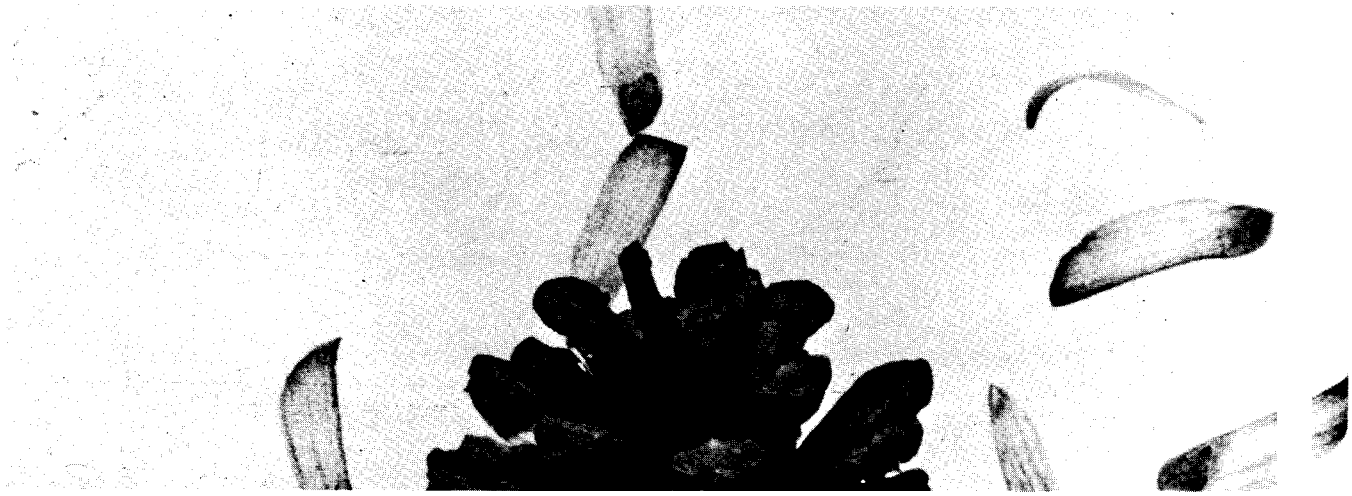

CONE ANALYSIS OF SOUTHERN PINES A GUIDEBOOK

General Technical Report SE-13
USDA-Forest Service
Southeastern Forest Experiment Station
Asheville, North Carolina
and
Southeastern Area, State and Private Forestry
Atlanta, Georgia

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PREFACE

The information in this publication was presented at two Cone Analysis Workshops organized by Dr Earl W Belcher, Jr, Director of the Eastern Tree Seed Laboratory, during November 1976 at Macon, Georgia. These workshops were presented by personnel from the Southeastern Area, State and Private Forestry. and the Southeastern Forest Experiment Station The authors of this workbook are those who developed the workshop and conducted training sessions.

This guidebook contains the information essential for cone analysis and interpretation of the results Cone Analysis Service (CAS) is also available at cost from the Eastern Tree Seed Laboratory at Macon, Georgia

CONE ANALYSIS OF SOUTHERN PINES
A Guidebook

by
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I. INTRODUCTION

Southern pine tree improvement programs require an ample supply of Improved seeds,[1] but productron from southern pine **seed orchards** has often been disappointing If high productron is to be maintained, yields must be monitored and causes of seed losses must be Identified. Techniques for determining seed efficiency were first used for red pine, *Pinus resinosa* Art.. by Lyons (1956) Bramlett (1972b, 1974) modified and further developed a procedure. known as **cone analysis,** for evaluating production efficiency in southern pine seed orchards

Cone analysis provides information needed to evaluate seed productron and seed orchard management Actual seed yield of individual **cones** is compared to the potential seed yield Productivity can then be expressed in terms of seed efficiency. One can determine in which stages of seed development certain losses occur, and the types of seed failures can be identified and quantified.

This guidebook outlines the basic cone-analysis procedure, the factors Involved in losses of seed, and the interpretation and value of the results It is a stepwise guide for those who wish to conduct their own cone analyses More detailed discussions may be found in the publications listed in the Literature Cited section Tree Improvement workers who do not want to analyze cones themselves can obtain this service at cost from the Eastern Tree Seed Laboratory This guidebook will help them understand the analytical results they receive

[1]Terms in boldface type are defined in a glossary at the end of this guidebook

II. CONE AND SEED DEVELOPMENT AND MORPHOLOGY

A basic knowledge of cone and seed development and morphology is essential to understand cone analysis.

Pine trees produce two types of **strobili:** male or pollen-producing **catkins** and female or ovule-producing cones The primordia of the **female cones** are initiated in the summer The following spring **female flowers** emerge in an erect position from protective bud scales on the tips of new shoots (fig. 1A). The female flowers are composed of many soft, fleshy scales spirally attached at right angles to a central axis. Pairs of **ovules** originate as small protuberances at the bases of scales (fig. 1 B). Not all scales produce functional ovules; only those in the central region of the cone have the potential to produce ovules and eventually seeds. These are called **fertile scales.** The lower scales and those at the tip of the cone are infertile and never bear seeds **(infertile scales).**

At the time of **pollination,** a single layer of cells **(integument)** covers the ovule. Pollination occurs when the **pollen grains** are carried by the wind from the male catkins to the female flowers. Pollen grains enter the ovule (fig. 1C) through an opening (the **micropyle)** in the **seedcoat.** Once in contact with the ovule. pollen grains germinate and **pollen tubes** grow from the pollen chamber into the ovule tissue **(nucellus) (fig. *1*** D). The pollen tube develops slowly during the first growing season, and the winter is over before it reaches the mature egg cell of the ovule. For more than 1 full year. the ovule remains only a fraction of the size of a fully developed seed. During the year after pollination, the female flowers are called **conelets.**

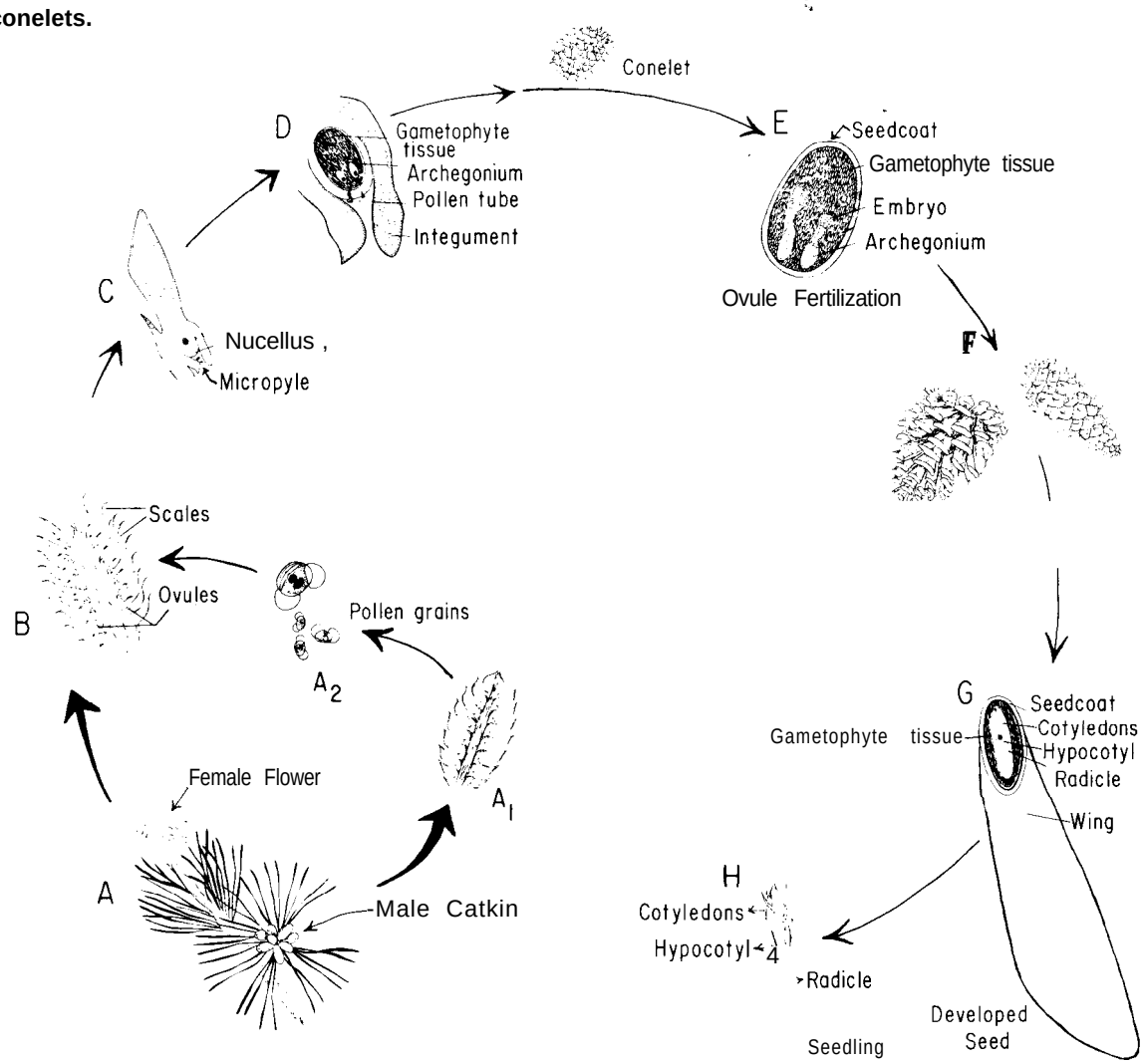


Figure 1. -Pine seed formation. Drawing adapted from one provided by Carolina Biological Supply Company,

When vegetative growth starts in the second spring, the ovule also resumes development and enlarges. From 12 to 16 months after pollination, depending on pine species, the pollen tubes grow far enough into the ovule to reach specialized structures **(archegonia)** containing the egg cells (fig. 1 E). Sperm cells from the pollen tubes fertilize the eggs, and embryos (2N tissues) form. The gametophyte tissue surrounds the embryo and provides the nutritional reserves for the seed (1N tissues). During this time, the conelet enlarges rapidly to form a full-sized cone. Seeds are full sized at the time of **fertilization,** but seeds and cones continue to mature until late summer or early fall

A mature, fully developed seed (fig. 1 G) has an embryo composed of Immature needles **(cotyledons),** immature stem **(hypocotyl),** and an immature root **(radicle).** The gametophyte tissue enveloping the embryo provides the food reserves for **germination.** The hardened seedcoat protects the seed

The sequence of pine seed development – from flower primordia formatron to seed fall – is illustrated in figure 2 This development spans parts of three growing seasons.

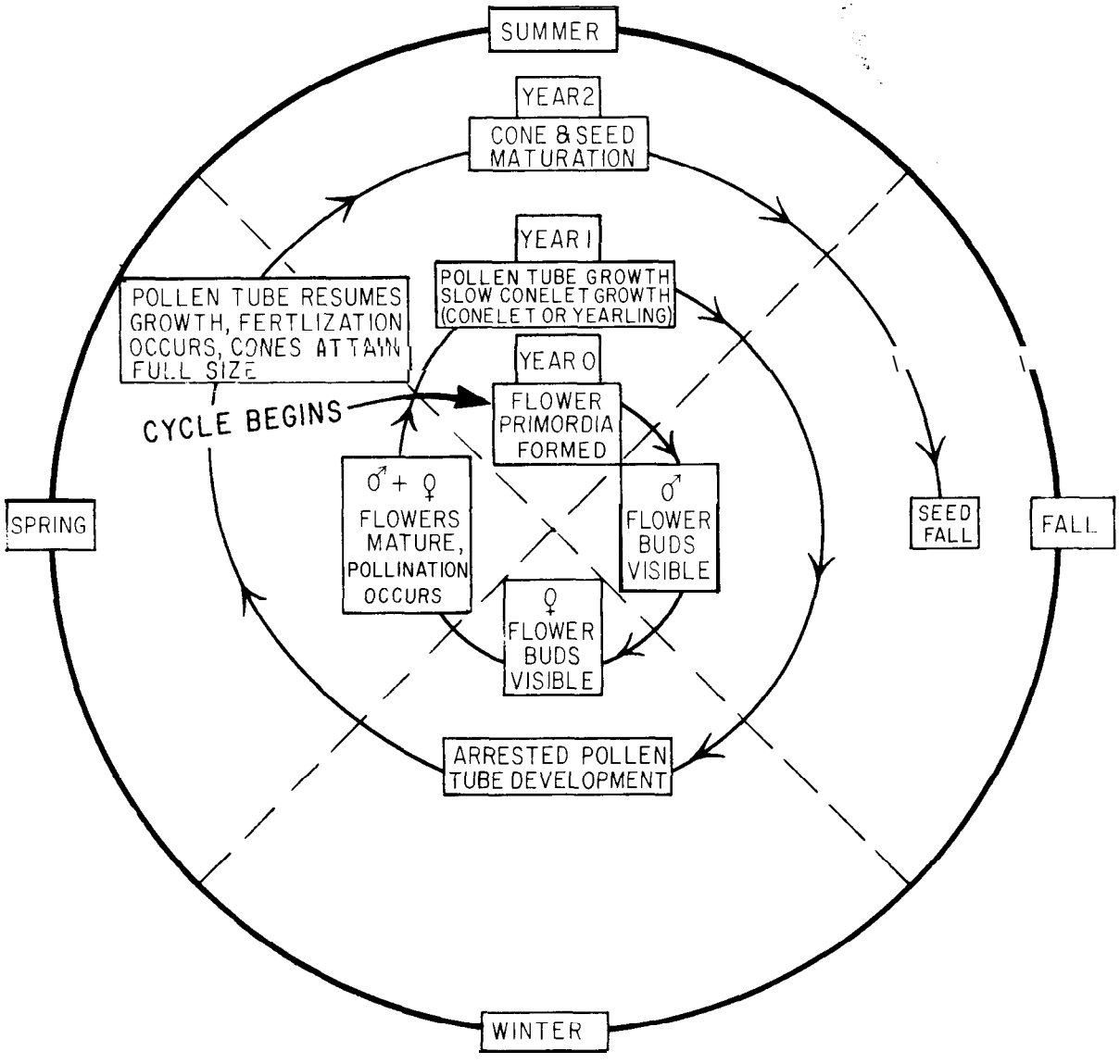


Figure 2.-The cycle for flowering and seed production in southern pines.

III. GENERAL GUIDELINES FOR CONE SELECTION

The method and number of cones to select for analysis depend on the analytical objectives, the planting design, anc variation wrthrn the orchard

Overall performance of the orchard can be evaluated by selecting cones from throughout the orchard. Exclude all insect-damaged cones, but do Include cones of all sizes that are normally harvested and extracted Such a bulk sample can be used for comparisons with other orchards or with other years for the same orchard A sample of from 25 to 100 cones per orchard is normally sufficient to evaluate overall seed efficiency However, with such selectron. no conclusions can be reached about individual clones.

If seed efficiencies must be estimated for rndrvrdual clones, cones have to be collected separately from specrfrc clones From limited data, it appears that ***10*** or more cones per clone are required If individual **ramets** are sampled wrthrn a clone then three to five cones per ramet will probably be necessary Additional research and experience will provide a better basis for specrfrc cone sample numbers

How well the sample represents orchard conditions depends on the variation within the orchard. Morphological traits such as cone length, number of scales, and **seed potential** vary relatively little, and only small samples are required to estimate these traits with specified reliability. Seed-yield traits, however, are more difficult to estimate because multiple factors (pollination, Insects, etc.) contribute to the variation. Seed and ovule losses must be attributed to specrfrc agents tc identify problems in orchard management, and such losses can be highly variable. Since seed losses are less wher management is good, seed-yield traits can be estimated from relatively small cone samples in a high-yielding, well-managed orchard The very orchards where problems exist and where information needs are greatest are the most difficult to assess and require the largest cone samples

IV.: RECOGNIZING ABORTED OVULES AND SEEDS

In order to understand the details of the cone analysis procedure, it is first necessary to be able to distinguish a developed seed from an **aborted ovule.** Ovule abortion may occur during the first or second year of development. First- and second-year aborted ovules can be distinguished from developed seeds fairly easily by the methods given in this section

FIRST-YEAR ABORTED OVULES

This class of ovule abortion occurs on fertile scales during the conelet stage – before cone enlargement begins in the second growing season The seed wing develops normally, and these ovules have been called "wings without seeds" Rudimentary, nonfunctronal ovules may develop with wings on some of the larger infertile scales. Infertile scales can be recognized by their narrow bases (fig. 3). The nonfunctronal ovule never has the capacity of becoming a seed and appears as a small dark spot at the base of the wing (Lyons 1956). First-year aborted ovules are noticeably larger than rudimentary ovules (figs 4. 5A and 5B). Some infertile scales at the base of a cone may produce wings with no evidence of even a rudimentary ovule. The smallest infertile scales at the very base of the cone may not produce wings.

SECOND-YEAR ABORTED OVULES

Ovules that abort during the second growing season are always larger than those that abort during the first year They may have a partially developed seedcoat (figs 4B and 5C) These ovules are usually considerably smaller than developed seeds, because they generally abort early in the second year of development Some ovules abort in late spring or early summer, these may be as large in outline as fully developed seeds but their seedcoats are collapsed. These ovules are frequently necrotic or shriveled and usually are attached to the cone scale with resin (fig. 5D).

DEVELOPED SEEDS

Developed seeds are full sized and have complete seedcoats (figs. 3C and D; 4C; 5E, right, and F). These can be placed in specrfrc classes based on their internal appearance on a radiograph (X-ray picture).

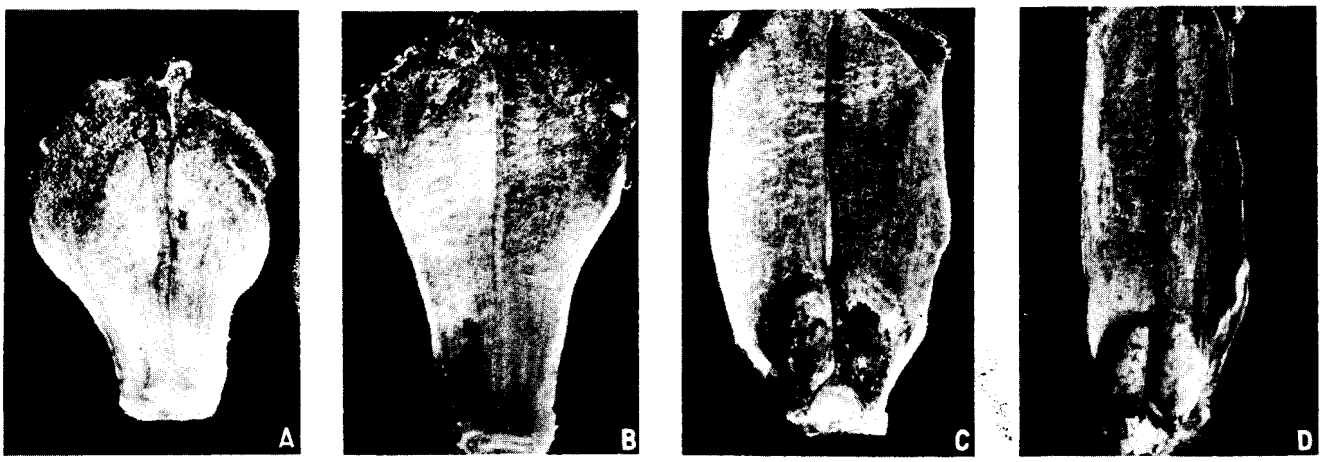


Figure 3.-Cone scales- (A & B) infertile scales with narrow base and no functional ovules, (C & D) fertile scales containing two developed seeds with mature seedcoat.

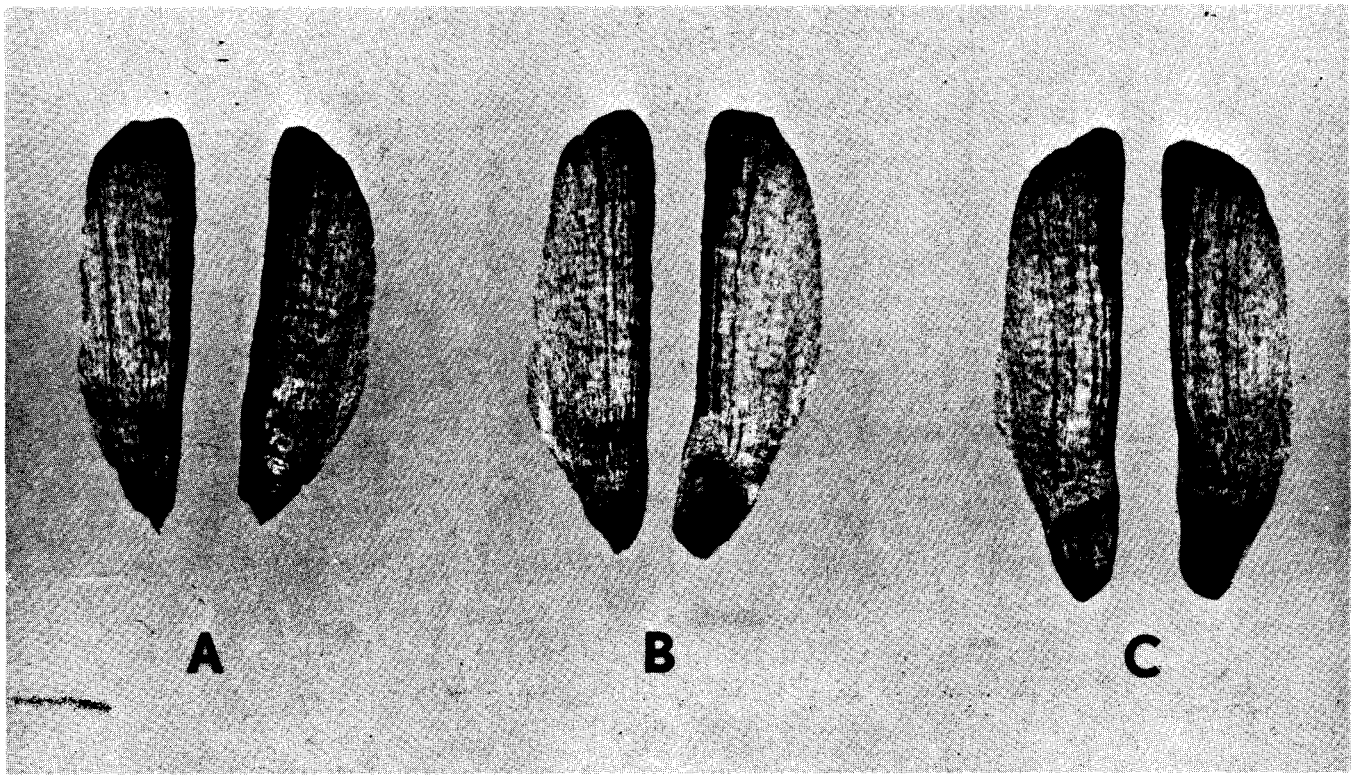


Figure 4.-Classification of aborted ovules and developed seeds: (A) first-year aborted ovules, (B) second-year aborted ovules, and (C) developed seeds.

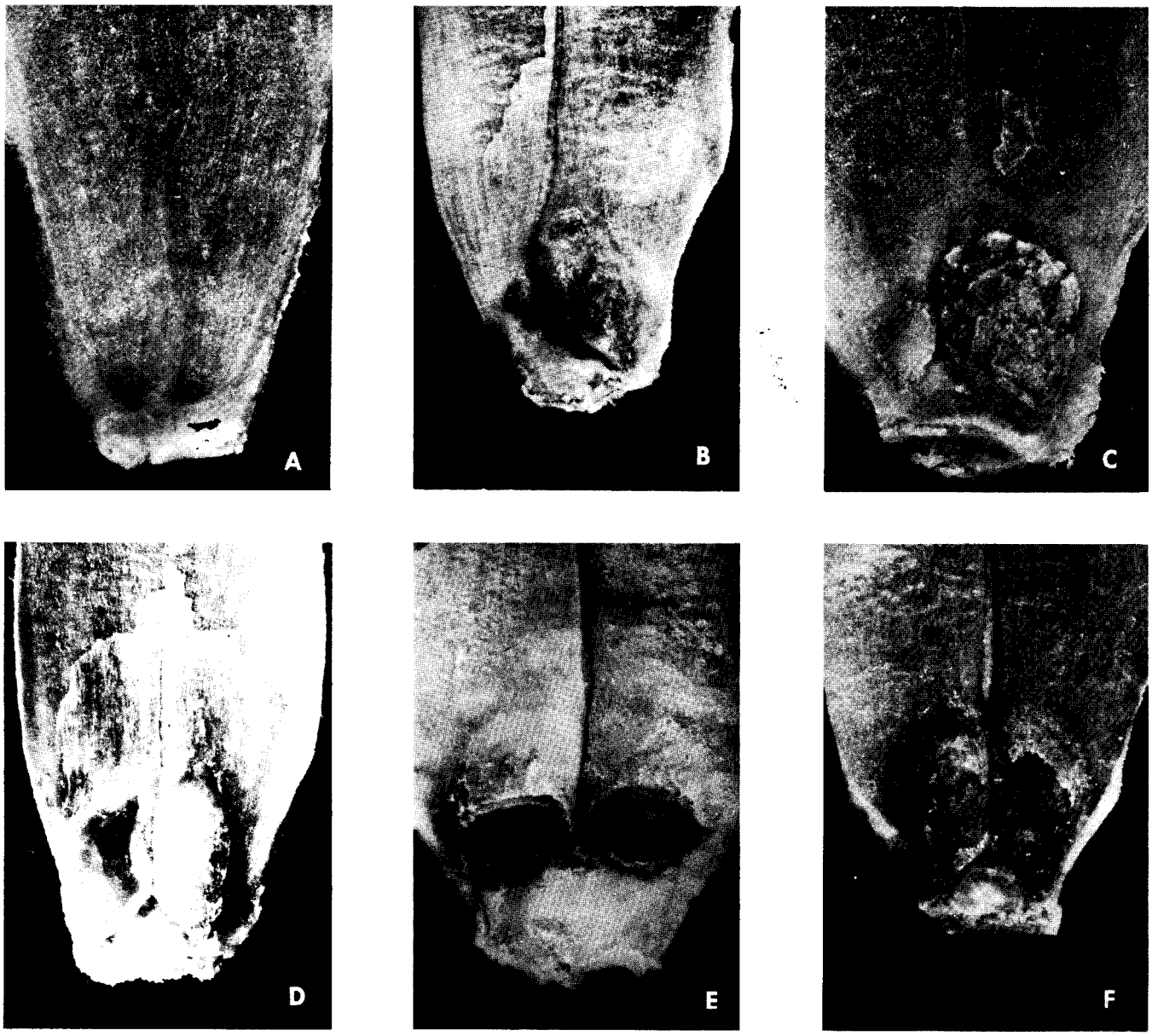


***Figure 5.**–Classification* **of ovules and seeds: (A) rudimentary ovules from infertile scale; (B) left. first-year aborted ovule;** *right, normal ovule; (C)* **left early second-year aborted ovule; right normal ovule; (D) left, typical** *seedbug* **induced second-year** *aborted* **ovule;** *right,* **normal ovule; (El sliced seed: left empty seed: right filled seed; (F) fully developed seeds.**

V. CONE-ANALYSIS PROCEDURE

In cone analysis, all loose seeds and aborted ovules are extracted from the cone by normal procedures Then cone scales re systematically removed and all the remaining ovules and seeds are collected The number of scales classed as fertile ıg 3) ıs used to determine the biological potential of the cone to produce seeds Ovules and seeds extracted and dissected om the cone are then classified by type (figs. 4 and 5) The seed efficiency of each cone ıs the number of filled seeds as a ercentage of the biological potential The causes for seed losses can be identified and quantified on radiographs The ex- ;nt of seed losses to each cause indicates where corrective measures might be taken to reduce seed mortality.

QUIPMENT NEEDS

Cone analysrs requires little specialized equtpment Essential needs are.

1. Paper collection bags for individual cones (bag size depends on tree species)
2. Cone-drying facility (90°-105° F [32°-40° C] and relative humidity below 50 percent)
3. Weighing balance accurate to 1 gram
4. Variable-speed 3/8-inch electric drill mounted ın a stationary horizontal position and adjusted to run at the slowest speed
5. Drill bits of various sizes (inches). 1/4, 5/16, 3/8, 7/16, 1/2, 5/8.
6. Grafting knife
7. Leather work gloves
8. Metric ruler
9. Containers for soaking cones
10. Germination supplies and equipment
11. X-ray supplies and equipment

ROCEDURE

The stepwise procedure for cone analysis ıs presented ın a flow chart ın figure 6 Bits of data that should be recorded are dicated ın the chart, the columns mentioned are those on the sample data sheet ın the Appendix Some morphological arts are considered optional (shaded columns on data sheet) and are not necessary for computation of the most Important ;ed and cone characteristics.

Two cone-analysis procedures are presented These are designated "Procedure A" and "Procedure B "During the ɔllection of data, there ıs one basic difference ın the two procedures With Procedure A, the extracted and dissected ;veloped seeds are combined (steps 9A and 15A – columns 46-61) With Procedure B, the extracted developed seeds tep 9B – columns 46-61) are kept separate from the dissected developed seeds (step 15B – columns 62-77) from the ɔne. Each procedure has certain merits and limitations, which are summarized below

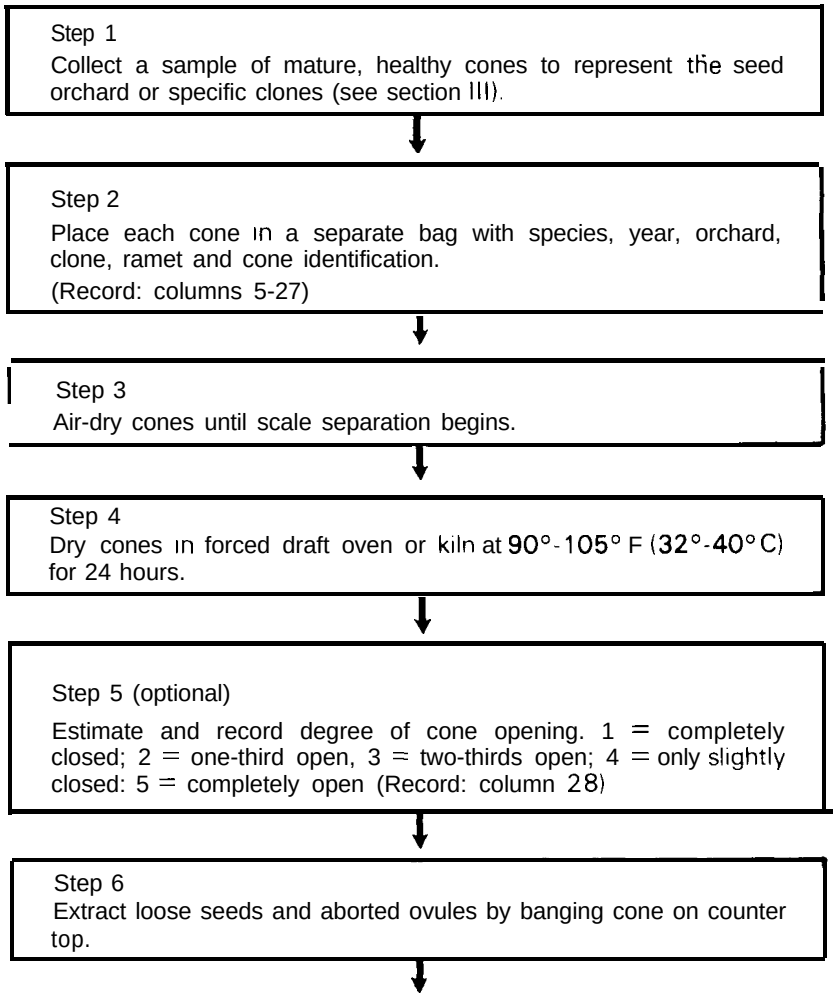
Procedure A. – This procedure evaluates the yield and the extraction and germination efficiencies of all the seeds ın the ature cone The developed seeds extracted from the cone by normal procedures are combined with the developed seeds covered by removing the cone scales (dissection) All seeds are then radiographed and classified as described ın section The advantage of the procedure ıs that only a single radiograph ıs required for each cone Once the cone analysrs ıs ;gun on a particular cone, the extractron. dissection, and seed classification can be completed at one time Also, the ımary emphasis ıs on the number of seeds actually produced ın the cone, rather than just on those seeds extracted from e cone under laboratory conditions. This procedure ıs simplest for small organizations doing their own cone analysrs Ger- ınation percentage using Procedure A ıs based on the ratio of the number of germinated seeds to the total number of led seeds produced by the cone.

Procedure B. – This is the procedure currently being used by the Eastern Tree Seed Laboratory for its Cone Analysis Service (CAS) In this procedure the seeds extracted after forced drying are radiographed, and tests of their germination are begun before the remaining steps rn the analysrs are completed. Because germination testing is the step that requires the most time, this procedure can substantially reduce the number of weeks needed to complete the analysis. This saving is particularly valuable when many cones are to be analyzed.

The germination percentage in Procedure B is the percentage of the extracted filled seeds that produce a normal seedling. Extraction efficiency by this procedure is the percentage of all filled seeds in the cone that were extracted after drying (Karrfalt and Belcher 1977).

Before starting cone analysis, the user must decide which procedure to follow. Once a procedure is decided upon, the steps and computations indicated for that procedure must be followed

Figure 6.–Flow chart for the steps in cone analysis. At several points steps are different for Procedure A than for Procedure B.



Step 7

Separate developed seeds from aborted ovules (see section IV).

Step 8

Count and record number of extracted, developed seeds. (Record. columns 43-45)

Step 9A

Place developed seeds in a labeled envelope.

Step 9B

a. Place developed seeds in a labeled envelope for X-ray examination.

b Radiograph seeds in envelope

c Begin germination procedures (see step 18)

d Classify seed images on radiograph (see section VI) (Record columns 46-61)

Step 10

Separate first-year aborted ovules from second-year aborted ovules (see section IV) Record only number of second-year aborted ovules (Record columns 41-42) Discard all aborted ovules

Step 1 1

Soak cone in water until scales close

Step 12 (optional)

Measure cone length to nearest millimeter (Record columns 29-31)

Step 13

Dissect cone beginning at base of cone, remove lower scales with a knife point (fig 7) As cone axis is exposed, use a drill with bit diameters of 1/4 inch to 5/8 inch to remove cone axis This facilitates scale removal Alternate drilling (1 inch or less at a time) and scale removal (fig 8) Match drill bit diameter with that of the cone axis

Step 14

Separate scales into groups: (1) lower infertile scales, occurring at the base of the cone (figs. 3A, B and 5A); (2) fertile scales, occurring in the central two-thirds of the cone and having two functional ovules or developed seeds (figs. 3C and D); and (3) upper infertile scales, occurring at the apex of the cone and not having the capacity to produce seed.

Step 15A

a. Take developed seeds from removed cone scales and add to previously extracted seeds in the envelope (step 9A).

b. Radiograph envelope containing seeds.

c. Classify seed images on radiograph (see section VI). (Record: columns 46-61)

Step 15B

a. Take developed seeds from removed cone scales and place in separate envelope.

b. Radiograph envelope containing seeds.

c. Classify seed images on radiograph (see section VI). (Record: columns 62-77)

Step 16

Count any remaining second-year aborted ovules and add to number previously extracted (step 10). (Record: columns 41-42)

Step 17

Determine the number of first-year aborted ovules by subtracting the total number of second-year aborted ovules plus the total number of developed seeds from two times the number of fertile scales on the cone. (Record: columns 38-40)[2]

Step 18

Place all developed seeds in germination test using standard germination testing procedures.

Step 19

Summarize data and compute important seed production indicator values (see section VII).

[2] *First-year aborted ovules may be counted as the scales are removed by observing the location of the scale where the seed is produced. Scales with first-year aborted ovules do not have the depression formed in the scale where the seed was located.*

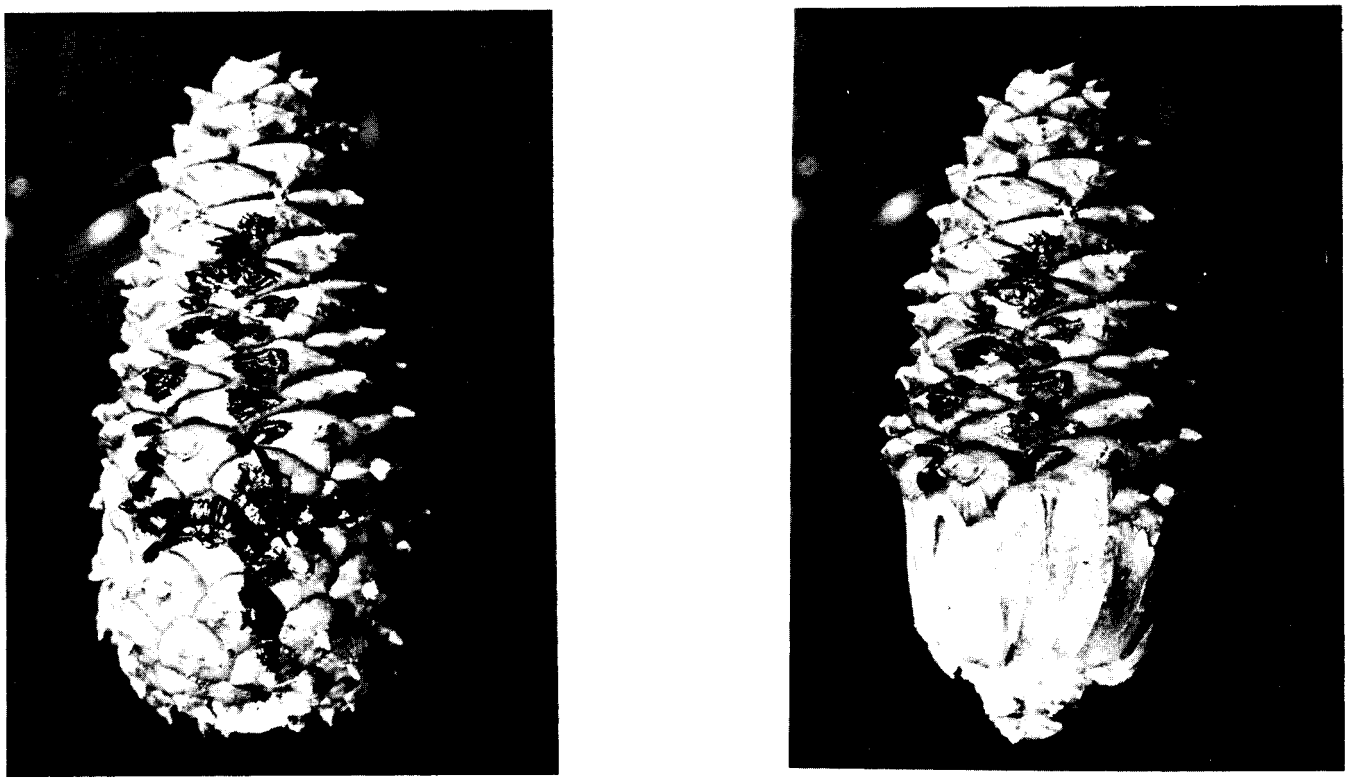


Figure 7.—(A) Shortleaf pine cone at harvest. (B) Same cone with lower infertile scales removed. Remaining scales are all fertile except for a few infertile scales at the cone tip.

Figure 8.—Cone scale removal. Scale removal is alternated withdrilling out short distances of the cone axis.

VI. CAUSES OF OVULE AND SEED LOSSES

After first-year aborted ovules and second-year aborted ovules have been separated from developed seeds, the causes for seed losses can be identified. The cause for abortion of an bvule often must be Inferred from the time at which abortion occurred. Developed seeds are classified by the appearance of the embryo and gametophyte tissue on a radiograph (fig. 9). Classes of ovules and seeds are defined, and causes for losses are summarized in tabular form in the Appendix.

FIRST-YEAR ABORTED OVULES

There are two causes of ovule abortion in the first Year – lack of pollen and seedbug damage

Lack of viable pollen. - Pollen is an obvious requirement for the production of healthy cones and viable seeds (Sarvas 1962) For productron of viable seeds, two pollination requirements must be met. Pollen must be available when female flowers are receptive, and it must germinate and grow into the ovule tissue Female flower receptivity begins when the opening between the scales is large enough for pollen to enter and extends from a few days to more than a week. Once the scales have closed, additional pollen cannot enter the ovule. If no pollen is present or if the pollen fails to germinate, the ovule aborts early in the first Year of cone development (Bramlett and Johnson 1975; McWilliam 1959, Sarvas 1962).

Seedbug ***feeding.*** – Feeding by nymphs of the leaffooted pine seedbug, *Leptoglossus corculus* (Say), often causes abortion of a high percentage of ovules (DeBarr and Kormanrk 1975; DeBarr and Ebel 1973; DeBarr and others 1975) Ovules destroyed by seedbugs cannot easily be distinguished from ovules aborting from lack of pollen.

Losses to seedbugs can be estimated by protecting some cones in screened cages and comparing yields of protected with unprotected cones With this technique, the impact of seedbugs can be separated from losses to physiological and environmental factors

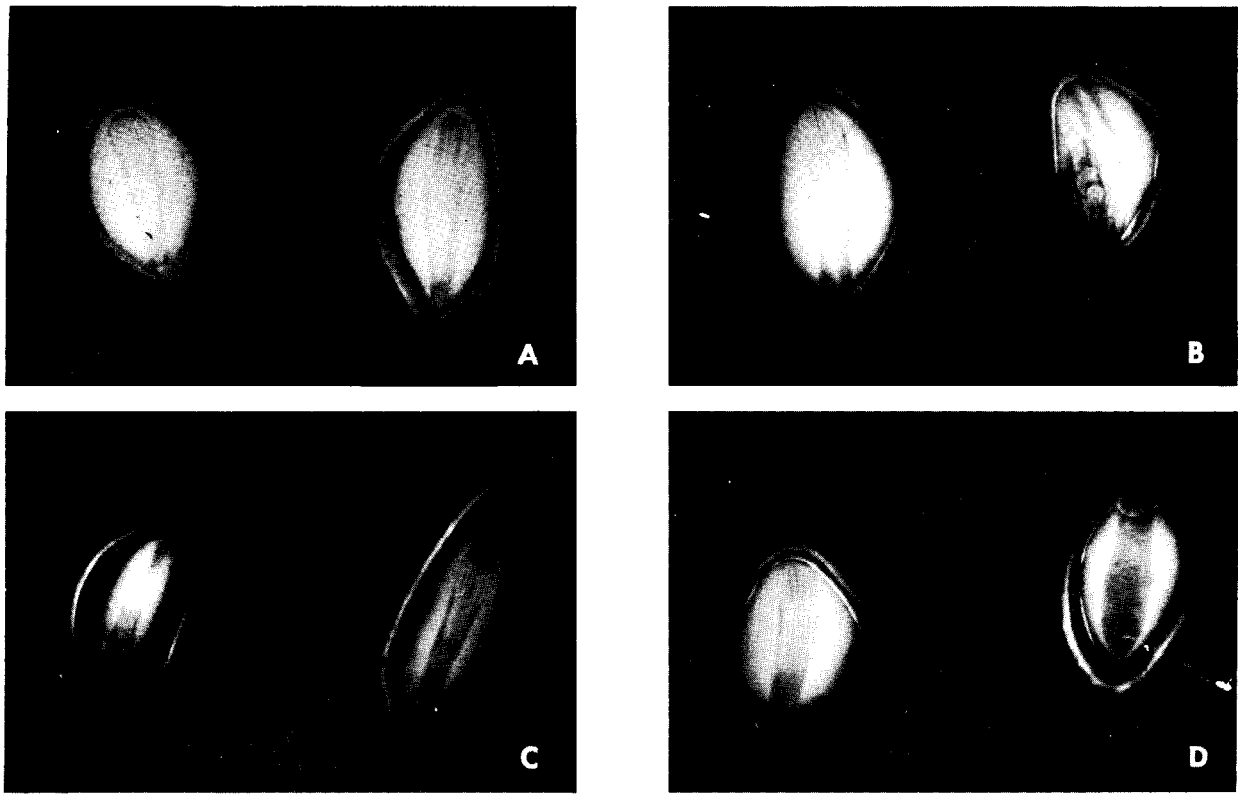


Figure 9.-Classification of radiographic images of developed seeds: (A) filled (potentially sound] seeds; (B) mechanically damaged seeds; (C & D) malformed seeds-C. incomplete gametophyte: D, distorted embryo (left); missing embryo (right); (continued)

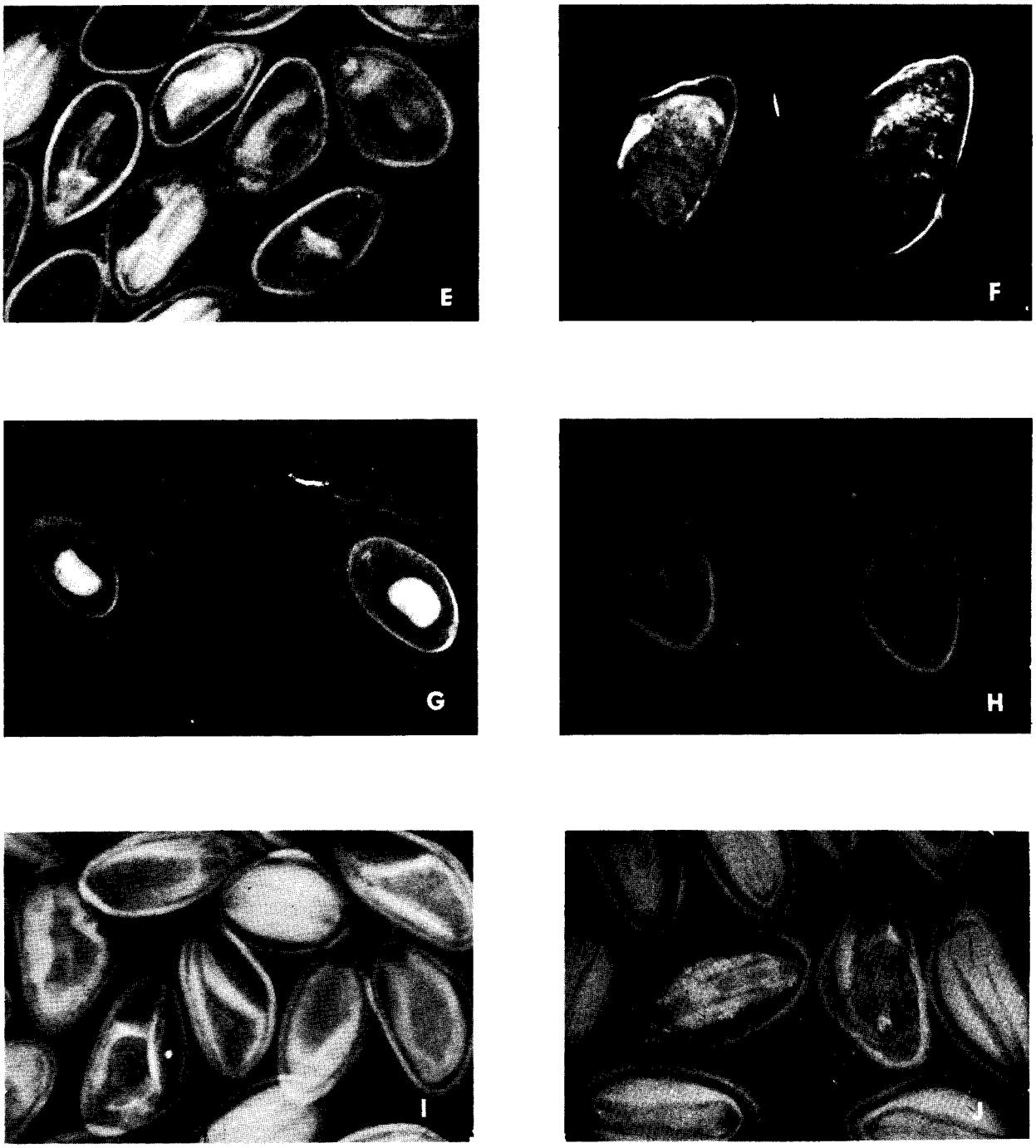


Figure **9** *(continued).–(E)* **damaged by** *seedbug; (F)* **destroyed by seedworm;** *(G)* **destroyed by seed** *chalcid; (H) empty* **seeds and** *(I & J)* **damaged by fungi**

SECOND-YEAR ABORTED OVULES

Seedbug **feeding.** – In extensive studies of shortleaf (*P.* **echinata** Mill.) and loblolly (*P.* **taeda** L.) pines (DeBarr 1974a), slash pine (*P. elliottii* Engelm. var. *elliottii*) (DeBarr and others 1975). and Virginia pine (**P. virginiana** Mill.) (Bramlett and Moyer 1973). second-year ovule abortion has clearly been shown to result almost entirely from feeding by the leaffooted pine seedbug in early summer (fig. 10). These ovules appear resinous, collapsed, or necrotic on the mature cone scales (figs. 4B; 5C, left; and 5D, right).

Developmental problems.-Some ovules stop developing during the second year after they have developed a seedcoat. These "miniature seeds" may be oniy slightly larger than first-year aborted ovules or up to one-half the normal size for seeds of the species. These are all empty. The cause of this type of ovule mortality is unknown, but it apparently results from a block in the normal developmental sequence prior to fertilization. The frequency of second-year aborted ovules due to developmental problems has been quite low in production seed orchards.

DEVELOPED SEEDS

Filled, partially filled, and empty developed seeds are readily distinguishable on radiographs. In addition, several types of damage can be identified on radiographs (fig. 9).

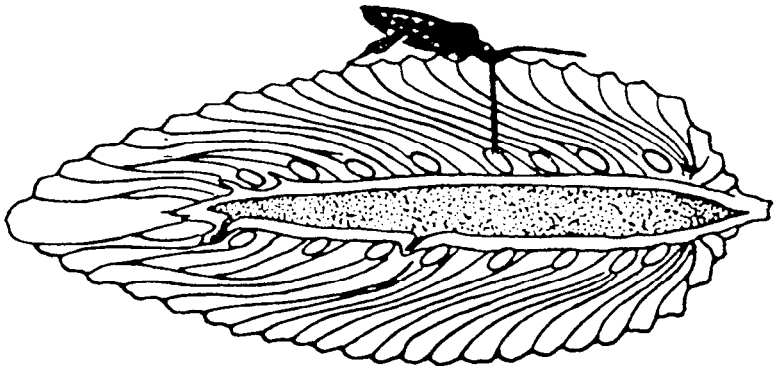


Figure 10.–Diagram of a cone showing how the leaffooted pine seedbug penetrates alternate layers of cone scales and seed wings to puncture seeds lying along the cone axis. (From DeBarr 1970)

Filled Seeds

Filled seeds (potentially sound seeds) are those that have healthy undamaged gametophyte tissue. a normal embryo. and no evidence of insect or fungal damage. These seed structures are readily distinguished on a radiograph (fig. 9A). Potentially sound seeds may be mechanically damaged at any stage of extraction (fig. 9B). Since the primary purpose of cone analysis is to judge production efficiency in the seed orchards, mechanically damaged seeds should be classed as filled.

Partially Filled Seeds

Seedbug.-The leaffooted pine seedbug and the shieldbacked pine seedbug, ***Tetyra bipunctata*** (H.-S.), feed on second-year cones, damaging and destroying seeds (DeBarr 1967). Damage to fully developed seeds can often be identified on radiographs by the appearance of the gametophyte tissue (DeBarr 1970). Typical seedbug feeding removes irregularly shaped portions of gametophyte tissue (fig. 9E). Extended feeding on a single seed often removes most of the gametophyte tissue and embryo, leaving only a shriveled remnant in the seedcoat.

Seedworm.-Three species of seedworms, *Laspeyresia* spp.. infest seeds of the southern pines (Ebel and others 1975). Since seedworm-damaged seeds generally are stuck to the cone scales with excrement and silk webbing, most are detected and counted as the individual cone scales are dissected during cone analysis. The few seeds included in the extracted sample are easily detected on the radiograph (fig. 9F). Seedworm larvae are not present in the seeds, and the exit holes are large. The gametophyte tissue has been replaced by larvel excrement and boring frass that looks granular on the radiograph and often is formed into concentric rings (DeBarr 1970)

Seed chalcid-A seed infested by a seed chalcid, *Megastigmus atedius* Walker, lacks gametophyte tissue and contains a single larva that is readily visible on the radiograph (fig. 9G). To date, the only pine growing in the Southern United States known to be infested by this seed chalcid is eastern white pine (*P. strobus* L.) (Speers 1975)

Fungi.–Fungal damage is recognizable on radiographs as (1) cloudy or hazy semicircular areas generally at the margin of the gametophyte tissue (fig. 9I), (2) partial to complete disorganization of both the gametophyte and embryonic trssues (fig. 9J), and (3) all gametophyte tissue and embryo shrunken or "mummified." Fungal damage may have the same appearance as advanced seedbug damage on the radiograph. Dissectron and close examination of infected seeds have revealed fungi in the gametophyte trssues, embryo, or both. The mechanisms of fungal penetration are not known, but the presence of fungi in seeds is known to reduce the germination percentage and to increase damping-off of seedlings (Miller *1976).*

Ma/formed seeds.–Seeds with incomplete embryos, distorted embryos, or incomplete gametophytes are classed as malformed. Missing or malformed embryos show up clearly on radiographs (fig. 9C and D). These malformations have physiological causes. In some malformed seeds, the gametophyte tisse does not completely fill the seedcoat and no internal insect, disease, or mechanical damage is evident (fig. 9C). In these seeds, approximately one-half to three-quarters of the normal amount of gametophyte tissue is normally present. Seeds with incomplete gametophytes occur in cones harvested before maturity. Malformed seeds occur at a low frequency in most lots and rarely exceed 1 to 2 percent for most trees.

Empty Seeds

Seeds that contain only a remnant of gametophyte or embryo tissue (fig. 9H) are classed as empty. In the trade. empty southern pine seeds are often called "pops." There are two known causes of empty seeds. First, each pine parent carries some recessive lethal genes (Bramlett and Pepper 1974; Franklin 1969). When two recessive lethal genes for the same trait are present, the embryo dies and the gametophyte tissue does not develop. The result is an empty seedcoat with only a remnant of the dead embryo present. Second, seedbug feeding during the early stages of embryo and gametophyte development will produce a completely empty seedcoat. The gametophyte tissue is completely destroyed by digestive enzvmes of the insect.

VII. SUMMARIZING DATA

To interpret the information obtained from the cone analysis, the data must be summarized. The summary should be tabulated according to trees, clones, or other categories of interest, For the following items, a numerical average can be computed:

Cone opening (CO)
Length (LT)
Lower infertile scales (LI)
Filled seeds (FL)
Malformed seeds (MS)
Seedbug-damaged seeds (SB)

Upper infertile scales (UI)
Fertile scales (FS)
First-year aborted ovules (A1)
Second-year aborted ovules (A21
Extracted seeds (ES)

Seedworm-destroyed seeds (SW)
Seed chalcid-destroyed seeds (SC)
Fungal-damaged seeds (FN)
Empty seeds (EM)
Germinating number of seeds (GN)

From the basic summary of the raw data, the key indicators of seed performance are calculated as shown below. Numbers in parentheses are the columns on the suggested data form in the Appendix.

Indicator **Computation**

1. **Seed potential (SP) = 2 x Fertile scales or 2 x (3436)**

2. **Total developed seeds (TS) = Seedbug-damaged seeds + seedworm-destroyed seeds + seed chalcid-destroyed seeds + fungal-damaged seeds + malformed seeds + empty seeds + filled seeds** *or* **(4647 and 62-63) + (48-49 and 64-65) + (50-51 and 66-67) + (52-53 and 68-69) + (54-55 and 70-71) + (56-58 and 72-74) + (59-61 and 75-77)**

3. **Percent developed seeds =** $\frac{\text{Total developed seeds}}{\text{Seed potential}} \times 100$ *or* $\frac{TS}{SP} \times 100$

4. **Percent filled seeds =** $\frac{\text{Filled seeds}}{\text{Total developed seeds}} \times 100$ *or* $\frac{(59\text{-}61 \text{ and } 75\text{-}77)}{TS} \times 100$

5. **Percent known insect-damaged seeds =**

$$\frac{\text{Seedbugdamaged seeds + seedworm-destroyed seeds + seed chalcid-destroyed seeds}}{\text{Total developed seeds}} \times 100 \textit{ or}$$

$$\frac{(4647 \text{ and } 62\text{-}63) + (48\text{-}49 \text{ and } 64\text{-}65) + (50\text{-}51 \text{ and } 66\text{-}67)}{TS} \times 100$$

6. **Percent malformed seeds =** $\frac{\text{Malformed seeds}}{\text{Total developed seeds}} \times 100$ *or* $\frac{(54\text{-}55 \text{ and } 70\text{-}71)}{TS} \times 100$

7. **Percent fungal-damaged seeds =** $\frac{\text{Fungal-damaged seeds}}{\text{Total developed seeds}} \times 100$ *or* $\frac{(52\text{-}53 \text{ and } 68\text{-}69)}{TS} \times 100$

8. **Percent first-year aborted ovules =** $\frac{\text{First-year aborted ovules}}{\text{Seed potential}} \times 100$ *or* $\frac{(38\text{-}40)}{SP} \times 100$

9. **Percent second-year aborted ovules =** $\frac{\text{Second-year aborted ovules}}{\text{Seed potential}} \times 100$ *or* $\frac{(41\text{-}42)}{SP} \times 100$

10. **Seed efficiency (SE) =** $\frac{\text{Total filled seeds}}{\text{Seed potential}}$ *or* $\frac{(59\text{-}61 \text{ and } 7577)}{SP} \times 100$

11. Extraction efficiency (EE) =

Procedure A: $\frac{\text{Extracted developed seeds}}{\text{Total developed seeds}}$ or $\frac{(43\text{-}45)}{\text{TS}} \times 100$

Procedure B: $\frac{\text{Extracted filled seeds}}{\text{Total filled seeds}}$ or $\frac{(59\text{-}61)}{(59\text{-}61 \text{ and } 75\text{-}77)} \times 100$

12. Germination percentage (GP) =

Procedure A: $\frac{\text{Germinated seeds}}{\text{Total filled seeds}} \times 100$ or $\frac{(78\text{-}80)}{(59\text{-}61 \text{ and } 75\text{-}77)} \times 100$

Procedure B: $\frac{\text{Germinated seeds}}{\text{Extracted filled seeds}} \times 100$ or $\frac{(78\text{-}80)}{(59\text{-}61)} \times 100$

13. Seedling efficiency = $\frac{\text{Seed efficiency x Extraction efficiency x Germinatidn percentage}}{10{,}000}$ or $\frac{\text{SE x EE x GP}}{10{,}000}$

VIII. -DATA INTERPRETATION AND UTILITY

The purpose of cone analysis is to evaluate seed production and to identify when and why potential seeds are lost. Table 1 shows typical values for key indicators in a well-managed seed orchard. It also shows values that may indicate need for corrective action.

The seed potential defines the biological limit for the number of seeds produced by each cone. Thus, each tree species has an average seed potential and a range of observed values based upon the number of fertile scales per cone. For example, the seed potential for cones from a slash pine seed orchard averaged 170. Loblolly pines had an average seed potential of 155, and Virginia and shortleaf pines had averages of 88 and 87 (Bramlett 1974). Variation in seed potential is relatively small within specific clones. Seed potentials in a given orchard, however, may differ from the average for the species, depending on the particular clones in the sample.

Aborted ovules are potential seeds that die before the formatron of a normal seedcoat. For all the southern pines, ovule abortion can be a serious cause of low seed yields The two known causes of first-year aborted ovules (lack of pollen and seedbug damage) are influenced by the orchard age. In young orchards, female flowering of many clones usually begins before male catkin productron is adequate to ensure complete pollination. Unless there is a supply of pollen from surrounding stands, many ovules die within 2 months from lack of viable pollen. In contrast, older seed orchards have an abundant pollen supply but also often have large seedbug populations that cause extensive first-year ovule abortion in unprotected conelets. Conclusive separation of Insect from pollination problems requires comparison of full seed yields from cones with and without screened cages that exclude Insects.

Second-year aborted ovules are, for practical purposes, a direct indication of seedbug feeding. Other agents seldom cause second-year abortions exceeding 1 to 2 percent of the seed potential. Second-year ovule abortion may reach 50 percent, but seedbug control may be warranted when the loss approaches 10 percent of the seed potential To prevent second-year ovule abortion, insecticide applications must be timed to reduce populations of leaffooted pine seedbugs dur-

ing the spring when the conelets are rapidly enlarging to full-srzed cones. Seedbug feeding on full-sized cones causes empty or damaged seeds rather than aborted ovules

Total developed seed is simply the number of normal, full-srzed seeds in each cone. Obviously, the orchard manager strives to maximize developed seed productron With adequate pollination and effective insect control. the developed seed yields should approach 80 to 90 percent of the seed potential

Yields of developed seeds are meaningless without considering seed quality. Using radiography. the actual number of filled seeds per cone is determined Then seed efficiency is expressed as the ratio of filled to potential seed. Thus. seed efficiency measures the productivity of a cone in relation to its biological capacity The average seed efficiency for a clone or an orchard is the single most Important measure of seed production It can be used to compare the relative performance of two pine species with different seed potentials For example, if a shortleaf pine produces an average of 45 filled seeds out of a potential of 90, the seed efficiency would be 50 percent However, 45 filled seeds in a slash pine cone with a Ventral of 180 indicates a seed efficiency of only 25 percent

TABLE 1.– KEY INDICATORS FOR **DATA INTERPRETATION**

Cone characteristic and threshold level (percent) [1]	Interpretation
1. First-year aborted ovules	
O-10	Good management practice
11-19	Evaluate possible insect or pollination problems
20+	Identify and correct insect or pollination problems
2. Second-year aborted ovules	
o-5	Good management practice
6-9	Evaluate seedbug control program
10+	Increase seedbug control
3. Percent filled seeds	
85+	Good management practice
50-84	Evaluate possible seedbug or inbreeding problems
Below 50	Identify and correct seedbug or inbreeding problems
4. Insect-damaged seeds	
O-10	Good management practice
11-19	Evaluate insect problem and control program
20+	Improve or increase control program
5. Seed efficiency	
55+	Good management practice
35-54	Evaluate causes of seed loss
Below 35	Identify causes of seed loss and begin correction
6. Extraction efficiency	
90+	Good management practice
Below 90	Identify and correct improper conditions or immature cone collections
7. Germination percentage	
90+	Good management practice
80-89	Identify cause (May be: undetected seedbug damage or mishandling of seed)
Below 80	Identify and correct problem (May be: improper germination, undetected seedbug damage, mishandling of seed)
8. Seedling efficiency	
50+	Good management practice
26-49	Identify cause – poor germination, immature cones
Below 25	Identify and correct cause

[1] Each organization may wish to modify these thresholds to meet its targets

The biological maximum for seed efficiency may be as high as 80 percent for individual cones, but values this high are seldom observed under seed-orchard conditions. Seed efficiencies greater than 70 percent have occurred when screen-wire cages were used to protect slash pine cones from Insects (DeBarr and others 1975)

Seed efficiency values of 35 to 54 percent indicate that some potential seeds are being lost. Yields could be Increased by identifying the specific causes of seed losses and prescribing corrective procedures within economic constraints Seed efficiencies of less than 35 percent indicate excessive losses The causes should be identified and the problems corrected as soon as possible

Developed seeds not classified as "filled" are worthless Thus, filled seed percentage simply expresses the ratio of filled to developed seeds Filled seed percentages seldom exceed 90 percent in commercial operations, but greater values have been reported with controlled pollinations or effective seedbug control (Bramlett and Pepper 1974, Merkel and others 1976) Wind-pollinated cones from orchards should average 85 percent or more filled seeds Lower values indicate losses in some or all of the classes identified from the radiographs Individual-loss classes exceeding 10 percent warrant corrective measures

Insects. particularly seedbugs, destroy a large percentage of the developed seeds In cone analysis, losses are attributed to seedbugs, seedworms, and seed chalcids. Usually, some seeds classed as "empty" are also the result of seedbug feeding Therefore, as recognized seedbug losses Increase. the number of empty seeds often rises (DeBarr 1974b; DeBarr and Ebel 1973).

Malformed seed losses (Incomplete gametophyte tissue, distorted embryo, missing embryo) seldom exceed 10 percent of the developed seeds in well-managed orchards. Incomplete gametophytes apparently result from retarded development or Inadequate nutrition. Cones collected before maturity produce seeds with incomplete gametophytes. Malformed seeds occur in sample lots at such low frequencies that these types of losses are of only minor importance.

Fungus damage to southern pine seeds has only recently been recognized as a potential problem. The Importance of fungal damage is undetermined. Part or all of the seeds from cones with external symptoms of fungal damage may be infected by fungi. Corrective measures are not yet available to prevent seed destruction by fungi.

The causes of empty seeds are difficult to identify Lethal genes in the embryo, seedbugs. and possibly fungi cause empty seeds. In wind-pollinated seeds from an orchard, as many as 15 percent of the developed seeds may be empty This value would reflect some effects of self-pollination within the clones in the orchard (Kraus 1975); the proportion of empty seeds is normally high after self-pollination. Lethal genes in embryos cannot be eliminated easily This genetically controlled system minimizes inbreeding and favors outcrossing in pines

If more than 15 percent of the developed seeds are empty, seedbug damage should be suspected and control programs should be reevaluated. Seedbug damage can be reduced with Insecticides.

Extraction efficiency measures the ease with which developed seeds are removed from the cones. It is the percentage of developed seeds extracted by kiln or air-drying the cones. It should be noted that this extraction is done in a laboratory – that the results are not necessarily the same as would be obtained by *commercial* extraction. Percentages below 90 Indicate poor extraction technique or immature cones.

Germination percentage measures the viability of seeds classified as "filled" (potentially sound). This value normally averages 90 percent or better Lower germination percentages may indicate Improper classification of some seeds in the lot (Rowan and DeBarr 1974). Germination percentages are useful for comparing the viability of collections from different trees or from subsequent years.

Seedling efficiency is simply the product of the three efficiency values – seed efficiency, extraction efficiency, and germination percentage. It reflects the total yields of the cone, seed, and seedling crops from the orchard. If any one of the efficiency values is low, the overall efficiency of the orchard is reduced. A maximum realistic goal for production orchards is a seedling efficiency of about 55 percent. Such would be the case if seed efficiency = 70 percent, extraction efficiency = 90 percent, and germination percentage = 90 percent.

Cone analysis does not estimate all seed losses; it only measures seed losses from surviving cones. Cone losses from the time of flower Initiation to cone maturity are not considered. Often 50 percent or more of the seed crop is lost during this period. By combining cone crop life tables with cone analysis, the total seed production for an orchard can be evaluated (Fatzinger 1975; DeBarr and Barber 1975; Bramlett 1972a; Ebel and Yates 1974).

IX. FOR ADDITIONAL INFORMATION

For help with known insect problems or assistance in identifying insect problems, contact:

STATE FOREST ENTOMOLOGIST

Alabama Forestry Commission
5 13 Madison Ave.
Montgomery, AL 36104
205/832-5897

Arkansas Forestry Commission
3821 W. Roosevelt Rd.
Little Rock, AR 72204
501/371-1736

Florida Division of Forestry
Collins Building
Tallahassee, FL 32304
904/488-7936

Georgia Forestry Commission
P.O. Box 819
Macon, GA 3 1202
912/744-3241

Kentucky Division of Forestry
207 Holmes St.
Frankfort, KY 40601
502/564-4496

Louisiana Forestry Commission
P.O. Box 1628
Baton Rouge, LA 70721
504/389-7121

Mississippi Forestry Commission
908 Robert E. Lee Building
Jackson, MS 39201
601/354-7124

N.C. Dep. of Natural Resources
P.O. Box 27687
Raleigh, NC 2761 1
919/733-4141

Oklahoma Forestry Division
Capitol Building
Oklahoma City, OK 73 105
405/521-3886

S.C. State Commission of Forestry
P.O. Box 2 1707
Columbia, SC 29202
803/758-2261

Tennessee Division of Forestry
2611 West End Ave.
Nashville, TN 37203
615/741-3326

Texas Forest Service
P.O. Box 310
Lufkin, TX 75901
713/632-7761

Virginia Division of Forestry
P.O. Box 3758
Charlottesville, VA 22903
804/977-6555

FOREST INSECT AND DISEASE MANAGEMENT ZONE OFFICE

Forest Insect & Disease Management
Seed Orchard and Nursery Insects
P.O. Box 5895
Asheville, NC 28803
704/258-2850 x625

Forest Insect & Disease Management
Seed Orchard and Nursery Insects
2500 Shreveport Highway
Pineville, LA 7 1360
318/445-6511

For questions on the use of pesticides, consult:
Pesticide Specialist
Resource Protection
Suite 706
1720 Peachtree Rd., NW
Atlanta, GA 30309
404/881-3734

For further information on cone analysis or on the service supplied, contact:
Eastern Tree Seed Laboratory
P.O. Box 819
Macon, GA 3 1202
912/744-3311

X. GLOSSARY

Many of the definitions given below are based on those in the 'Glossary for Forest Tree Improvement Workers' (Snyder 1972) and 'Seeds of Woody Plants in the United States" (USDA Forest Service 19741

ABORTED OVULE:

FIRST-YEAR Any potential seed that aborts (ceases development) during the first growing season or conelet stage of pine seed productlon

SECOND-YEAR Any potential seed that aborts (ceases development) during the second growing season before fertilization or seed-coat hardening

ARCHEGONIUM: Female sex organ in the ovule of pine

CATKIN: The male strobilus which produces pollen

CLONE: Group of genetically identical plants produced by vegetatively propagating a single plant over one or more generations; accomplished by grafting, rooting, air-layering, or tissue culture Compare ortet. ramet

CONE: The female reproductive structure of pines, consisting of a central axis supporting bracts, each of which subtends a scale bearing naked seeds

CONE ANALYSIS: A systematic procedure to evaluate the seed productlon potential and efficiency of pine cones

CONE AXIS: Central core of the cone

CONELET: Immature female cone of pines from time of female "flower" scale closure after pollination until the initiation of rapid development of the cone a few months b-fore maturity

COTYLEDONS: First leaves developed in the embryo of a seed They become functional leaves after germination; Immature leaves

FEMALE CONE: See cone

FEMALE FLOWER: The pine female strobllus (cone) prior to pollination

FERTILE SCALE: A cone scale that is capable of producing seed, generally Includes most scales in the upper one-half to two-thirds of the cone

FERTILIZATION: The union of the egg (1 N) within the ovule and the sperm (1N) from the pollen tube to form the embryo (2N)

FILLED SEED (POTENTIALLY SOUND) Mature ovule containing an embryo and nutritive tissue enclosed in layers of protective tissue (seedcoat), capable under suitable conditions of development into a plant similar to the one that produced it

GAMETOPHYTE TISSUE: Haploid material (1N) surrounding the embryo, provides nutritional reserves for the seed of pine

GERMINATION: Sufficient development of plant parts from a seed to indicate a potential for developing into a normal plant

HYPOCOTYL: All of the axis of an embryo or stem of a seedling between the cotyledons and the radicle; an immature stem

INFERTILE SCALE: A pine cone scale Incapable of producing seed, located at the base and apex of the cone

INTEGUMENT: A single layer of tissue enclosing the pine ovule and nucellus After fertilization, it develops into the seedcoat

MICROPYLE: Small opening in the Integument of an ovule through which the pollen grain normally passes to reach the pollen chamber

NUCELLUS: Mass of thin-walled cells that composes the central and main part of the body of an ovule. It is surrounded by the Integument

OVULE: A female structure which contains an egg cell and develops into a seed

POLLEN GRAIN: The winged male fertilizing structure

POLLEN TUBE: An elongate outgrowth of the germinating pollen grain.

POLLINATION: The transfer of pollen to the receptive female flower

POTENTIALLY SOUND SEED: Seed filled with apparently undamaged tissue on which viability has not been tested

RADICLE: Portion of the axis of an embryo from which the root develops. Immature root

RAMET: Independent member of a clone

SEED: "In conifers" the structure formed after fertilization; initially consists of seedcoat, embryo, and gametophyte storage tissue

SEEDCOAT: Protective layer on a seed derived from an Integument

SEED ORCHARD: A plantation consisting of clones or seedlings from selected trees and cultured for early and abundant seed productlon

SEED POTENTIAL: Two times the number of fertile cone scales or the maximum number of seeds a cone is capable of producing

STROBILUS: Male or female fruiting body of pines

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XII. APPENDIX

CONE ANALYSIS DATA CARD CODING AND COLUMN NUMBERS

ITEM	CODE	COLUMN	
Test number	TN	1-4	Identification Input
Year	YR	5-6	
Species	SP	7-8	
Orchard	OR	9-10	
Clone	CL	11-17	
Ramet	RA	18-25	
Cone number	CN	26-27	
Cone opening	co	26	Optional Data
Cone length	LT	29-31	
Lower infertile scales	LI	32-33	
Upper infertile scales	UI	37	
Fertile scales	FS	34-36	
First-year aborted ovules	A I	38-40	
Second-year aborted ovules	A2	41-42	
Extracted seeds	ES	43-45	Developed Seeds
Soedbug-damaged seeds	SB	46-47.62-63	
Seedworm-destroyed seeds	SW	48-49,64-65	
Seed chalcid-destroyed seeds	SC	50-51,66-67	
Fungal-damaged seeds	FN	52-53.68-69	
Malformed seeds	MS	54-55,70-71	
Empty seeds	EM	56-58,72-74	
Filled seeds	FL	59-61,75-77	
Germinated seeds	GN	78-80	

CONE ANALYSIS DATA FORM

	TN	YR	SP	OR	CL	RA	CN	CS	LT (MM)	LI	FS	LU	AI	A2	ES	PROCEDURE A EXTRACTED & DISSECTED							PROCEDURE B-EXTRACTED							PROCEDURE B-DISSECTED							GN
										SCALES			ABORTED OVULES																								
	1-4	5-6	7-8	9-10	11-17	18-25	26-27	28	29-31	32-33	34-36	37	38-39	40-42	43-45	46-49	50-53	54-55	56-58	59-61	62-63	64-67	68-69	70-71	72-74	75-77	78-80										
	IDENTIFICATION INPUT							OPTIONAL DATA								DEVELOPED SEEDS																					

CLASSIFICATION OF OVULES AND SEEDS

Class	Columns on data card: Procedure A	Columns on data card: Procedure B	Identity[1]	Cause	Comment
A. Aborted ovules					
1. First-Year	38-40	38-40	Small undeveloped ovules with normally developed wing (V)	a. Lack of viable pollen b. Seedbug feeding	Requires caged cones to establish cause
2. Second-Year	41-42	41-42	Flattened seed often resinous (V)	a. Seedbug feeding b. Developmental problems	Usually due to seedbug attack
B. Developed seeds					
1. Filled	59-61	69-61 75-77	Full-sized seed with complete seedcoat (V), embryo, and a gametophyte that fills the seedcoat (R)	--	includes mechanically damaged filled seed; does not change classification
2. Partially filled					
Seedbug-damaged	46-47	46-47 62-63	Irregular areas of missing gametophyte tissue (R)	Seedbug feeding	--
Fungal-damaged	52-53	52-63 68-69	Cloudy or hazy semicircular areas at margin of gametophyte tissue, disorganization or shrunken or mummified gametophyte tissue (R)	Fungi	--
Malformed	54-55	54-55 70-71	Missing or damaged embryo or gametophyte tissue does not fill seedcoat (R)	Physiological	Early cone harvest will cause incomplete gametophyte tissue
3. Empty					
Seedworm-destroyed	48-49	48-49 64-66	Seed filled with granular material (R) Entry-exit hole in seedcoat (V)	Seedworm feeding	--
Seed chalcid-destroyed	50-51	50-51 66-67	Larva in seedcoat (R)	Seed chalcid feeding	Limited to eastern white pine seed
Empty	56-58	66-58 72-74	Seedcoat empty or containing a shriveled remnant of tissue (R)	a. Seedbug feeding b. Recessive lethal genes	Requires caged cones to establish cause

[1] The designation "V" indicates identifying character visible to unaided eye. "R" indicates visible only on radiograph.

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