TECHNIQUE AND INTERPRETATION IN TREE SEED RADIOGRAPHY

by Howard B. Kriebel1

The study of internal seed structure by radiography requires techniques which will give good definition. To establish the best procedures, we conducted a series of experiments in which we manipulated the principal controllable variables affecting the quality of X-radiographs: namely, focus-to-film distance, film speed (grain), exposure time, kilovoltage, and milliamperage. The results presented here will eliminate the need for repetition by others interested in moving directly to research applications of X-radiography. A few examples will illustrate the interpretation of detail and perhaps suggest how radiographs can contribute to an understanding of the biology of seed formation.

The physics of X-rays and fundamental principles of radiography are clearly described in illustrated publications by Eastman Kodak (1957, 1959, 1960) and General Electric (1957, 1963). Pictures obtained by this diagnostic method are, strictly speaking, radiographs and not "X-ray photographs", because light is not the type of radiant energy involved except in certain special methods.

This exposure to radiation is for diagnostic purposes only. It will usually be applied at a very low dosage level, with so-called "soft" X-rays, rather than the "hard" X-rays used for inducing changes in somatic or reproductive cells. The X-ray machine used should be designed for radiography and for operation at low kilovoltages.

Studies of radiation sensitivity of tree seeds, especially of pines, have given some indication of minimum dosage levels of X-rays which will affect germinability or induce somatic changes in the plant. These studies have included investigation of certain seed properties and conditions influencing radiosensitivity (Baldwin 1936; Simak and Gustafsson 1953; Gustafsson and Simak 1958; Suszka *et al.* 1960; Simak *et al.* 1961; Ohba and Simak 1961; Ohba 1961; Snyder *et al.* 1961; Yim 1963). Results obtained with various conditions of moisture content, seed ripeness, post-treatment storage time, and other factors indicate that in pines the 100 to 600r range is the lowest at which any depressing effect on germination rate or time can be observed. On the basis of present knowledge, the 1 to 3r dosage required for most coniferous seed radiography appears to present a very low risk of tissue damage or induced mutations.

Various aspects of the application of X-ray analysis to tree seed testing have been discussed in the literature. The technique is useful for evaluating seed maturity and quality, insect infestation, and mechanical damage. In certain species the technique has been refined for viability testing by the use of organic and inorganic seed-impregnating contrast agents. These applications have been discussed by various workers, including Muller-Olsen and Simak 1954; Simak 1955, 1957; Muller-Olsen *et al.* 1956; Gustafsson and Simak 1956; Rohmeder 1957; Hagner and Simak 1958; Nekrasov and Smirnova 1961; Swaminathan and Kamra 1961; Simak and Kamra 1963; Kamra 1963a, 1963b, 1964a, 1964b; Hansen and Muelder 1963. Results of a study by Klaehn and Wheeler (1961) indicated that examination of remnants of the embryo and the collapsed female gametophyte in so-called "empty" seed may provide information on the degree of incompatibility in species crosses and selfings.

Equipment

X-ray units designed specifically for seed radiography, in particular for checking stored grain for insect infestation, were formerly available, but their production has been discontinued. The two principal types of machines now available for radiography are the medical and industrial units. Medical types are mainly designed for very rapid exposures with high current and are generally not as suitable for tree seed radiography as are some of the industrial units.

The type used in these studies was a General Electric LC-90 Industrial X-Ray Unit with a CSI-25 tube. This unit has a range of 0-40 kvp, 1-5 ma, has a nominal focal spot size of 2 mm. and 0.030 beryllium filtration. We have enclosed it in a lead housing large enough to permit a maximum focus-film distance of 28 inches.

Films

Table 1 lists the characteristics of some X-ray films suitable for tree seed radiography. It is not intended to be a complete list of all brands or types available. Data are from publications of Eastman Kodak Company and Picker X-Ray Corporation. Medium and coarser grain fast films are also available, but these types are less suitable for seed radiography.

Experimental Procedure and Results

Experiments with radiographic technique ineluded the following variables:

[1]Professor of Forest Genetics and Acting Chairman, Department of Forestry, Ohio Agricultural Research and Development Center, Wooster, Ohio.

Table 1.--Grain, contrast, and speed of some x-ray films suitable for seed radiography

GROUP	GRAIN	CONTRAST	RELATIVE SPEED 1/	BRAND TYPE
I	ULTRAFINE	VERY HIGH	VERY SLOW (10)	KODAK R (SINGLE-COATED)
II	ULTRAFINE	HIGH	SLOW (20)	KODAK R ANSCO SUPERAY HD
III	VERY FINE	HIGH	SLOW (40)	KODAK M ANSCO SUPERAY B DUPONT 510 ILFORD F
IV	FINE	FAIRLY HIGH	MEDIUM (150)	KODAK AA ANSCO SUPERAY A DUPONT 506 ILFORD B AND C

1/ NUMBERS ARE FOR KODAK FILMS, AS RATED BY EASTMAN KODAK FOR ALUMINUM AND OTHER LIGHT ALLOYS, NO SCREENS, 80 KV.

Type of film (5 types, groups II, III, IV)
Kilovoltage (10, 12½, 15, 17½, 20, 25, 30)
Milliamperage (1, 3, 5)
Focus-film distance (5½", 9", 12½", 19", 28")
Time (15 to 360 seconds)

It was not necessary to include every possible combination of factors. It soon became evident, for instance, that the higher range of kilovoltage could only be used at the longest distance, with an exposure of very short duration. The number of practical treatment combinations for any particular type of film is therefore rather limited.

Under constant conditions of kilovoltage, milliamperage, and target-film distance, image intensity varies in direct linear proportion to exposure time. This relationship can be measured by recording dosage in roentgens per unit of time. Figures 1 and 2 illustrate results obtained at 3 ma using distances of 19.5 and 28 inches, and kilovolt peaks of 15, 17.5, and 20. Repetitive readings were taken with a Victoreen Model 570 Condenser R-Meter with an interchangeable ionization chamber.

Radiation intensity varies inversely with the square of the focus-film distance, if other factors are constant. Actual dosage curves obtained with

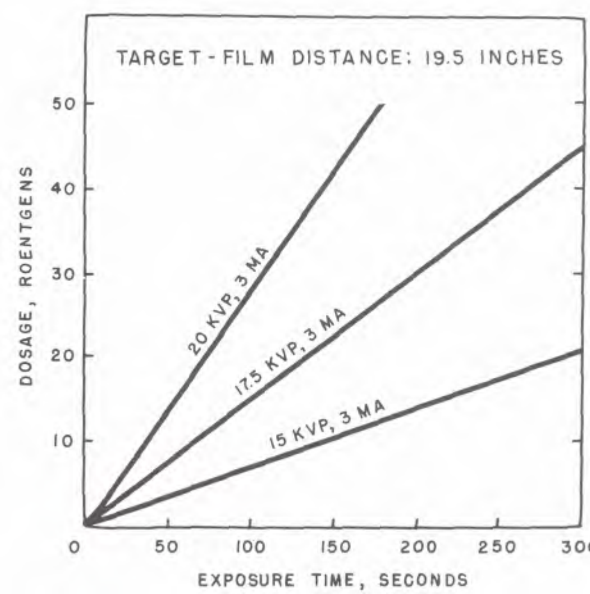


FIGURE 1. — The relationship of radiation intensity at the film plane to exposure time and kvp, at 3 ma and 19.5 inches target-film distance (GE CSI-25 tube, 2 mm. focal spot, 0.030 beryllium filtration).

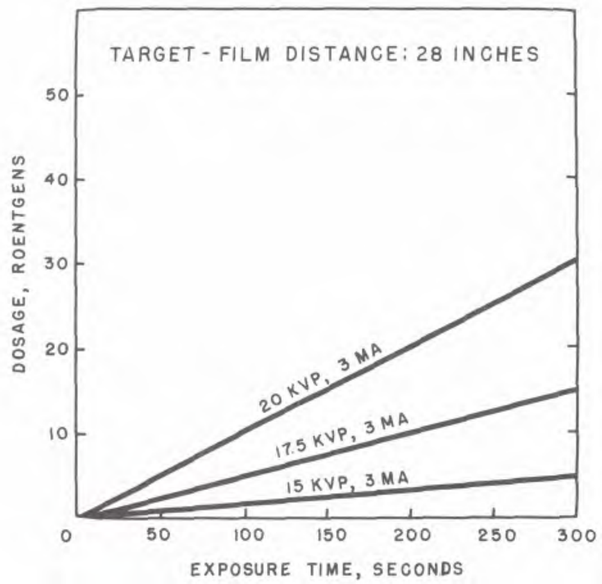


FIGURE 2. — The relationship of radiation intensity at the film plane to exposure time and kvp, at 3 ma and 28 inches target-film distance (GE CSI-25 tube, 2 mm. focal spot, 0.030 beryllium filtration).

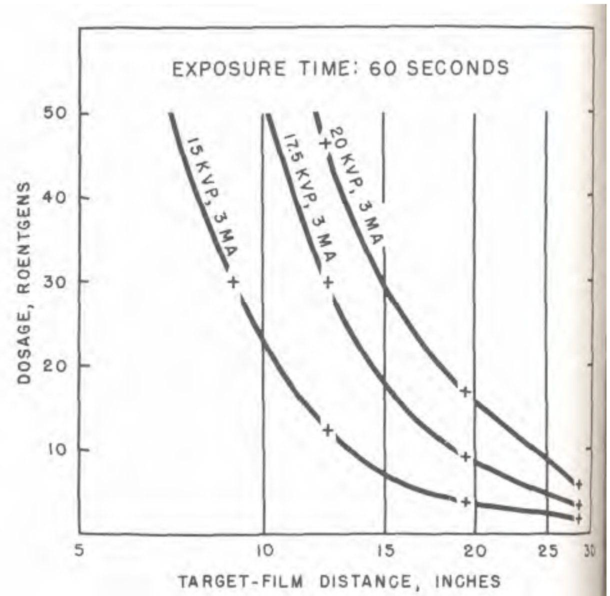


FIGURE 3. — The relationship of radiation intensity at the film plane to target film distance and kvp, at 3 ma with a 60-second exposure (GE CSI-25 tube, 2 mm. focal spot, 0.030 beryllium filtration).

the meter are shown in figures 3 and 4, for combinations of 3 and 5 ma with 15, 17.5 and 20 kvp for a 60-second exposure.

Theoretically, the milliamperage required for a given exposure is inversely proportional to the exposure time. This means that the product of milliamperage and time always remains constant for a given radiographic result, if no other factors are changed (Eastman Kodak Co. 1960). Under this rule an increase in milliamperage should increase exposure, other factors being equal. The rule holds true at the high kilovoltages normally used in industrial and medical radiography. However, when the kilovoltage is less than 22 or 23, there is a reversal, at least with the General Electric LC-90, CSI-25 unit. A greater intensity of radiation is obtained at 15 or 20 kvp with 3 ma than with 5 ma. This relationship can be seen in figures 3 and 4. Dosages are higher in each curve at any distance at 3 ma than at 5 ma, although as the kilovoltage increases, the difference decreases.

There is noticeably less contrast in images made at 20 kvp than in those made at 15 kvp. High contrast seems to give the best definition for seed radiography, and for this reason and in order to minimize dosage, 15 kvp appears to be about the optimum level, at around 3 ma. Also for reasons of definition and dosage level, we consider our maximum obtainable distance of 28 inches to be most satisfactory for coniferous seed.

We have, therefore, selected 15 kvp, 3 ma, and 28 inches as constants for most of our pine seed radiography. The level of the other variable, time,

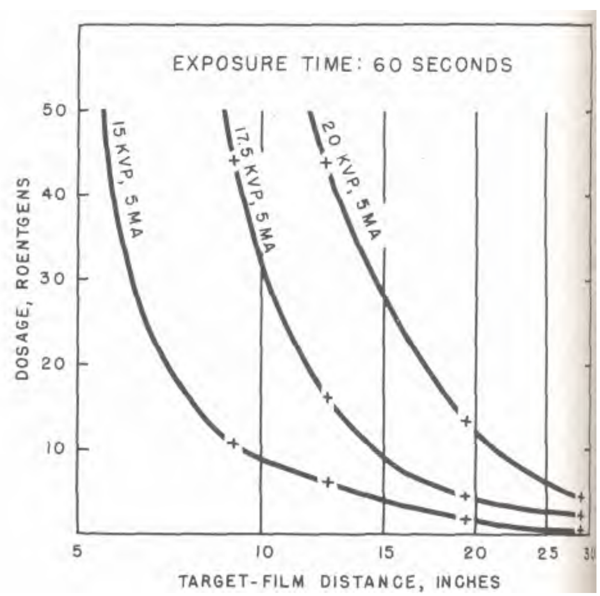


FIGURE 4. — The relationship of radiation intensity at the film plane to target-film distance and kvp, at 5 ma with a 60-second exposure (GE CSI-25 tube, 2 mm. focal spot, 0.030 beryllium filtration).

is determined by the type of film used. Optimum exposure times for films tested under these conditions are presented in figure 5. These values are based on inspection of several hundred radiographs by 2 observers at 2½ magnifications.

Very little gain in definition of detail is obtained by using a "very fine" or "ultrafine" grain film

FIGURE 5. — Optimum exposure times for high definition in white pine seed radiographs, with dosage levels, using various films at 15 kvp, 3 ma and 28 inches focus-film distance (GE CSI-25 tube, 2 mm. focal spot, 0.030 beryllium filtration).

in place of a "fine" grain film (table 1). Sharpness of image detail is less dependent on film grain than it is upon the distance between subject and film plane. If the seeds are placed directly on top of the shielded film, nothing can be done to reduce this distance. With large seeds, the undesirable effect of greater subject-to-film distance is offset by the large size of the seed image. Film grain is a relatively unimportant consideration under these conditions. Radiographs of very small seeds, on the other hand, may require inspection at 5 to 10 or more magnifications. In this case there may be an advantage in the use of the finer grain films, with longer exposure time and higher radiation dosage. Of course, if the seed is not to be carried through germination, dosage level is of no importance.

Examples of Interpretation of Seed Radiographs

Figure 6A is typical of detail obtainable in radiographs of white pine seeds under conditions just described. This is seed from an intraspecific cross. All filled seeds are normal and fully developed.

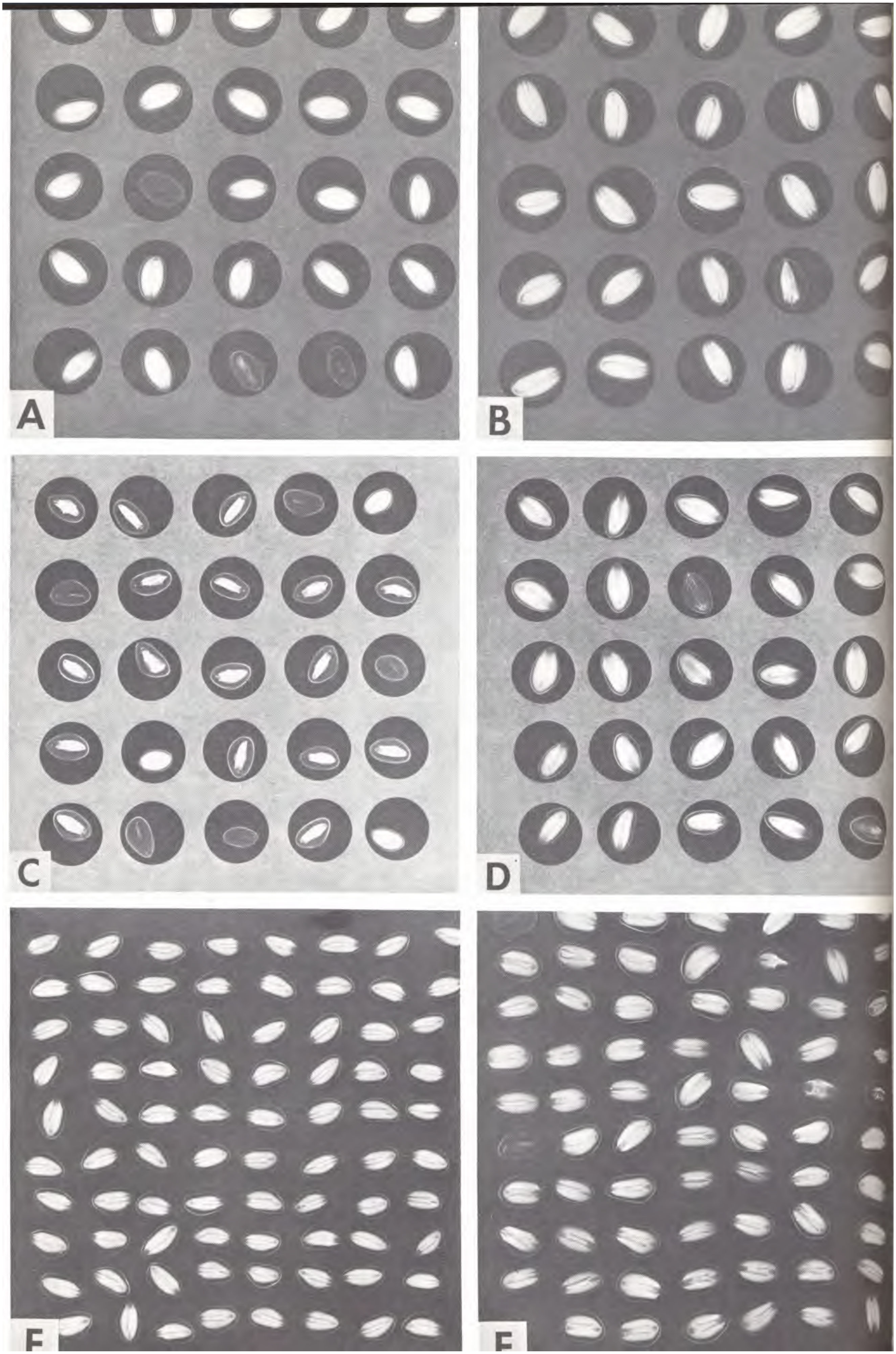
In our crossing program, sound seed fromself -ing of *Pinus strobus* look just the same as the seed in figure 6A. The entire seed lot, of course, has a much higher proportion of empty seed than would be obtained from outcrossing, but defective or poorly developed seeds have seldom been found after selling white pine. The filled seeds have the same high germination capacity as cross-pollinated seeds, they germinate promptly, and exhibit slight inbreeding depression in the seedling stage. Apparently the incompatibility reaction is lethal at an early stage. These results could be quite different from those obtained in other breeding programs and could be expected to vary with the species or species group and the degree of inbreeding already present in the selfed tree.

The quality of white pine hybrid seed, as judged from radiographs, has frequently been better than the quality of seed from crosses within the species *Pinus strobus*. *As* in P. *strobus* sells, in hybrids we commonly find an "all or none" response: well-developed seed, or empty seed. Figure 6B is an example. This is filled seed of a cross of P. *strobus x monticola.* All embryos are well-developed, although one seed has an imperfect endosperm. Germinative capacity was very high in this case, although in some hybrid seed lots germinative capacity is low in seed that looks normal and well-developed.

Figure 6C illustrates an unusual condition in an intraspecific *Pinus strobus* cross. The embryos were developing normally, but in most seeds the endosperm degenerated. In other seed lots from the same mother tree but different fathers, this condition did not exist. However most of these other seed lots did have some seed with incompletely developed endosperm. One cross with this mother tree produced very small seed.

Seed from trees growing in a severe climate, as found at high altitude near timberline or at extreme northern limits of the species range, may be immature at the end of the growing season. The seed of *Pinus aristata* shown in figure 6D is an illustration. Not a single embryo is fully developed, and nearly all seeds have multiple embryos. Yet the seeds germinated rapidly and had a high degree of germinability after 90 days of stratification. Evidently embryo development continued during stratification. Simak and Gustaffson (1959) reported similar results from stratification of immature seed of *Pinus sylvestris.*

As a last example, we can compare two 10-year-old seed samples of *Pinus lambertiana* from two different trees, each of a different provenance. The seed in figure 6E was collected from a tree at an elevation of 6,000 feet in Eldorado County, Calif., and the seed in figure 6F from a tree at an unknown elevation in the Sequoia National Forest.


A
B
C
D

The seeds in figure 6F were significantly heavier than the seeds in figure 6E, although the radiograph shows that many of them are polyembryonic. Some have grayish embryos which are probably necrotic. The radiographs suggest that it might be advisable to X-ray seed samples from mountainous regions before including them in provenance tests. Comparisons of trees grown from two such dissimilar .seed lots may be justified, but at least it would be desirable to know that such an internal condition exists.

Literature Cited

Baldwin, H. I. 1936. X-ray treatment of tree seeds. J. Forest. 34: 1069-1070.

Eastman Kodak Co., X-Ray Division. 1957. Radiography in modern industry. 136 pp., Rochester, N.Y.

_______.1959. Radiography in modern industry. Suppl. 2. 24 pp. Rochester, N. Y.

Eastman Kodak Co., Medical Division. 1960. The fundamentals of radiography. 76 pp. Rochester, N.Y.

General Electric Co., X-Ray Dept. 1957. X-ray tech-niquiz. 31 pp. Milwaukee 1, Wis.

_______. 1963. The story of X-ray. 62 pp. Milwaukee, Wis.

Gustafsson, Ake and Milan Simak. 1956. X-ray diagnostics and seed quality in forestry. 12th Congr., I.U.F.R.O., Oxford., 15 pp

______and _______. 1958. Effect of X- and ν-rays on conifer seed. Medd. Fr. Statens Skogsforskningsinst. 48 (5) : 1-20.

Hagner, S. and M. Simak. 1958. Stratifierings-forsok coed fro av *Pinus cembra*. (Stratification experiments with seed of *P. cembra*). Norrlands Skogsv-Forb. Tidskr. 2: 227-275.

Hansen, J. H. and D. W. Muelder. 1963. Testing of redwood seed for silvicultural research by X-ray photography. Forest Sci. 9(4) : 470-475.

Kamra, S. K. 1963a. Determination of mechanical damage on Scots pine seed with X-ray contrast method. Stud. Forest. Suecica No. 8, 20 pp., Royal Coll. Forest., Stockholm.

_________.1963b. Studies on a suitable contrast agent for the X-ray radiography on Norway spruce seed. Int. Seed Test. Assoc. Proc. 28 (2) : 197-201.

_______. 1964a. The use of X-rays in seed testing. Int. Seed Test. Assoc. Proc. 29 (1) : 71-79.

______1964b. Determination of seed quality by X-rays. Advancing Frontiers Plant Sci. 9: 119-130.

Klaehn, F. U. and W. P. Wheeler. 1961. X-ray study of artificial crosses in *Picea abies* (L.) Karst. and *Picea glauca* (Moench) Voss. Silvae Genet. 10: 71-77.

Mueller-Olsen, Carl and Milan Simak. 1954. X-ray photography employed in germination analysis of Scots pine *(Pines silvestris L.)* Medd. fr. Statens Skogsforskningsinst. 44 (6) : 1-19.

_____, _____, and Ake Gustafsson. 1956. Germination analyses by the X-ray method: *Picea abies* (L.) Karst. Medd. fr. Statens Skogsforskningsinst.

Nekrasov, V. I. and N. G. Smirnova. 1961. Use of the X-ray method for studying the development of the seeds of introduced trees. (Rus) Moscow Glav. Bot. Sad. B, 43: 47-52. Bibliogr. Agr. 26(8) : No. 61930, 1962.

Ohba, K. 1961. Radiation sensitivity of pine *(Pines densiflora* *)* seeds of different water content. Hereditas 47(2) : 283-294.

_____, and M. Simak. 1961. Effect of X-rays on seeds of Scots pine from different provenances *(Pisses .silvestris L.)*. Silvae Genet. 10: 84-90.

Rohmeder, E. 1957. Die Rontgenfotografie im Dienst der forstlichen Saatgutbeurteilung. Allg. Forstz. 12(8/9) : 103-110.

Simak, Milan. 1955. Bestamning av insektskador pa granfro medelst rontgenfotografering. (Insect damages on seeds of Norway spruce determined by X-ray photography.) Norrlands Skogsvardsforbunds Tidskr. 3: 299-310.

_____. 1957. Grobarhetstestning av talifro med rontgenkontrastmetod. (The X-ray contrast method for seed testing.) Medd. fr. Statens Skogsforskningsinst. 47(4) : 3-21.

____ and Ake Gustafsson. 1953. X-ray photography and sensitivity in forest tree species. Hereditas 39: 458-468.

____ and Ake Gustafsson. 1959. Rontgenanalys och det norrlandska tallfroets kvalitetsforbattring. Svenska Skogsvardsforeningens Tidskr. 3: 475-486.

_____ and S. K. Kamra. 1963. Comparative studies on Scots pine seed germinability with tetrazolium and X-ray contrast methods, Int. Seed Test. Assoc. Proc. 28(1) : 3-18.

_____, K. Ohba and B. Suszka. 1961. Effect of X-irradiation on seeds of different weight from individual trees of Scots pine *(Pinus sylvestris* *)*. Bet. Notiser 114(3) : 300-212.

Snyder, E. G., H. C. Grigsby and J. U. Hidalgo. 1961. X-radiation of southern pine seed at various moisture contents. Silvae Genet. 10: 125-131.

Suszka, B., K. Ohba and M. Simak. 1960. Uber das Wachstum von Kiefernsamlingen aus rontgenbestrahltem Samen. (The growth of pine seedlings from seed irradiated with X-rays.) Medd. fr. Statens Skogsforskningsinst. 49(9): 3-18.

Swaminathan, M. S. and S. K. Kamra. 1961. X-ray analysis of the anatomy and viability of seeds of some economic plants. Indian. J. Genetics & Plant Breeding 21(20) : 129-135.

Yim, Kyong Bin. 1963. Sensitivity of pine seed to neutron, gamma and X-ray irradiation. FAO/FORGEN-63, Vol. I, 1/6: 1-9.

FIGURE 6. - Examples of pine seed radiographs. A, P. strobus x strobus (1513, 687 x 1281) ; B, *strobus x monticola* (142(6, 1278 x 625) ; *C, P. strobus x strobus* (1509, 685 x 1281) ; *D, P. aristata* (1550, Hoosier Pass, Colo.) ; *E, P. lambertiana* (1455, Eldorado Co., Calif.) ; *F, P. lambertiana* 1456, Sequoia N. F., Calif.).