3. Mr. Eden was also asked to give other advantages of this procedure. He answered that it shows up the obviously bad seeds such as those destroyed by insects and disease; you can see which seeds had actually deteriorated during germination and it is a good potential for determining what percentage of your seed is bad. It also shows up cracks, and you can run controls on various equipment to see if it is damaging the seed.

USE OF X-RAY TECHNIQUE FOR DETERMINING SOUND SEED

by C. J. Eden, Forester California Department of Conservation

The amount of work the California Division of Forestry has been doing on radiograph evaluation is rather limited. Actually we have only just started getting our feet wet. We plan on going in a little deeper, but it will have to be a gradual involvement because of regular work commitments. Trying to outguess the radiographs has proven to be quite challenging and it is rather tempting to spend more time than can be spared to this interesting work.

'A small germination laboratory is in use at the Davis Headquarters Forest Nursery. Germination, purity, and seed per pound tests are run here for all seed sown by the Division in its four nurseries. The operation of the laboratory is one of the responsibilities of the Forester I assigned to the Davis Nursery. Work on radiograph evaluation has been done primarily by this individual.

Our first contact with the use of radiographs was through Dr. R. W. Stark, Associate Professor of Entomology, Department of Entomology and Parasitology, University of California, Berkeley, California. In a cooperative study between the University of California and the California Division of Forestry, Dr. Stark began to work on "bad seed" detection methods based on European use of X-rays in seed examination. Dr. Stark's work brought to our attention the studies of Simak 1/ and others in the use of radiograph evaluations for determining viable seed. In the spring of 1962 we worked with fresh seed and felt good correlation was obtained between evaluations and germination results obtained with a germination chamber. Evaluations and germination tests agreed 74 times out of a total of **97** seeds. (Table 1)

In the spring of 1963 some work was done with stored seed and even with the use of Ba Cl2 as an impregnating chemical we were not satisfied with our results. Simak has pointed out ".. Ba Ql2 is no universal agent for an X-ray contrast analysis. Seed of different species react differently to the same chemical substance." With no treatment, agreement between evaluations and germination tests was obtained only 54 times out of a total of 98 seeds. The use of Ba Cl2 increased agreement to 183 times out of a total of 297 seeds. (Table 2)

1/ Simak, Milan,?, The x-ray contrast method for seed testing. Medd. fr. Stat. skogforskn. - inst. Bd 47:4.

Table 1. Numbers of times, by species, radiograph evaluation for germination agreed with results obtained in a germination chamber; fresh seed.

| Species | Lot No. | No. of times agreed | No. of evaluations |
|--|---------|--|----------------------------------|
| Pinus ponderosa 248 Pseudotsuga menziessii 1660 Abies concolor 268 Pinus radiata 1784 | | 19 18 34 <u>3</u> Total 74 | 25 24 42 <u>6</u> 97 |

Table 2. Numbers of times, by method of treatment and species, radiograph evaluation agreed with results in a germination chamber; stored seed.

| Species | Lot No. | Type of treatment | No | of times agreed | No. of evaluations |
|---------------------------|---------|-----------------------------|-------|-----------------|-----------------------|
| Pinus ponderosa | 1812 | None 10% BaClo. | | 28 | 48 |
| | | one hour soak 10% BaCl, | | 34 | 50 |
| | | two hour soak 20% BaCl2, | | 34 | 50 |
| | | two hour soak | | 31 | 50 |
| Pseudotsuga menziessii | 1660 | None 10% baCl2 | | 26 | 50 |
| | | one hour soak 10% BaCl2 | | 25 | 49 |
| | | two hour soak 20% BaCl2 | | 30 | 49 |
| | | two hour soak | | 29 | 49 |
| | | | Total | 237 | 395 |

Our work, being of an exploratory nature, has been entirely of individual seed evaluation on radiographs, with later individual testing; then comparing our evaluations with the actual germination result. Work has been done on ponderosa pine, Pinus ponderosa; Douglas fir, Pseudotsuga menziessii; white fir, Abies concolor; Monterey pine, Pinus raddata; and Sierra redwood. Sequoia gigantea. Sierra redwood seed is extremely difficult to work with because of its small size. White fir seed gives considerable trouble because of the pitch pockets.

I have several slides to show covering some of the work we have done. The slides do not show the radiographs clearly, but perhaps they will give you an idea of how the various species show up on the radiographs. Titles of slides are as follows:

- 1. Equipment used in evaluation of radiographs: Kodak Deluxe Transparency Illuminator, 10 x 10. Model 12; and Luxo Magnifying Lamp.
- 2. Radiograph of Pinus ponderosa, Lot 248, fresh seed, w/o magnification.
- 3. Radiograph of P. ponderosa, Lot 248, fresh seed, w/magnification.
- 4. Radiograph of Abies concolor, Lot 268, fresh seed, w/o magnification.
- 5. Radiograph of A. concolor, Lot 268, fresh seed, w/ magnification.
- Radiograph of Pseudotsuga menziessii, Lot 1660, fresh seed, w/o magnification.
- 7. Radiograph of P. menziessii, Lot 1660, fresh seed, w/ magnification.
- 8. Radiograph of Sequoia gigantea, Lot 1457, fresh seed, w/ magnification.
- 9. Radiograph of P. ponderosa, Lot 1812, stored seed, w/ magnification: Tater 1 to 50 -- no treatment; 51 to 100 -- 10% BaC12, one hour soak; 101 to 149 -- 10% BaC12, two hour soak.
- Radiograph of P. ponderosa, Lot 1812, stored seed, w/ magnification: seeds number 160 to 209 -- 20% BaCl2, two hour soak.
- 11. Radiograph of P. menziessii, Lot 1660, stored seed, w/ magnification: seeds number 1 to 50 -- no treatment; 51 to 99 -- 10% BaC12, one hour soak; and 100 to 149 - 10% BaC12, two hour soak.
- 12. Radiograph of P. menziessii, Lot 1660, stored seed, w/ magnification: seed numbers 150 to 199 -- 20% BaC12, two hour soak.

Radiographs as viewed through an opaque projector show the seed anatomy in better detail. As we look at a few radiographs the seed which did not germinate in tests run later in a germination chamber will be pointed out. You can note, in the radiographs taken of stored seed, that the dead tissue was not readily impregnated with BaC12, even though various concentrations and tine periods of soak were used. In Simak's work with Pinus sylvestrus it was found that BaC12 was absorbed by dead tissue of the seed. After being X•rayed, this tissue showed up as a dark coloration on the radiograph. In contrast live tissue was light in color.

Titles of radiographs are as follows:

- Pinus ponderosa, Lot 248, fresh seed, seed numbers 132 to 150 and 160 to 260. (six seeds, numbers 267 to 272, of P. radiate, Lot 1784, fresh seed, are also shown.)
- 2. Pseudotsuga menziessii, Lot 1660, fresh seed, seed numbers 1 to 131.
- 3. A. concolor, Lot 268, fresh seed, seed numbers 1 to 125.
- 4. S. gigantea, Lot 1457, fresh seed.

- 5. P. ponderosa, Lot 1812, stored seed: seed numbers 1 to 50 -- no treatment; 51 to 100 -- 105 BaC12, one hour soak; and 101 to 149 -- 10; 'o BaC12, two hour soak.
- 6. P. ponderosa, Lot 1812, stored seed; seed numbers 160 to 209 20% BaC12, two hour soak.
- 7. P. menziessii, Lot 1660, stored seed: seed numbers 1 to 50 -- no treatment; 51 to 99 -- 10% BaC12, one hour soak; 101 to 149 -- 10% BaC12, two hour soak.
- P. Menziessii, Lot 1660, stored seed; seed numbers 150 to 199, 20% BaC12, two hour soak.

An attempt has been made to show in detail, by drawings copied from the radiographs, certain anatomical characteristics which could possibly show why an individual seed did not geminate. These drawings can be viewed through the use of the opaque projector.

Titles of drawings are as follows:

- 1. P. ponderosa, Lot 248, fresh seed, seed numbers 132, 138, 146, and 150.
- 2. P. ponderosa, Lot 248, fresh seed, seed numbers 161 and 164.
- 3. P. menziessii, Lot 1660, fresh seed, seed numbers 4, 11, 13, and 19.
- 4. A. concolor, Lot 268, fresh seed, seed numbers 1, 4, 5, and 6.
- 5. A. concolor, Lot 268, fresh seed, seed numbers 7, 9, 11, and le

Our rather meagre work in the field of radiograph evaluations for the germination potential of seeds has given two primary conclusions. They are:

- 1. With experience a person should be able to evaluate radiographs of fresh seed amd come up with a germination percent very close to one arrived at through the use of a germination chamber.
- 2. Work with stored seed has not given satisfactory results because of the inability of distinguishing between live and dead tissue.

Even if radiographs cannot be used effectively for viable seed counting they should be of use in insect detection, detection of damage caused by the various extracting and cleaning methods, and evaluating percentages of anatomically unsound seed. It is also possible that stages of maturity can be deter• mined by embryo development.

The Committee adjourned for lunch and then took a tour of the nursery grounds.

The meeting was again called to order at 3:15 p.m.