calculated. A decision must also be made as to how many years' supply of seeds will be maintained as a safety margin: 1 year's? 3 years'?

Assumptions:

1. You must grow 2 million *Acacia nilotica* and 3 million *Pinus wallichiana* seedlings each year.
2. A 3-year supply of seeds will be stored.
3. Number of seeds per kilogram (kg) is *7,000* for *A. nilotica* and *26,000* for *l? wallichiana.*
4. For every three seeds planted, only two will produce a plantable seedling.
5. The seeds will be stored in large plastic bottles that hold 10 kg each. The bottles are 80 centimeters (cm) tall and 40 cm in diameter.
6. Ten percent of the cold storage space is in aisles, etc.

Calculate:

1. How many kilograms of each species are to be stored?
2. How many bottles and cubic meters of storage space will be required?
3. Repeat calculation 2 if storage is in boxes 40 by 40 by 40 cm. Each box will hold 6.5 kg of seeds.
4. What are the minimum cold storage dimensions needed to store the seeds in calculations 2 and 3 above?

Answers:

1. *A. nilotica:* (2,000,000) (3) ÷ *(0.67)* = 8,955,224
   8,955,224 ÷ 7,000 = 1,279.3 kg
   P. wallichiana: (3,000,000) *(3)* ÷ *(0.67)* = 13,432,836
   13,432,836 ÷ 26,000 = 516.6 kg
2. *A. nilotica:* 128 bottles (127. 93)
   Bottle volume = (0.1257) (0.8) = 0.10 m^3,
   Space needed = (0.4) (0.4) (0.8) (128) = 16.4 m^3
   P. wallichiana: 52 bottles (51.7)
   Space needed = 6.6 m^3
3. *A. nilotica:* 197 boxes (196.8)
   Space needed = (0.4) (0.4) (0.4) (197) = 12.6 ma
   P. wallichiana: 80 boxes (79.5)
   Space needed = 5.1 m^3
   But square boxes need air space. A 10-cm space on four sides increases space needed to 19.7 m^3 and 8.0 m^3.
4. Bottles: (16.4 + 6.6). ÷ 0.9 = 25.6 m^3
   Boxes: (12.6 + 5.1) ÷ 0.9 = 19.7 m^3; 30.8 m^3 with spaces.

Exercise 5 — Sampling

Objective:

To learn the basic methods of sampling bulk lots and some special applications for tree seeds.

Methods:

1. Mix each lot thoroughly either with a mechanical mixer or by hand. To do the latter, spread the seeds out on a smooth surface and mix by scooping from side to side. Then pour back and forth between two containers.

2. Determine the proper size of the submitted sample (twice the working sample).
3. Draw samples using the following equipment/methods:
   a. Seed trier
   b. Mechanical divider
   c. Division
   d. Extended hand
4. Weigh each sample to the nearest gram, place in a plastic bag, and label.
5. Save these bagged samples for later measurements of purity, weight, and moisture.

Supplies:

A seed trier, a mechanical divider, a spatula, a spoon, plastic bags (15 by 15 cm), marking pens, and laboratory balances.

Exercise 6 -Moisture, Purity, and Weight

Objective:

To carry out the basic steps in measuring moisture, purity, and weight of a submitted sample.

Methods:

1. Moisture
   a. Use submitted samples drawn in the sampling exercise.
   b. Use a spoon or spatula to draw two subsamples of 4 to 5 g each. Place samples in drying cans. Follow guidelines in Bonner (1981b).
   c. Weigh to the nearest 0.01 g and dry in ovens for 17 hours at 103 "C.
   d. Cool in desiccators and reweigh. If desiccators are not available, use rapid-weigh techniques to obtain dry weight.
   e. Calculate moisture as a percentage of wet weight:
   $$\text{percent moisture} = \frac{\text{wet wt.} - \text{dry wt.}}{\text{wet wt.}} (100)$$
2. Purity
   a. Reduce the remainder of the submitted sample to the proper working sample size. To determine the proper size, take at least 2,500 seeds up to a maximum of 1,000 g. Table 19 [no equivalent table in Student Outline] may be used to determine sample size also.
   b. Weigh the working sample (see 3.5.1.A in ISTA 1985).
   c. Divide the sample into the following components:
      (1) Pure seeds
      (2) Other seeds (other species)
      (3) Inert matter (includes seed parts)
   d. Weigh each component and express as a percentage of the working sample weight:
   $$\text{percent pure seed} = \frac{\text{wt. of pure seed}}{\text{wt. of entire sample}} (100)$$
3. Weight
   a. Use the pure seed component from the purity test.
   b. Either weigh and count the entire pure seed component or use smaller replicates (the usual method).
   c. Replicate method:
      (1) Randomly count out 8 replicates of 100 seeds each.
      (2) Weigh each replicate to the same number of decimal places used in the purity determination.
      (3) Obtain the mean weight of 100 seeds and multiply by 10 for 1,000-seed weight.

(4) Convert to pure seeds per kilogram as follows:

$$\frac{1{,}000{,}000}{\text{wt. of 1,000 seeds}} = \text{seeds per kg}$$

d. In official testing, variation would be estimated as follows:

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

(2) Standard deviation (σ) = $\sqrt{\text{variance}}$

(3) Coefficient of variation (CV) = $\left(\frac{\sigma}{\bar{x}}\right)(100)$
($\bar{x}$ = mean wt. of 100 seeds, see 3.c above)

(4) If CV is 4.0 or less, the answer in "3.c" above is acceptable. If CV is more than 4.0, take 8 more replicates and repeat the process, using all 16 replicates in the calculations.

Supplies:

A spatula, a spoon, laboratory balances, an oven, drying cans, desiccators, forceps, and pencil and paper,

Exercise 7 — Calibration of Electric Moisture Meters

Objective:

To demonstrate a simple method for developing calibration charts for electric moisture meters. These methods will work with any type of meter.

Methods:

1. Draw 4 to 5 kg of seeds from a bulk lot of the desired species; mix well.
2. Separate into 10 random samples of about 400 g each.
3. Adjust moisture in these samples to span the range of moisture content that will be encountered (approximately 5 to 20 percent). Do this by drying several samples (vary drying conditions) and by adding water to others (vary the amount of water).
4. Place each sample in a plastic bag and place the bag in a cooler for 1 week to allow complete moisture equilibration.
5. After 1 week, remove the samples and let them come to room temperature (2 to 3 hours).
6. Take a meter reading on the driest lot according to the manufacturer's instructions. Record the value and immediately draw two 5-g subsamples for oven determinations of moisture content. Follow previous instructions.
7. Repeat step 6 with the other samples in the order of ascending moisture content.
8. Plot data on a graph: oven moisture percentage vs. meter reading. Use this curve to relate future meter readings to actual moisture content for this particular species only.
9. For more accurate calibration, fit a regression curve (oven moisture percentage on meter readings) and calculate values for a calibration table. More than 10 observations should be available for a regression, so another 10 samples should be drawn for a repeat of the entire process.
10. This procedure must be done separately for each species to be tested.

Supplies:

An electric meter, a spoon, laboratory balances, an oven, weighing dishes, desiccators, plastic bags, and graph paper.

Exercise 8 — Germination Tests

Objective:

To learn the basic steps of a germination test and to carry out simple tests on some important species. Because of the length of this course, a full test may not be possible. By starting a few samples before talking about testing at length, some germination should occur and be available for evaluation.

Methods:

1. Presoak seed samples in tapwater at room temperature (27 "C) for 15 to 24 hours.
2. Divide samples into 2 replicates of 20 to 50 seeds each, depending on the species. If *Eucalyptus* spp. seeds are used, weigh out two replicates according to ISTA (1985) rules.
3. Half the class will surface-sterilize their samples with a lo-percent chlorine bleach solution, and the other half will not treat theirs. Treatment will consist of a 5- to lo-minute soak, followed by rinsing in running tapwater.
4. Hard-seeded species (e.g., *Acacia)* will be scarified with a knife, file, or sandpaper on the radicle end as determined in Exercise 1.
5. Place replicates in glass or plastic dishes on moist filter paper or other suitable media. Paper should be moist, but not moist enough to leave "free water" in a depression made by mashing down on the paper with a finger. Put dish covers on; if there are no covers, use plastic wrap.
6. Label all dishes and place them in a germinator or constant temperature room if available. If these facilities are not available, place the dishes on a table under lights in the center of the room. If good lights are not available, place the dishes near windows that allow good natural light.
7. Check dishes every day for moisture; add water if they dry out. Germination may become evident in about 7 days. Record normal germination, abnormal germination, and evidence of insect or disease problems.

Supplies:

A knife, small file, or sandpaper for scarification, chlorine bleach, germination blotters, dishes (10 per student), a glass-marking pen, and laboratory balances. A germinator or constant temperature room is desirable but not necessary.

Suggested species:

Pinus, Acacia or another legume, *Eucalyptus,* and two indigenous species.

Exercise 9 — Scarification

Objective:

To demonstrate the relative effectiveness of simple scarification techniques that can be used in seed testing.

Methods:

1. Count out 120 seeds of a hard-seeded species and divide them into 8 samples of 15 seeds each.
2. Scarify 2 replicates of 15 by each of the following procedures:
   a. Rub hand files or similar abrasive devices across the seedcoat enough to cut a notch in the seed.
   b. Use hand clippers, shears, or a knife to cut through the seedcoat along one side.
   c. Sandpaper the seed enough to cut through the seedcoat on the radicle end.
   d. The other two samples will be the untreated controls.

3. Place the scarified samples on moist blotters in dishes and cover as in the germination test. Place all dishes in the germinator if there is space. If not, place them on a table with good lighting and leave them for observation through the rest of the course.
4. Periodically count the number of germinating seeds and the number of swollen seeds. This latter condition confirms that water uptake has occurred, but something else may be blocking germination. Report both conditions as a percentage of the total number of seeds in the test.

Supplies:

Four glass or plastic dishes, germination blotters, a hand file, clippers or shears, rough sandpaper, and marking pens.

Exercise 10 -Rapid Test: Tetrazolium Staining

Objective:

To learn basic techniques of the tetrazolium (TZ) stain test for viability.

Methods:

1. Draw two 50-seed samples from seeds that have been soaking in tapwater for 24 hours.
2. Prepare a l-percent solution of TZ by dissolving 10 g of a TZ salt (chloride or bromide) in 1,000 mL of distilled water (pH 6.5 to 7.0). If the pH of the water is outside this range, a buffered solution must be prepared as follows:
   a. Prepare two solutions:
      (1) Solution 1 -Dissolve 9.078 g KH_2PO_4 in 1,000 mL of water.
      (2) Solution 2 -Dissolve 11.876 g $Na_2HPO4 \cdot 2H_20$ in 1,000 mL of water.
   b. Mix two parts of solution 1 with three parts of solution 2.
   c. Dissolve 10 g of TZ salt in 1,000 mL of the buffer solution to make a l-percent solution.
3. Carefully cut open the imbibed seeds to fully expose the embryo. The embryo may be completely removed, as in the excised embryo test.
4. Completely immerse the embryos in TZ solution in dishes and incubate them in the dark at 30 °C for 15 to 24 hours (depending on the species and seed condition).
5. For evaluation, decant the TZ solution, rinse seeds in water, and examine the embryos on a wet surface. Moderate red staining generally indicates viable tissues, heavy red staining indicates damaged tissues, and the absence of any staining indicates nonviable tissues. Stain interpretations may vary by species. See ISTA (1985) for guidelines.
6. Compare results with other rapid test results or germination test results.

Supplies:

Dissecting equipment, dishes, tetrazolium salt, buffers (if necessary), and a constant-temperature dark incubator.

Exercise ll-Rapid Tests: Cutting and Excised Embryo

Objective:

To learn techniques of the cutting test for viability estimation and of embryo removal for the embryo excision test.

Methods:

1. Cutting Test
   a. Draw samples of 50 seeds from each of several seedlots and divide them into sublots of 25 seeds each.
   b. Cut the seeds in half, using a transverse cut through the center of the seeds. Categorize seeds as either viable, damaged by insects or disease, or empty. Average the results from the sublots.
2. Excised Embryo Test
   a. Draw samples of 50 seeds from each of several seedlots that have been soaking in tapwater for 24 to 48 hours at room temperature and divide into sublots of 25 as before.
   b. Using razor blades or scalpels, carefully cut through each seedcoat and endosperm (if present), and expose the embryo.
   c. Carefully "tease" the embryo out of the surrounding tissues with dissecting needles or other sharp-pointed instruments. Avoid damaging the embryo.
   d. Carefully place the excised embryo on moist filter paper in a covered dish, such as a petri dish. Maintain at 20 °C in light until an evaluation can be made (usually within 14 days).
   e. Diseased or damaged embryos should not be placed in the dishes. Empty seeds should be categorized as such and not replaced in the test.
   f. The working surface and all instruments should be disinfected to reduce mold infections with a 50-percent ethanol solution. Instruments should be "dipped" between each dissection.
   g. Embryos should be categorized within 14 days as follows:
      (1) Viable
         (a) germinating embryos
         (b) embryos with one or more cotyledons exhibiting growth or greening
         (c) embryos remaining firm, slightly enlarged, and either white or yellow according to species
      (2) Nonviable
         (a) embryos that rapidly develop severe mold, deteriorate, and decay
         (b) degenerated embryos
         (c) embryos exhibiting extreme brown or black discoloration, an off-gray color, or white watery appearance
         (d) seeds in which the embryo is dead, missing, or deformed
   h. Compare your results with the cutting test results.

Supplies:

Single-edged razor blades, scalpels, dissecting needles, dishes, filter paper, and ethanol.

Exercise 12 — Seed Health Testing

Objective:

To learn basic techniques of seed health testing.

Background:

Health testing of seeds is important for three reasons:
1. Seed-borne inoculum may cause diseases in the field.
2. Imported seedlots may introduce new diseases, so tests to meet quarantine regulations may be required.
3. Seed health testing may aid in seedling evaluation and help determine causes for poor germination or field establishment. It supplements the germination test.

Seed Health refers primarily to the presence or absence of disease-causing organisms (e.g., fungi, bacteria, and viruses) and animal pests (e.g., eelworms and insects). However, physiological conditions such as trace element deficiency may be involved.

Incubation maintains seeds in an environment favorable to the development of pathogens or symptoms.

Pretreatment is any physical or chemical laboratory treatment of the working sample preceding incubation that is done solely to facilitate testing.
Treatment is any process, physical or chemical, to which a seedlot is submitted.

Sample:

1. Entire submitted sample may be the working sample, depending on the test.
2. The working sample is normally 400 pure seeds or an equivalent weight.
3. Sampling rules are followed.
4. Replicates containing a specified number of seeds, if required, are taken at random for a subsample after thorough mixing.

General directions:

1. Use different methods of testing depending on factors such as pathogen or condition being investigated, species of seeds, and purpose of test. See ISTA (1966, 1985).
2. Examine the working sample with or without incubation.
   a. Examine without incubation. (This method provides no indication of the viability of the pathogen.)
      (1) Examine the sample with a stereomicroscope for general evidence of diseases or pests.
      (2) Examine imbibed seeds. Immerse the working sample to make fruiting bodies, symptoms, or pests more easily visible and to encourage the release of spores. Examine with stereomicroscope after imbibition.
      (3) Examine organisms removed by washing. Immerse the working sample in water with a wetting agent, or in alcohol, and shake to remove spores, hyphae, nematodes, etc. Examine the excess liquid with a compound microscope.
   b. Examine after incubation.
      (1) After a specific period of incubation, examine the working sample. Note the presence of disease organisms or pests on or in seeds or seedlings. Use blotters, sand, or agar for incubation media.
      (2) Use blotters when required to grow the pathogens from the seeds or to examine the seedlings. Seeds may or may not be pretreated. Space widely to avoid secondary spread of organisms. Use light as necessary to stimulate sporulation. Examine with a microscope.
      (3) Sand or artificial composts can be used for certain pathogens. Seeds are not usually pretreated, but they are widely spaced on the medium. Incubation is favorable for symptom expression.
      (4) Use agar plates to obtain identifiable growth of organisms from seeds.
         (a) Sterility is required; seeds are normally pretreated and spaced.
         (b) Identify characteristic colonies and spores by microscopy.
         (c) Use lighting and germination inhibitors.
3. Examine growing plants. Grow plants from seeds and examine them for disease symptoms to determine the presence of bacteria, fungi, or viruses. Use inoculum from the test seedlot to test for infection of healthy seedlings.

Calculations and Expression of Results:

1. Express results as a percentage of seeds affected or as number of organisms in the weight of sample examined.
2. Report results on the ISTA certificate.
   a. Report test method.
   b. Report pretreatments.
   c. Absence of health test does not imply satisfactory health condition.

Specific Test Example-Pitch Canker Fungus:

1. Adapted from Anderson (1986b).
2. Blotter Method, 400-seed sample.

a. Pentachloronitrobenzene (PCNB) Broth
Combine peptone, 15 g; $MgSO_4 7H_2O$, 5 g; KH_2PO_4, 1 g; terraclor, 1 g with 1 L of distilled H_2O. Stir well using magnetic stirrer. Autoclave for 15 minutes. After autoclaving, place flask on magnetic stirrer and stir slowly until solution cools to room temperature or slightly warmer. Add 1 g of streptomycin sulfate and 1 to 2 g of neomycin sulfate under sterile conditions, and stir.

b. Place 25 seeds on blue blotter paper in plastic containers. Crush the seeds with a sterilized piece of plastic cut to fit the plastic box opening. Spray seeds and blotter paper with PCNB broth.

c. Incubate 14 days at 20 °C or until colonies are 2 cm in diameter.

d. Inspect all seeds for slow-growing, granular white colonies. Check each suspected colony using a light microscope at 100 to 400 magnification for microconidia and polyphialids. Select fungus from seed surface, not the blotter surface. Split the seeds into 4 groups of 100 for reporting purposes.

3. Agar Method

a. Prepare fresh potato dextrose agar (PDA) (makes 1 L)
(1) Clean and dice one medium-sized potato.
(2) Put diced potato in beaker with 500 mL of distilled H_2O. Run through autoclave.
(3) In flask, add 20 g of dextrose and 17 g of agar to 500 mL of distilled H_2O.
(4) Put dextrose/agar solution on magnetic stirrer and low heat.
(5) Strain cooked potatoes through two layers of cheesecloth to obtain at least 200 mL of slurry.
(6) Note amount of slurry, and pour slurry in flask with dextrose/agar solution.
(7) Add enough distilled H_2O to make total slurry solution amount to 500 mL (i.e., if there are 200 mL of slurry, add 300 mL of distilled H_2O).
(8) Put solution in autoclave and run for 15 minutes. To acidify media, add 20 drops of 50 percent lactic acid to obtain a pH of 4.7.

b. Isolating external seed fungi, 25-seed sample
(1) Place the whole seeds on acidified PDA (pH 4.7).
(2) Incubate 14 days at 20 "C.
(3) If possible, observe fungal growth daily and identify the fungi.

c. Isolating internal seed fungi, 25-seed sample
(1) Surface sterilize the whole seeds in 70 percent ethanol for 10 minutes. Stir the seeds every 2 minutes.
(2) Under sterile conditions, cut each seed open and remove half the center material.
(3) Place the seed half (center material) on acidified PDA (pH 4.7) using sterile technique.
(4) Incubate 14 days at 20 "C.
(5) If possible, observe fungal growth daily and identify the fungi.

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Appendix

Final Evaluation Questionnaire

At the end of the training course, most sponsoring agencies and instructors would like an indication of the effectiveness of the course from the students. It should always be our goal to improve content and relevance of the course, as well as the method of presentation to the students. A questionnaire for this purpose is presented in the following pages. This particular questionnaire was prepared by the USDA Office of International Cooperation and Development for the numerous training courses that they sponsor. All of it may not be relevant to every presentation of this material; instructors should review the questionnaire and choose those parts that apply. The authors of this manual have used this questionnaire and recommend it to others for improvement of their future uses of this material.

Course name and number _____

SHORT COURSE EVALUATION FORM

PRECOURSE INFORMATION

1. Who discussed the general objectives of the course with you before you arrived at the course site? _____

_____

2. How clear were the course objectives? (Please circle answer.)

1	2	3	4	5
Very clear	Clear	Somewhat clear	Somewhat unclear	Unclear

In what ways did the course differ from what you expected? _____

_____

_____

4. Please describe how you were selected for participation in this course.

_____

_____

ORIENTATION

How helpful was your initial orientation at the course location?

Not helpful 1 2 3 4 5 Extremely helpful

Did not attend

Comments on precourse information and orientation: _____

_____

_____

COURSE ADMINISTRATION/LOGISTICS

Please indicate your satisfaction with the following support arrangements at the course location:

	Not at ail satisfied					Extremely satisfied
	1	2	3	4	5	
Housing accommodations	1	2	3	4	5	
Training facilities	1	2	3	4	5	
Transportation	1	2	3	4	5	
Administrative and logistic help	1	2	3	4	5	
Arranged social activities	1	2	3	4	5	

Please comment on the above arrangements: _____

_____

FIELD TRIP **ADMINISTRATION/LOGISTICS**

How adequate were the following field trip arrangements?

	Poor	1	2	3	4	5	Excellent
Preparatory information		1	**2**	**3**	4	5	
Transportation		1	**2**	**3**	**4**	**5**	
Lodging		**1**	**2**	**3**	4	**5**	
Helpfulness of instructors accompanying you		1	**2**	**3**	**4**	**5**	
Helpfulness of people you met in the field		1	**2**	**3**	**4**	5	
Overall coordination		1	**2**	**3**	**4**	5	

Please comment on the above arrangements.

COURSE CONTENT

1. At the beginning of the course or during the course, did you discuss with the instructors how the course content would meet your specific needs?

____Yes ___No

If yes, did you find the discusson helpful?

____Yes ___No

2. To what extent did you reach the following objectives through this course?

	Not achieved	1	2	3	4	5	Fully achieved
Objective 1		1	2	3	4	5	
Objective 2		1	2	3	4	5	
Objective 3		1	2	3	4	5	
Objective 4		1	2	3	4	5	
Objective 5		1	2	3	4	5	

3. Which objectives were most appropriate for your professional development? Please explain:

_____

_____

4. Was the level of presentation of the subject matter:

simple? _____ About right? complex?

5. Which aspects of the subject matter covered in the course will you use most when you return to your job? Please explain: _____

_____

6. Which aspects of the subject matter will you use least when returning to your job? Please explain: _____

_____

7. Based on your own needs, what course topics would you recommend be expanded? _____

Shortened? _____

Omitted? _____

Added? _____

8. What aspects of the course were most relevant to your country's conditions and to your role in your country's development? Please explain: _____

9. Did the field trip provide you with practical applications of the course content? Please explain:

COURSE DESIGN AND DELIVERY

1. During the course, was the daily schedule:

Too	About	Too
____short?	-right?	____long?

2. Was the overall length of the course:

Too	About	Too
____short?	-right?	____long?

3. Which of the following training methods were used to help you learn in this course? (Please circle those that were used.)

a. Lecturettes

b. Large group discussions

c. Small group discussions

d. Case studies

e. Practical hands-on experience in the field

f. Classroom/laboratory exercises and experiments

g. Role plays/simulation

h. Individual consultations with instructors

i. Other _____

4. Which of these methods most helped your learning and ability to use the skills? (List in the order of priority for helping you learn.)

a. _____

b. _____

c. _____

d. _____

e. _____

5. Were any of these methods used too much? ____Yes ____No

If yes, please explain: _____

_____

_____

Were any of these methods used too little? ____Yes ____No

If yes, please explain: _____

_____

_____

7. Could the course have been presented more effectively to meet your needs? Yes ____No

If yes, please explain: _____

_____

_____

8. How useful were the following types of materials used in the course? (Please circle the appropriate number.)

	Not at all useful					Extremely useful
Written materials (manuals, handouts, texts)	1	2	3	4	5	
Audiovisual materials	1	2	3	4	5	
Computer-assisted instruction (ii used)		2	3	4	5	

9. Please give examples of the most effective materials.

_____

_____

IO. Please give examples of the least effective materials.

_____

_____

INSTRUCTORS

Name of first instructor: _____

Please rate this instructor in the following areas:

	Poor 1	2	3	4	5 Excellent
Knowledge of subject matter	1	2	3	4	5
Training ability	1	2	3	4	5
Ability to relate material to your country	1	2	3	4	5
Overall effectiveness	1	2	3	4	5

Comments: _____

_____

_____

Name of second instructor: _____

Please rate this instructor in the following areas:

	Poor					Excellent
Knowledge of subject matter	1	2	3	4	5	
Training ability	1	2	3	4	5	
Ability to relate material to your country	1	2	3	4	5	
Overall effectiveness	1	2	3	4	5	

Comments: _____

_____

_____

Name of third instructor: _____

Please rate this instructor in the following areas:

	Poor					Excellent
Knowledge of subject matter	1	2	3	4	5	
Training ability	1	2	3	4	5	
Ability to relate material to your country	1	2	3	4	5	
Overall effectiveness	1	2	3	4	5	

Comments: _____

_____

_____

Name of fourth instructor: _____

Please rate this instructor in the following areas:

	Poor					Excellent
Knowledge of subject matter	1	2	3	4	**5**	
Training ability	1	2	3	4	5	
Ability to relate material to your country	1	2	3	4	5	
Overall effectiveness	**1**	2	3	4	5	

Comments: _____

_____

_____

OVERALL COURSE SATISFACTION

1. Would you recommend this course to other individuals with a background similar to yours? ___Yes ___No

Please explain why: _____

2. Please rate your overall satisfaction with the participation of your classmates.

Not at all satisfied 1 2 3 4 5 Extremely satisfied

Comments: _____

_____

_____

3. Please rate your overall satisfaction with this course:

Not at all satisfied 1 2 3 4 5 Extremely Satisfied

4. What final comments or suggestions do you have on this course?

_____

_____

_____

PARTICIPANT DATA

Home country: _____ Gender: - Male - Female

Main field of education: _____

Highest degree achieved: Secondary ___(BA/BS) ____(MA/MS) (Ph . D .)

Other: _____

Year in which highest degree was obtained: _____

Type of position currently occupied:

Scientific Administrative Technical Other

If you have managerial or administrative responsibilities, please describe them: _____

_____

_____

How long have you been working in your current position?. _____

Did you find language to be a problem in the course? Yes ____No.

If yes, please describe: _____

_____

_____

Bonner, F.T.; Vozzo, J.A.; Elam, W.W.; Land, S.B., Jr. 1994. Tree seed technology training course. Instructor's manual. Gen. Tech. Rep. SO-106. New Orleans, LA: U.S. Department of Agriculture, Forest Service, Southern Forest Experiment Station. 160 p.

A training manual that covers all aspects of tree seed technology from collection to sowing.

This publication reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by approrpiate State and/or Federal Agencies before they can be recommended.

Caution: Pesticides can be injurious to humans, domestic animals, desirable plants, and fish or other wildlife -if they are not handled or applied properly. Use all pesticides selectively and carefully. Follow recommended practices for the disposal of surplus pesticides and pesticide containers.