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Artificial Ripening of Douglas-Fir Cones

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DOUGLAS-FIR (*Pseudotsuga menziesii* [Mirb.] Franco) seed is seldom fully ripened on the tree before late August in the Pacific Northwest. Since natural seed fall may begin as early as the last week in that month, the cone collecting period is often short. Lengthening of this period is a worthwhile goal, for it would permit better organization of cone collection and allow skilled personnel to collect more of the crop. Artificial ripening of seed in cones picked in early August now appears to be a distinct possibility.

The idea that cones might be ripened off the tree has probably been advanced many times. Dr. J. W. Duffield, Industrial Forestry Association geneticist, has suggested that squirrels, in their storage methods, might provide conditions for ripening immature cones. The present study stems from an exploratory study of simulated squirrel caches in 1954 by the author and Richard C. Koenig on the H. J. Andrews Experimental Forest. Cones collected early and stored in sandy soil were found to have an appreciable percentage of germinable seed.

The Study

A study was begun during the summer of 1956 to determine whether seed from immature cones could be ripened artificially and, if so, whether the seed and seedlings produced were normal in size and weight. Five cone-bearing trees ranging from 50 to 100 feet tall were chosen near the Wind River Nursery in southwestern Washington; cones were collected from these trees on July 11 and at 10-day intervals until August 30. A collection planned for September 10 could not be made because many of the cones had opened and shed their seed.

Each collection consisted of 60 cones picked from over the whole crown of each tree. These were mixed and divided into 3 lots of 20 cones, each lot to be stored separately under 1 of 3 conditions:

1. *Dry storage.* Cones were spread on screen trays and stored in racks in a shed that was open to the air on all sides. Temperatures varied between 60° and 80°F.

2. *Damp storage.* Cones were stored between layers of damp peat moss in powder boxes in a dark cellar, where temperatures were uniformly about 63°F. Peat moss was chosen to inhibit mold development.

3. *Wet storage.* Cones were stored in hardware cloth enclosures and immersed in well-aerated water (about 56°F.) of the fish ladder on Trout Creek Dam, near the Wind River Nursery. This treatment was begun with the August 1 collection, whereas the other two treatments were begun with the July 11 collection.

The various lots were kept in storage until September 30 in order to allow the damp-stored cones to mature. Then they were stored in air until October 11, when they were kiln dried at 110°F. maximum temperature.

After drying, some of the cone scales remained closed in some lots. In order to eliminate any possible bias from the failure of some cone lots to shed their seed uniformly, all seed was extracted by hand by breaking the scales apart where necessary. Except for occasional small seeds in the tip or base of the cone, every seed was extracted.

The lots were cleaned in a Dakota blower to remove as much of the debris as possible without losing any of the seed. Then, a random sample of 200 seeds was counted by hand from each lot for a germination test. An additional sam-

[1]Cooperators in the study were Forrest Deffenbacher and James Hutchins of the U. S. Forest Service Nursery, Carson, Wash., who collected and extracted the seed. Germination tests were made at the Cooperative Seed Testing Laboratory, Corvallis, Ore. William Scheuner made observations of the seedlings as a student project at Oregon State College. Seed weights were determined by Peter H. Geiger, U. S. Forest Service.

ple was set aside for a later seed weight test.

The germination test was carried out with four 50-seed sub-samples from each lot placed on moist river sand in plastic dishes. Seeds were prechilled for 2 weeks at 3°C., then they were germinated at alternating temperatures of 20° and 30°C. for 7 weeks. To watch for evidence of possible abnormal development in lots from early collections, half the germinated seedlings from each lot were planted in flats in river sand and grown in a greenhouse for 5 weeks.

Finally, in order to test whether or not the seed had gained weight during storage, five kernels from each dry-stored and damp-stored seed lot were decoated and dried at 80°C. for 16 hours to obtain ovendry weight. Weights were not obtained for wet-stored seeds.

Germination, seedling data, and seed weight were tested by analysis of variance.

During the course of the study, supplementary examinations were made to determine differences in embryo development for the three storage methods. Seeds from each lot were dissected August 2 and August 23.

For cones receiving normal dry storage, "endosperms" (female gametophytes plus integuments) were usually shrunken and embryos shriveled shortly after picking. Embryos in cones receiving wet storage seemed to be developing normally on August 23, except that some from the early collected lots appeared to be slightly more yellow than normal.

TABLE 1.—GERMINATION PERCENTAGES OF SEED FROM CONES COLLECTED AT 10-DAY INTERVALS AND STORED UNDER DRY, DAMP, AND WET CONDITIONS

Cone storage method	Tree No.	Collection date: July 11	July 21	Aug. 1	Aug. 10	Aug. 20	Aug. 30
Dry	1	0	0	0	0	6	28
	2	0	0	0	0	48	32
	3	0	0	0	0	8	40
	4	0	0	5	0	4	35
	5	0	0	0	0	45	50
	Average	0	0	1	0	22	36
Damp	1	0	0	22	38	18	18
	2	0	0	6	39	48	33
	3	0	0	27	14	15	32
	4	0	2	59	38	23	24
	5	0	4	40	30	48	40
	Average	0	1	31	32	30	29
Wet[1]	1	--	--	0	0	0	2
	2	--	--	0	0	2	39
	3	--	--	0	0	0	0
	4	--	--	0	0	0	2
	5	--	--	0	0	0	39
	Average	--	--	0	0	0	17

[1]Treatment begun with August 1 collection.

Embryos continued to develop in all damp-stored lots. Except for the July 11 collection, embryos were nearly equal in length to those in freshly collected cones and slightly lighter in color. When examined on August 23, some developing embryos were even found in cones from the July 11 collection. Except in some of the lots from the July 11 collection, endosperms were firm.

There were large differences between the five parents in the percentage of seeds containing embryos.

Results

Germination. — Germination results were very clearcut. The damp-stored lots collected from August 1 on gave good germination (29 percent or better), whereas the dry-stored lots collected before August 30 did not. The wet storage method gave either poor or no germination (Table 1 and Fig. 1.).

The low average germination percentages over the whole study are explained by the sampling procedure. Wormy or otherwise defective cones were not culled. (Cones from individual trees showed insect damage ranging from 22 percent to 41 percent of the total.) During extraction and cleaning every seed in the cone was recovered, whether flat or hollow. A uniform procedure was necessary to keep the lots strictly comparable for germination tests. The fact that germination percentage in no case exceeded 59 percent points up the high proportion of poor seed usually removed by selective cone collection methods and

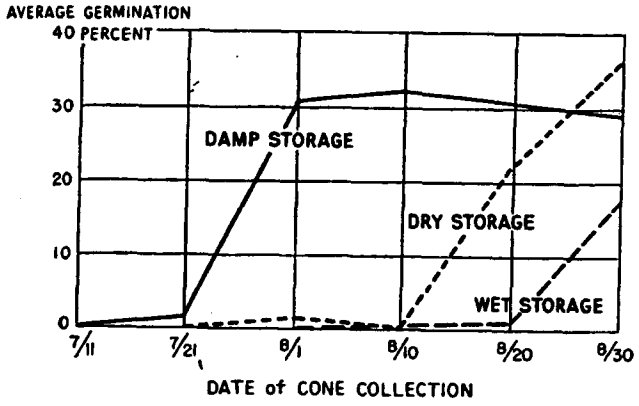

FIG. 1.—Average germination percentages of seed, by collection date, from cones stored in dry, damp, and wet storage.

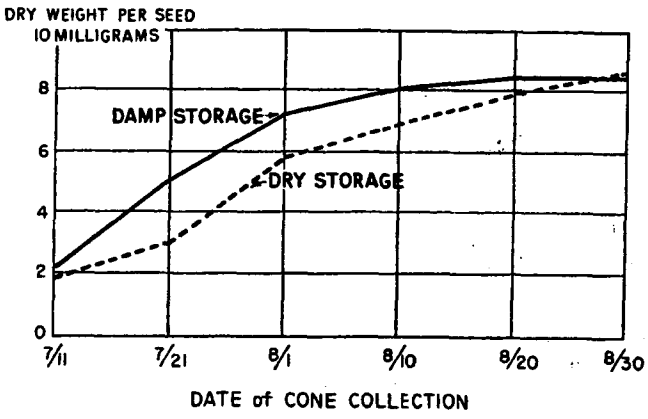

FIG. 2.—Oven-dry weight of decoated seed, by collection date, for damp and dry cone storage treatments.

effective cleaning in commercial operations. The germination percentages reported here represent true germination capacity of natural seed without any mechincal screening.

Considering these results in greater detail, dry cone storage gave no or insignificant germination for collections made from July 11 to August 10. The August 20 collection was significantly lower in germination than the August 30 collection (21.7 percent compared to 36.2 percent). However, there was considerable tree-to-tree variation. Seed obtained from one tree on August 20 germinated better than seed collected on August 30.

The germination of the damp-stored lots collected on July 11 was zero and 1.3 percent for the July 21 collection. Thereafter, germination climbed to 30 percent or higher, a percentage not significantly different from that obtained from dry storage with the August 30 collection. As in the case of dry storage, variation with parentage was evident; some trees showed their highest germination in lots collected as early as August 1, others not until August 30, and some at an intermediate date. No collection after August 1 gave a significantly higher average result than others.

Wet storage resulted in no germination for the collections of August 1 and August 10, only 0.3 percent for August 20, and 17.3 percent for the final collection on August 30. At best, wet storage was poorer than dry storage.

Seedling growth.—For the damp treatment, the seedlings showed slight but significant differences in growth as measured by length of cotyledons and epicotyl. On the average, epicotyl length was less by about 17 percent (0.48 inch vs. 0.58 inch) in the August 1 collection as compared with the August 30 collection. Cotyledon length also was less by about 14 percent (0.75 inch vs. 0.87 inch). The percentage of abnormal seedlings was very small in all collections. Data taken on relative numbers of seedlings with seed coat growth restrictions and abnormal stem color, and the numbers (few) lost to damping off showed no apparent relation to date of collection.

Seed weight.—Dry-weight comparison after storage of seed kernels from the same collection should indicate whether or not the seeds received nutrients from the cone while in storage. The dry-stored cones quickly became too dry for further development, whereas the damp-stored cones stayed fresh and green.

Kernels of seed from early collections placed in damp storage weighed appreciably more after storage than kernels from corresponding lots of dry-stored cones (Fig. 2). The average difference in seed weight was 1.4 milligrams for the July 21 collection but became progressively less for later collections, as would be expected. This difference represents weight gains from damp storage as much as 37 percent over corresponding dry-stored lots, particularly in the case of the July 21 collection.

An indication of possible loss of vitality resulting from early collection is provided by the comparison of dry weight of damp-stored lots collected on August 1 with that of lots collected when fully ripe on August 30. This difference averaged 1.2 milligrams per seed (14 percent) based on the average weight on August 30.

Discussion

The study fulfills the immediate purpose: to determine if seed in cones picked early in the season would ripen under some artificial treatment. The storage in damp peat moss proved to be effective whereas neither the more drastic wet treatment nor normal dry storage was effective.

With the possibility of early cone collection established, there remains the problem of finding a commercial method. Some further developments are suggested by this study. First, a successful method seems to depend on keeping the cones fresh until the seed ripens. The cones in this study that were individually packed in damp peat moss retained a fresh appearance for from 30 to 40 days. For instance, those collected on July 11 were fresh until August 20, but the scales had died by August 30. Cones collected on August 30 were still green when removed from storage September 30. Apparently the food necessary for continued seed development is available in the cones as early as August 1 and perhaps earlier, but the cones must be kept moist for seed maturation to take place.

It appears also that oxygen levels must be kept above those in well-aerated water. Lack of germination under wet storage conditions most likely was due to oxygen deficiencies. Any storage treatment based on wetting or spraying should be tested for possible oxygen deficiencies.

Mold may be one of the obstacles to extraction of seed from early cone collections. In this study, the earlier collections in damp storage became heavily molded between August 20 and October 11. For this reason, considerable differences were observed in various lots during seed extractions, even though the final germination percentages were similar. The wet-stored cones were by far the easiest to extract from because all the scales became widely extended. They showed no mold even though they were somewhat slimy when removed from the fish ladder September 30.

The dry-stored cones opened uniformly, but the scales were not spread widely enough for easy extraction. Removal of seed from the damp-stored lots was even more difficult because mold had caused many of the scales to remain closed. Later collections were not as badly affected and the August 30 collection particularly, which was still green at time of removal from storage on September 30, gave little difficulty. This points up the distinct possibility of eliminating such troubles from mold. As long as the cones remain alive they are free of mold. If they could be removed just before the scales die, mold might be avoided, and the cones would likely open without serious hindrance.

The second purpose of the study

was to determine whether or not the seed from early picked cones would produce good nursery stock. This is only partially answered. Small but statistically significant growth differences were found in the 5-week-old seedlings of the lots picked on August 30 and those from the lots picked earlier. Measurable differences in other characteristics were not found. Insufficient seed was available to make nursery tests.

Data from the oven-dry seed kernel weights give a picture consistent with seedling measurements. It is interesting that reductions in cotyledon growth, epicotyl growth, and dry seed weight between the August 1 and August 30 collections were 17, 14, and 14 percent, respectively. Whether this similarity in percentage between measurements is accidental or reflects a meaningful reduction in seedling vigor will require further testing for an answer.

In these measurements, the reduction in seed weight and seedling growth was much less for the August 10 collection than for the August 1 collection. Hence the risk of producing a lighter, less vigorous seedling would be reduced by collecting after August 10, which would still extend the cone collection period considerably.

The curves of oven-dry seed weight (Fig. 2) and germination percentages (Table 1) bear further inspection. Little germination occurred from either dry- or damp-stored seed until kernels averaged over 7 milligrams; fully matured seedlings were produced when the kernels averaged over 8 milligrams. Criteria have been sought for assessing the maturity of Douglas-fir seed during cone collection, such as floatation of cones in solvents, cone color, or embryo size. Since none has been developed so far, the leveling off of the curve of average seed kernel dry weight is suggested for further investigation as a possible criterion.

Summary

A study to determine whether or not Douglas-fir seed from cones picked early in the season could be artificially ripened was made on cones collected from five trees near the Wind River Nursery, Carson, Wash. Collections were begun on July 11 and continued at 10-day intervals until seedfall in early September. Of three storage methods tried, cones stored at about 63° in damp peat moss gave full germination for collections made on August 1 and later. Storage in a running stream gave lower germination than other methods. Normal dry storage of cones in air gave no appreciable germination for collections prior to August 20 and even this collection yielded only about two-thirds of the germination obtained from the collection on August 30. Hence the damp-storage method is shown to be effective in ripening the immature cones and their seeds.

Seeds in the damp-stored lots, however, were more difficult to extract due to mold on the cones. Seedlings from damp-stored lots picked as early as August 1 were apparently normal but showed slightly less cotyledon and epicotyl growth. Dry-weight measurements of the seed kernels showed that damp storage of cones collected on August 1 resulted in seed only slightly under full weight.

The artificial ripening of seed in immature cones by some commercial method of damp storage appears possible for Douglas-fir. This may greatly lengthen the period of cone collection for this species, or provide a method of handling cones inadvertently picked before they are fully ripe.