Radiography in Tree Seed Analysis Has New Twist

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The advantages of using soft X-ray technology in testing tree seed for insect (7, 8, 9) and mechanical (2) damage, viability (4), and embryo development (3) are well known. But despite these advantages, its use has been very limited in the United States for a number of reasons.

For one thing, the expense of film has been prohibitive in many cases. In addition, 1) the interpretation of images has required the talents of professional personnel; 2) constant viewing of backlighted films has produced eye problems, 3) the specialized equipment has not always been available. In short, X-ray use was complicated and time consuming (5, 6).

All this has changed. Soft X-ray equipment is now available that presents little more challenge than an ordinary Xerox machine. Instead of expensive X-ray film involving laborious processing and viewing on back-light equipment, the new equipment utilizes a radiographic paper producing and image that can be viewed with reflected light.[2] This paper has an emulsion coating on one surface which can be developed in 10 seconds in an instant processor. The soft X-ray machines (American made) come complete with double interlocks for safety.[3]

2 Faxitron by Field Emission Corp., McMinnville, Oregon and Norelco by Phillips Electronics Instruments, Mt. Vernon, New York.

3 Industrex 600 paper by Eastman Kodak Company, Rochester, New York.

Operation is simple (fig. 1). The instant X-ray paper is placed in a light-proof holder, emulsion side up, or a ready-pack can be used. Seed are placed on top of the holder, and the packet is given a radiation exposure. The exposed paper then goes into the instant processor, emulsion side down. The entire process takes less than 5 minutes.

However, as simple as the procedure may be, precautions must be taken to avoid ruining a good radiograph. Some of the more common mistakes are shown in Figure 2.

One must also remember that this process is not permanent. The paper needs to be chemically "fixed" if a permanent record is desired. However, experience at the Eastern Tree Seed Laboratory has shown that the paper fades very little in a year's time if placed in a dark envelope or protected by some other means from constant exposure to visible light.

The radiation dosage depends on thickness of the material to be x-rayed, variation in material density, moisture content, and details desired. Detailed radiographs of tree seed appear best with exposures between 10KV and 18KV, although higher kilovoltages can be used. The work illustrated here was done at a fixed exposure of 5ma and a distance of 40cm from the tube to the material. The dosage given varied by species and ranged from 0.5 - 2.0 roentgen.

X-ray evaluation of percentage of empty seed is presently made for every lot tested at the Eastern Tree Seed Laboratory. Upon request, these radiographs are color-coded with china markers and sent with a letter of explanation to the customer at cost. Detail on the paper, when used at low KV without screens, appears to be as good as that of Kodak type M film. Small size does not appear to be a problem as shown by the radiograph of Portulaca seed which has more than 3 million seed per pound (fig. 3). Other features that can be detected include mechanical damage, abnormally developed internal seed structures, thickness of seed coat, and seed viability when combined with a staining technique (fig. 4) or a contrast agent (1). X-ray is also helpful to evaluate insect damage and the morphology of internal seed structures (figs. 5a & b).

Unlike with X-ray films, photographs for reproduction can be made directly from the paper radiograph. Complicated photo equipment is unnecessary; pictures can be made with a polaroid camera as was done with several in this article.

1 Tree seed specialist at the Eastern Tree Seed Laboratory, Macon Ga. Laboratory is operated under cooperative agreement with Georgia Forestry Commission, Georgia Forest Research Council, Southern Forest Experiment Station, and Southeastern Area, State and Private Forestry, USDA Forest Service.

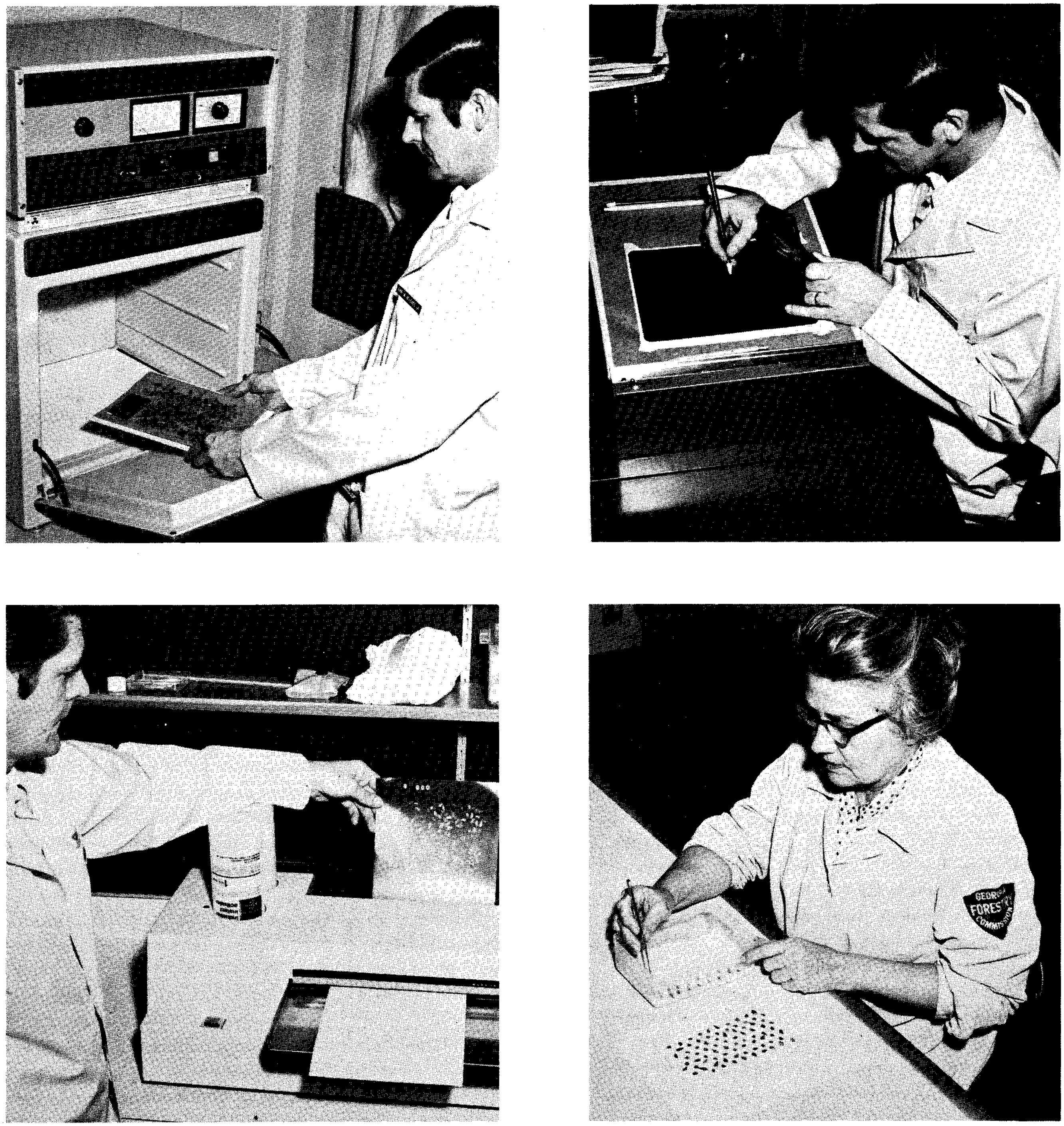

Figure 1.—Technique for making instant X-ray prints: *Upper left:* Author places X-ray paper with tree seeds into soft X-ray machine. X-ray paper is enclosed by a thin, light-proof paper envelope; *lower left:* The paper is processed in 10 seconds when placed in a Kodak instant processor; *upper right:* Radiographs are evaluated and scored with china markers. Although a light table is not necessary, it may help increase visual detail for close scrutiny; *lower right:* By using seed holders, you can transfer seed to germination dishes after X-raying. This permits direct comparison between germination and the radiographic image of the internal structure of the seed.

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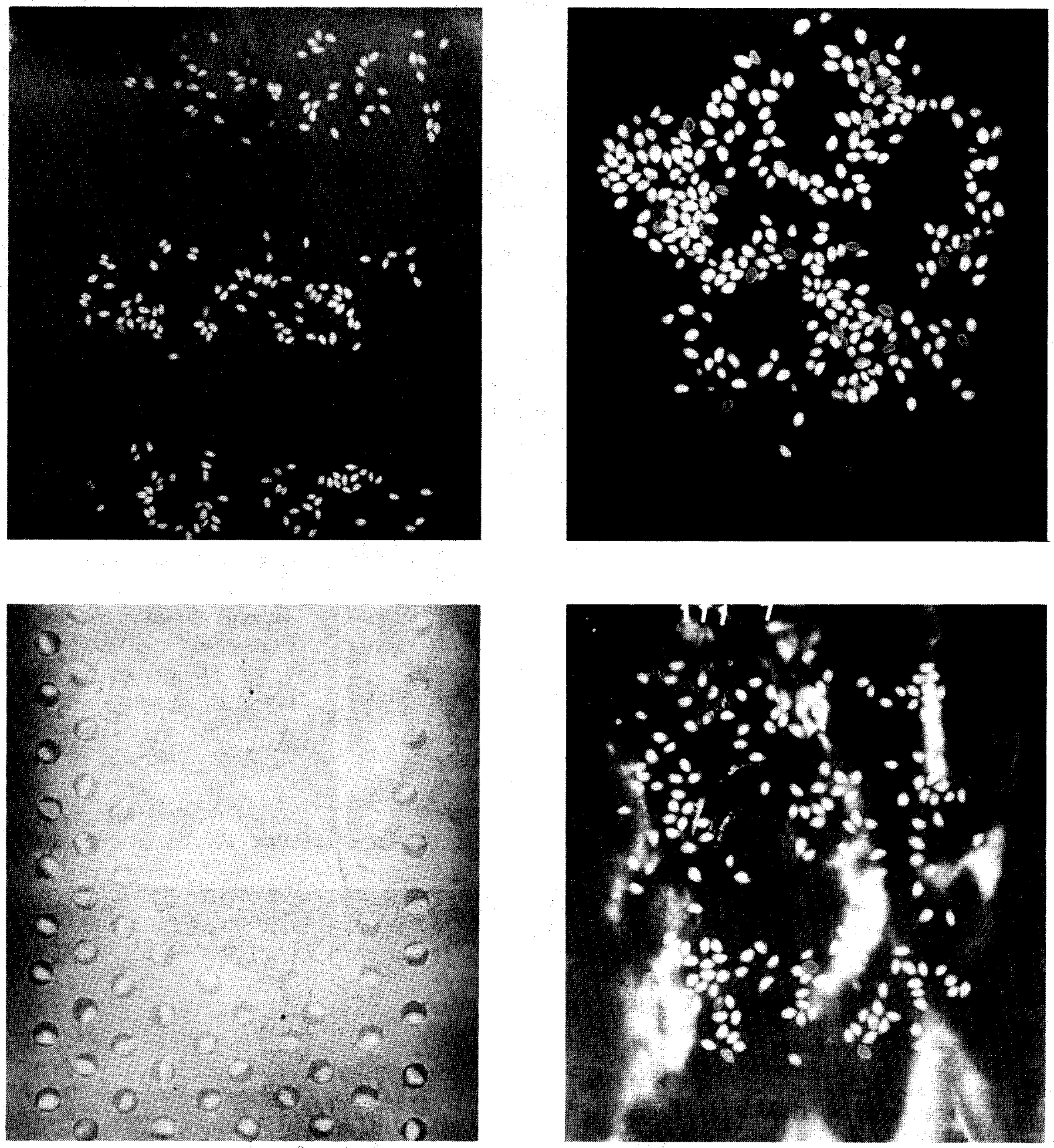

Figure 2.—Possible mistakes to be aware of: *Upper left:* radiograph which was unknowingly exposed to light during the final stages of loading the packet; *upper right:* radiograph which had been exposed to radiation with seed on the non-emulsion surface. (Notice the reverse image): *lower left:* radiograph developed with emulsion side up in processor. The only development is that made by chemicals existing on the rollers; *lower right:* poor radiograph caused by processing in old chemicals.

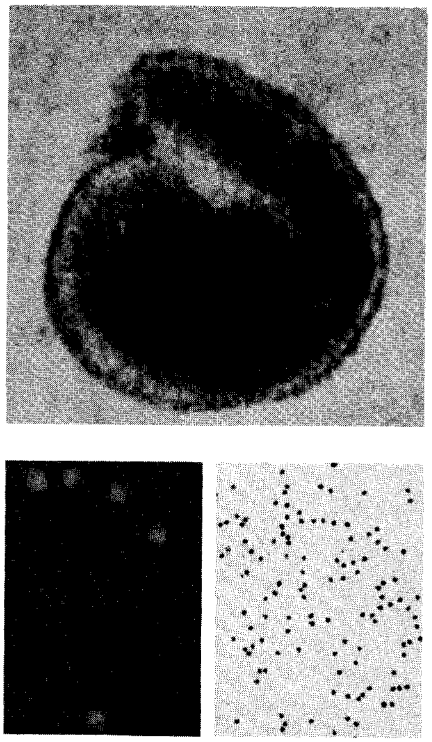

Figure 3—(LEFT) Portulaca seed. *Left:* X-ray 52X made from Kodak Type M film: *lower left:* X-ray on instamatic 600 paper 4X: *lower right:* photograph at a actual size of same seed. This species averages more than 3 million seed per pound.

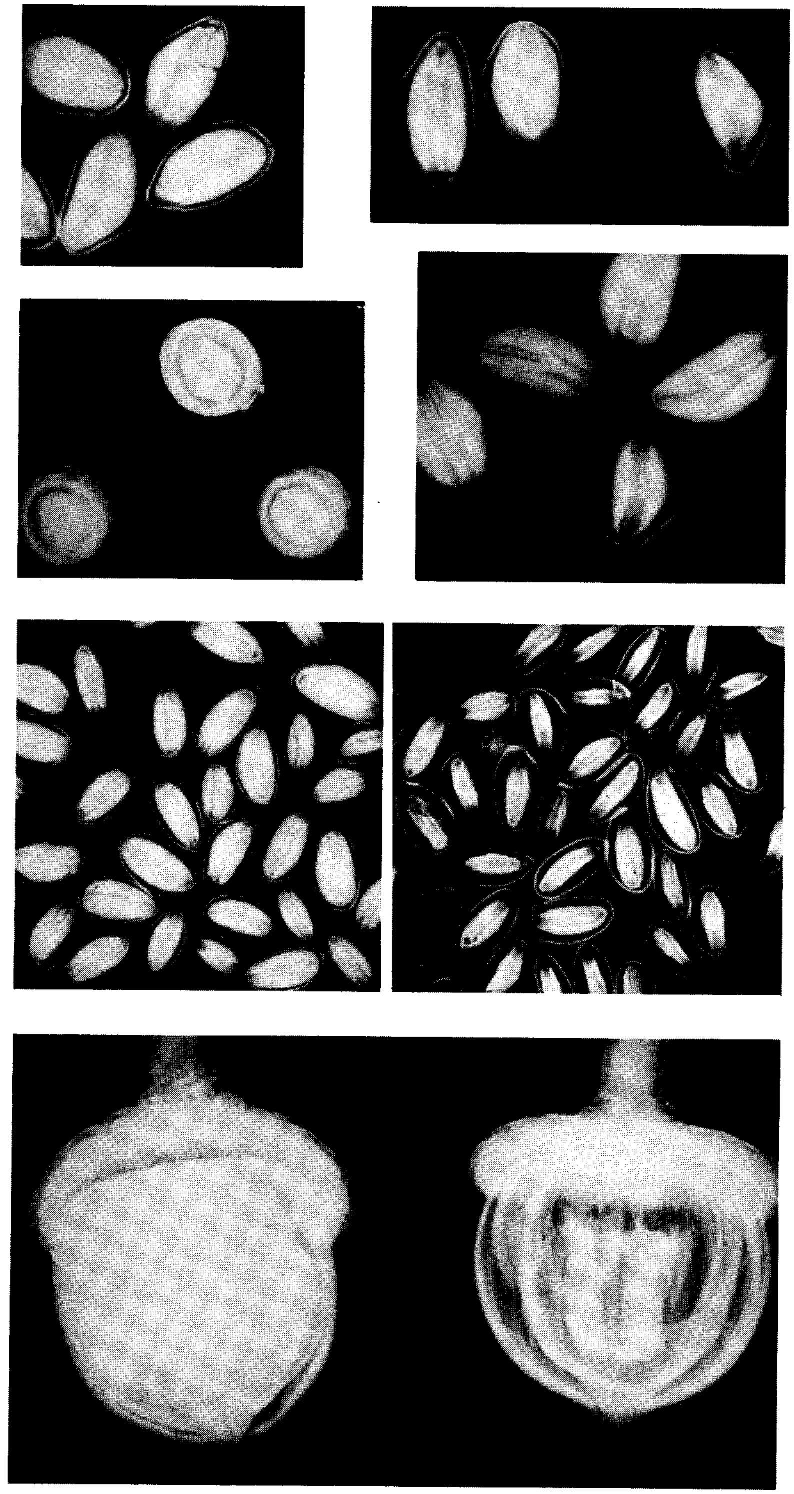

Figure 4.—(RIGHT) Tree seed evaluation (all X-rays in this figure were made at 5ma with a tube to film distance of 40 cm. They were all made on Kodak's Instant 600 Paper and increased to 2X with Polapan 52 Polaroid film in a P3 camera system). *Top left:* Mechanically damaged longleaf pine *(Pinus palustris)* seed made at 12½ Kv, 45 sec. *Top right:* Variations in internal anatomy of long-leaf pine seed are shown. From left to right, this radiograph shows a double embryo, a normal seed, and a seed with no embryo. *Second row left:* Black cherry *(Prunus serotina)* seed made at 12½ Kv, 90 sec. Compare the thickness of the seed coat of this seed with that of the longleaf above. *Second row right:* More longleaf pine seed variations. Dark seed are empty while the seed between the two dark ones had only a minute embryo in the embryo cavity. The seed at the top of this radiograph are normal in appearance. *Third row left:* Normal eastern white pine *(Pinus strobus)* made at 12½ Kv, 45 sec. This seed germinated about 95 percent. *Third row right:* Abnormal internal structure in eastern white pine seed. This lot germinated only 12 percent. *Bottom:* Northern red oak *(Quercus rubra)* acorn made at 12½ Kv, 90 sec. A full seed on the left and an empty seed on the right. Note the detail in the acorn's cap.

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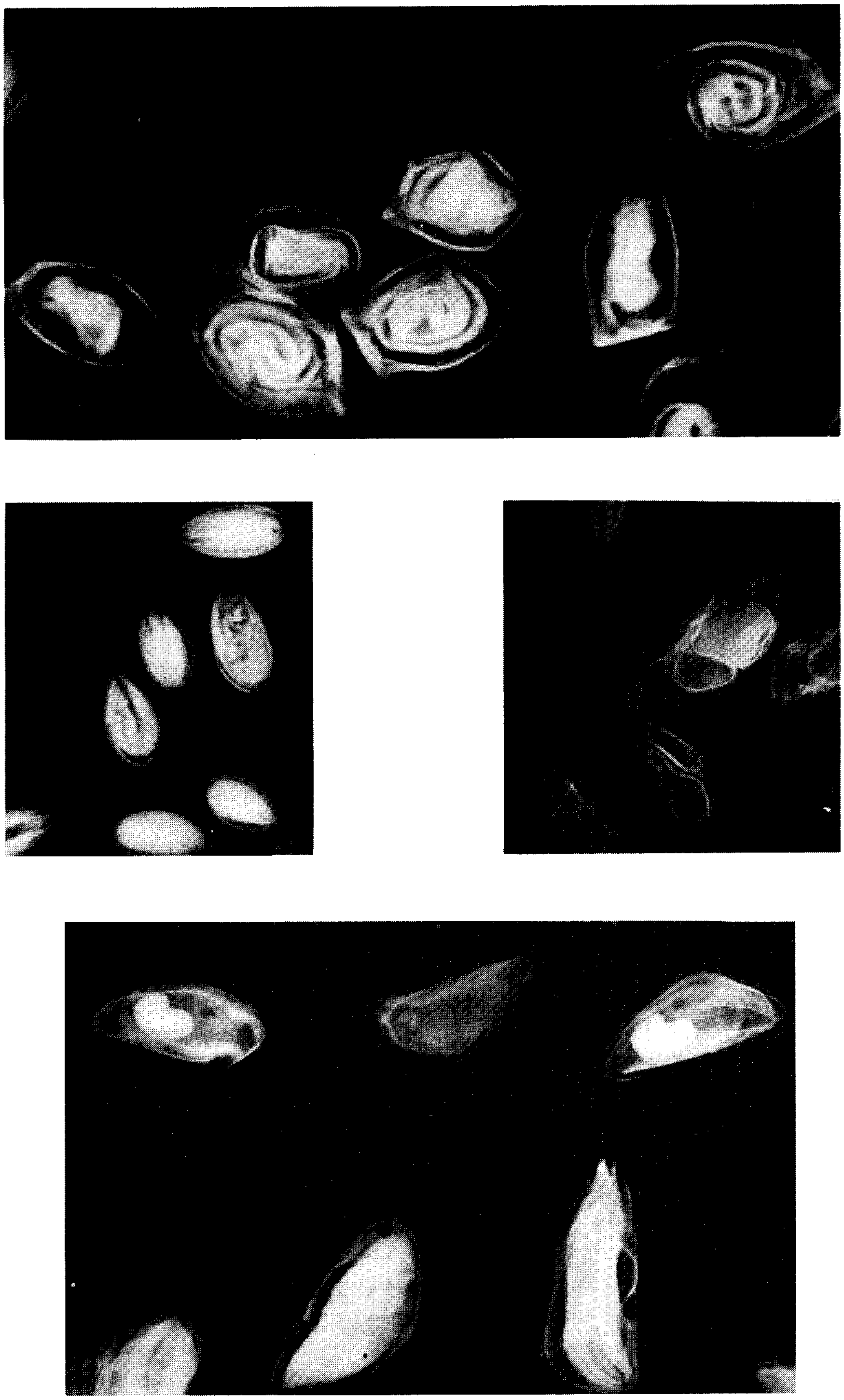
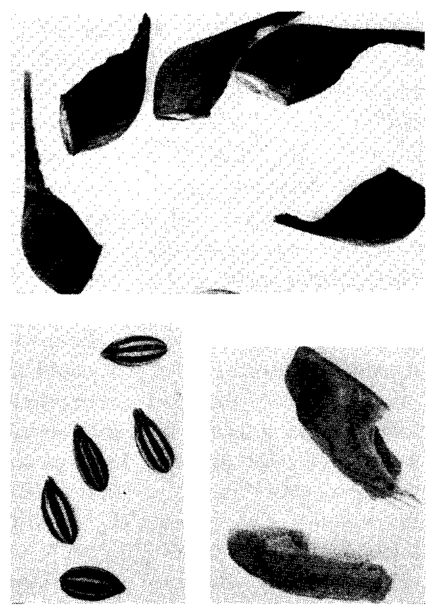


Figure—5,b. The internal picture tells much: *Top:* Sugar maple embryo curls within the seed coat. This is a characteristic of maples. (12½ Kv, 50 sec.) *Middle left:* Autumn olive has a leathery seed coat which is pliable when moist but becomes hard when dry. Radiograph shows two damaged embryos caused by pressure while the seed were wet. (12½ Kv, 60 sec.) *Middle right:* The yellow poplar is really a fruit called a samara which contains two seed. All seed are empty except one. This is the white area in the center of the radiograph. (12½ Kv, 60 sec.) *Bottom:* Seed of white fir *(Abies concolor)*. (12½ Kv, 50 sec.) Seed in the top two corners of this radiograph contain insect larvae. The seed between them is empty due to insect feeding.



Figure—5,a. External appearance indicates nothing of the internal structure: *Top*: Sugar maple *(Acer saccharum)*; *Botton left*: Autumn olive *(Elaeagnus umbellata) bottom right:* Yellow poplar *(Lirodendton tulipifera).*