

Artificial Ripening of Sugar Pine Seeds

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Seeds of sugar pine (*Pinus lambertiana* Dougl.) are shed from the cone usually during September and October. They become mature some time before the cones open. But the precise time of seed maturation is highly variable, depending on a number of factors, including weather conditions, locality, tree age, and tree vigor. To aid the cone collector in harvesting only cones with predominantly mature seeds, such indices of cone and seed maturity as cone specific gravity have been developed.^{1 2} If collection of immature cones could be followed by artificial ripening of the enclosed seeds, cone collecting operations could become more flexible; the cone collecting period could be lengthened; and immature cones from logging operations in fall could be used.

Artificial ripening of seeds has proved to be feasible in most of the conifers in which it has been investigated. Silen³ demonstrated that immature seeds of Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) could be artificially ripened in the cone by a damp storage method. He suggested the possibility of commercial ripening of seeds. Church and Sucoff⁴ reported that the viability of immature seeds of Virginia pine (*Pinus virginiana*

Mill.) increased if the seeds were left in the cones on felled trees. Later, Fenton and Sucoff⁵ found that ripening and subsequent germination of Virginia pine seeds collected between August 30 and September 20 and removed from the cone could be improved by prolonged cold dry storage in closed containers. Similarly, Schubert⁶ found that seeds of some western pines matured during cold storage after removal from the cone. Seeds of both ponderosa (*Pinus ponderosa* Laws.) and Jeffrey pine (*Pinus jeffreyi* Grev. & Balf.)—but not sugar pine—were capable of further maturation during prolonged storage. Schubert also reported that abnormal ponderosa and Jeffrey pine seedlings were obtained most often from immature fresh seeds and that the frequency of seedling abnormalities decreased during storage.

The studies cited suggest that immature seeds of some conifers reach a stage where they no longer depend on the tree or the cone for further development. In this study, immature sugar pine seeds at different developmental stages were permitted to develop either in the immature cone after removal from the tree, or after removal from the cone. The results suggest that ripening immature sugar pine cones artificially is possible.

Methods

Exploratory studies were begun during the summers of 1957 and 1958, and a full scale investigation was made in 1959. The conclusions from the data for all 3 years were essentially the same. Therefore only the data for 1959 are reported here because they were more complete.

¹ Fowells, H. A. *An index of ripeness for sugar pine seed*. U.S. Forest Serv. Calif. Forest & Range Exp. Sta. Res. Note 64, 5 pp. 1949.

² Schubert, G. H. *Effect of ripeness on the viability of sugar, Jeffrey and ponderosa pine seed*. Proc. 55th Annu. Meeting, Soc. Amer. Foresters 1955: 67-69. 1955.

³ Silen, R. R. *Artificial ripening of Douglas-fir cones*. J. Forestry 56: 410-413. 1958.

⁴ Church, T. W., Jr., and Sucoff, E. I. *Virginia pine seed viable two months before natural cone opening*. U.S. Forest Serv. NE. Forest Exp. Sta. Res. Note 102, 4 pp. 1960.

Seed collection.—About 25 cones were collected biweekly from each of three mature sugar pine trees located at 4,000 feet elevation in the University of California's Blodgett Forest, in the central Sierra Nevada of California. Cone collection began during the first week of June and continued until second week of October, when seeds were shed from the trees. As a measure of ripeness, green cone specific gravity was determined by the water displacement method within 2 days of collection. Cones then were segregated into

⁵ Fenton, R. H., and Sucoff, E. I. *Effects of storage treatments on the ripening and viability of Virginia pine seed*. U.S. Forest Serv. NE. Forest Exp. Sta. Res. Note NE-31, 6 pp. 1965.

⁶ *Op. cit.*

specific gravity classes. Two-thirds of the cones of a specific gravity class collected on a given day were opened by hand and their seeds pooled.

Maturity test.—Germination of fresh seeds measured their physiological maturity at the time of collection. Three 150-seed samples were randomly selected from each of the pooled seed lots immediately after removal from the cone, and planted in two parts vermiculite and three parts sand in a darkened room at a temperature of 25°C.

Germination was recorded every 2 days for 60 days. Germination is defined here as the resumption of growth of the embryo, recognized by a definite rupture of the seed coat and the appearance of the radicle or hypocotyl. The germination percentage was based only on filled seeds, and statistical significance determined after angular transformation of the data.⁷ Germination and seedling development were classified as normal or abnormal on the basis of the embryo development, and on seedling behavior during a 6-week period after germination.

Artificial ripening of the seed in the cone was investigated by randomly selecting 10 cones of each specific gravity class from each biweekly collection. These cones were dusted with 50 percent Captan, sealed individually in plastic bags, and stored in the dark at 10°C. within 2 days of cone collections. Preliminary studies had shown that storage at 10°C. reduced to some extent external and internal fungal contaminations not inhibited by the fungicide, but did not inhibit embryo development. The seeds from the stored

cones were hand extracted during the first week of October and three 150-seed samples from these seeds were tested for germination.

Artificial ripening of seeds removed from cones was studied by randomly selecting 800 seeds from cones of each specific gravity class collected on a given date. These seeds were first treated with 50 percent Captan and then stored in moist vermiculite at 10°C. within 3 days of cone collection. Three 150-seed samples from each of these seed lots were tested for germination, starting the first week of October.

Anatomical development of the seed extracted before, during, and after storage was investigated. During seed storage in the cone, one cone representing each of the specific gravity classes from each of the collection dates was selected randomly for analysis at the end of the first, the second, and the last week of storage. After seed was extracted from cones in each of the treatments, 50 seeds were randomly selected for anatomical studies. The dry weight of the female gametophyte plus embryo was determined for the remaining seeds.⁸

Finally, from each of the different specific gravity classes of cones collected on a given date, 100 seeds were selected at the end of the first, the second, and the last week of storage from the seed sample stored in moist vermiculite. Half of these seeds were used to determine the anatomical development during storage; the remainder was used to determine the dry weight of the female gametophyte plus embryo during and after storage in moist vermiculite.

Results

Changes in cone specific gravity.—In the first week of June the cones averaged 15 cm. in length and had specific gravities of 1.01 to 1.50 (fig. 1). By the fourth week of June—at the time of fertilization—specific gravity had decreased to 0.91 to 1.00. After fertilization the cones elongated rapidly, and the final mean cone length of 35.0 cm. was reached by the fourth week of July.

Concurrently, the specific gravity of the cones continued to decrease gradually until the end of August, when this drop became more rapid. Cones having specific gravities as high as 1.00 to 0.96 were found until the last week of August, but cones

of specific gravity of 0.80 or less were found only after the last week of August. By the time the cones began to open in October their mean specific gravity had dropped to less than 0.75. Specific gravity of mature sugar pine cones has been reported to be 0.80 or less.^{9 10}

Germination behavior of fresh seeds.—None of the seeds collected through the end of July germinated (table 1). However, some of the seeds collected during the second week in August did

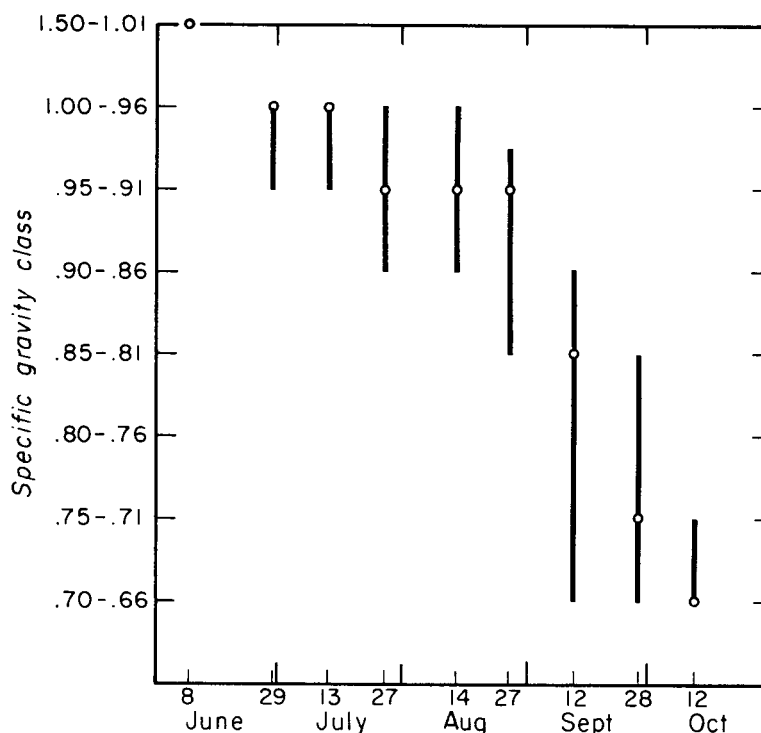
⁷ Snedecor, G. W. *Statistical methods*. 534 pp. Ames: Iowa State College Press. 1956.

⁹ Krugman, S. L. *Germination potential of sugar pine seed (Pinus lambertiana Dougl.) during maturation and associated biochemical changes*. 1961. (Ph.D. thesis on file at Univ. of Calif., Berkeley.)

⁹ *Op. cit.*

¹⁰ *op. cit.*

Figure 1.-
Specific gravity of freshly
collected sugar pine cones.
The mode and range of specific
gravity classes for each collection
date are shown.



germinate. The inability to germinate, speed of germination, and incidence of abnormal germination were related to specific gravity of the cone. Only 3 percent of the seeds from cones having a high specific gravity germinated. And all of these seeds germinated abnormally. In contrast, 25 percent of the seeds from cones having low specific gravity (less than 0.91) germinated. And about half of these seeds germinated abnormally. Furthermore, 90 percent of the germination was completed only after 16 days.

The type of abnormal germination also varied with specific gravity of the cone. The most prevalent abnormality among seeds from the heavier cones was reverse germination. The cotyledons protruded first, rather than the radicle, and these seedlings died within 2 weeks. In some seeds, both the radicle and the cotyledons appeared, but radicle growth ceased in a few days. All of these seedlings were dead within 4 weeks of planting. In the most prevalent form of abnormal germination among seeds from less dense cones, the cotyledons grew through the female gametophyte, but remained tightly restricted within it. These seedlings died within 6 weeks of germination. Schubert¹¹

¹¹ *op. cit.*

reported a similar type of abnormal germination in immature sugar pine seeds; Stone¹² encountered it in germinating mature sugar pine seeds that had been stored several years.

Germination of seeds collected August 27 was much better than germination of seeds collected August 14, and the seeds collected August 27 also germinated faster. Germination percentages of the seeds collected during the last week of August ranged from 67 to 82. Germination was 90 percent completed in 5 days, in contrast to those seeds collected on earlier dates. Abnormal germination was confined to seeds from cones having a specific gravity greater than 0.86. In the most prevalent form of abnormal germination, cotyledons were restricted within the female gametophyte. But only 40 percent of these abnormal seedlings eventually died, since the cotyledons would often grow out of the female gametophyte.

Germination of seeds collected during the second week of September increased slightly—ranging from 78 to 95 percent. All seeds collected in September that germinated did so normally.

¹² Stone, E. C. *Auxin and respiration changes during stratification of sugar pine, Pinus lambertiana Dougl., seed and their relation to subsequent embryo growth.* 1948. (Ph.D. thesis on file at Univ. of Calif.)

Germination behavior of stored seeds.—Seed storage in the cone or in moist vermiculite increased germination significantly—compared to nonstored seeds of the August 14 collections. But storage had no significant effect on earlier or later seed collections (tables 1, 2). None of the seeds collected before August 14 germinated.

For the August 14 collections, seed storage in the cone proved a better ripening method than moist vermiculite. For all cone specific gravity classes, seeds stored in the cone germinated better than vermiculite-stored seeds. After August 14 there were no significant differences in total germination between treatments.

Rate of germination of stored seeds collected August 14 varied with specific gravity of the cone. All stored seeds from cones having specific gravities less than 0.96 had higher germination than fresh seeds collected at the same time (tables 1, 2).

Total germination (normal + abnormal germination) of seeds extracted from cones having specific gravities of 0.90 to 0.86 increased from 25

percent for fresh seeds to 56 percent for seeds stored in vermiculite and 74 percent for seeds left in the cone.

Normal germination increased from 10 percent for fresh seeds to 35 and 39 percent for seeds stored in vermiculite and in the cone. And storage in the cone resulted in some germination among seeds from all cone specific gravities. Only 3 percent of the fresh seeds extracted from cones having specific gravities 0.95 to 0.91 germinated, contrasted to 50 and 65 percent for these seeds when stored in moist vermiculite and in the cone.

Fresh seeds and seeds stored in vermiculite from cones of the highest specific gravity class (1.00 to 0.96) collected on August 14 failed to germinate. But after storage in the cone 25 percent of these seeds germinated.

In the most common form of abnormal germination among stored seeds, the cotyledons grew through the female gametophyte. However, unlike the abnormal germination of fresh seeds, a small percentage of these abnormal seedlings did sur-

Table 1.--Type of germination of sugar pine seeds from cones with different specific gravities collected on various dates

Collection date	Cone specific gravity	Type of germination	
		Normal	Abnormal
		Percent	
June 8	(1/)	0	0
June 29	(1/)	0	0
July 13	(1/)	0	0
July 27	(1/)	0	0
August 14	1.00 - 0.96	0	0
August 14	.95 - .91	0	3
August 14	.90 - .86	10	15
August 27	1.00 - .96	65	2
August 27	.95 - .91	80	5
August 27	.90 - .86	80	2
August 27	.85 - .81	79	0
September 12	.90 - .86	80	0
September 12	.85 - .81	78	0
September 12	.80 - .76	95	0
September 12	.75 - .71	84	0
September 12	.70 - .66	83	0
September 28	.85 - .81	80	0
September 28	.80 - .76	86	0
September 28	.75 - .71	93	0
September 28	.70 - .66	90	0

¹ No germination by seeds collected on these dates regardless of cone specific gravity.

Table 2.--Type of germination of sugar pine seeds following ripening in the cone and in moist vermiculite at 10°C

Collection date	Cone specific gravity	Percent germination after storage			
		Cone		Vermiculite	
		Normal	Abnormal	Normal	Abnormal
		<i>Percent</i>			
June 8	(1/)	0	0	0	0
June 29	(1/)	0	0	0	0
July 13	(1/)	0	0	0	0
July 27	(1/)	0	0	0	0
August 14	1.00 - 0.96	5	20	0	0
August 14	.95 - .91	40	25	20	30
August 14	.90 - .86	39	34	35	21
August 27	1.00 - .96	81	7	73	10
August 27	.95 - .91	82	0	84	9
August 27	.90 - .86	80	0	82	0
August 27	.85 - .81	86	0	76	0
September 12	.90 - .86	83	0	83	0
September 12	.85 - .81	86	0	80	0
September 12	.80 - .76	87	0	93	0
September 12	.75 - .71	85	0	86	0
September 12	.70 - .66	89	0	83	0
September 28 ²	-- --	--	--	--	--

¹ No germination by seeds collected on these dates regardless of method of ripening.

² Seeds of this date were mature and needed no further ripening.

vive. For seeds collected August 14, and stored in the cone, about 10 percent of the seedlings having abnormal germination survived. In contrast, less than 4 percent of abnormally germinated seedlings produced by seed collected August 14 and stored in vermiculite survived. There was no relationship between cone specific gravity and survival after an abnormal germination.

Anatomical development of fresh seeds.—During the first week of June the female gametophyte was almost completely cellular in structure, but the archegonia had not yet formed. By the last week of June, all stages from unfertilized archegonia to early proembryos were present within a cone regardless of its specific gravity. By the second week of July seeds from all cones included well developed proembryos and corrosion cavities (embryo cavities).

During the last week of July, seeds from all cones included some embryos composed of 100 to 200 cells. And the remaining portions of the archegonia had collapsed. The corrosion cavity occupied one-third to one-half of the female gametophyte.

Only in the August 14 collections were there differences between embryos extracted from cones of different specific gravities. The cones having the highest specific gravity (1.00 to 0.96) had significantly shorter embryos than cones of lower specific gravities (table 3). Their average length was 2.4 mm., compared to 3.8 mm. and 4.2 mm. for embryos from cones having specific gravities of 0.95 to 0.91 and 0.90 to 0.86. Of the seeds extracted from cones having the lowest specific gravity (0.90 to 0.86), 15 percent included embryos whose mean length (9.1 mm.) equaled that of embryos in mature seeds collected 2 weeks later. Thus, the 10 percent normal germination of fresh seeds from these cones could have been from this small portion of apparently anatomically mature seeds.

Anatomical development of stored seeds.—The female gametophyte of seeds collected before August 14 disintegrated within the first week after removal from the cone and storage in moist vermiculite, and within 2 weeks in the stored cone. In no instance did the embryos from the pre-August collections complete their development

Table 3.--*Embryo length of seeds collected August 14 before and after 50 days of storage*

Cone specific gravity	Mean embryo length ¹		
	At time of collection	After storage ² ...	
		Cone	Vermiculite
		<i>Millimeters</i>	
1.00 - 0.96	2.4	4.8	(3/)
.95 - .91	3.8	6.3	5.3
.90 - .86	4.2	7.4	5.8

¹A difference of 0.8 mm. is significant

²Cones and seeds stored at 10°C.

³Female gametophyte and embryo disintegrated

after the seed had been removed from the tree.

In contrast, embryos from seed collected August 14 increased in length during both storage conditions (table 3). Seeds from cones with the lowest specific gravity (0.90 to 0.86) stored in the cone produced larger embryos than similar seeds stored in moist vermiculite. The smallest embryos found after storage were in seeds from cones having the highest specific gravity, 1.00 to 0.96. However, the final mean embryo length after both types of storage was less than the mean embryo length (8.1 mm.) from seeds collected during the last week of August.

Besides growing longer during storage, certain seeds of the August 14 collections became heavier (table 4). Seeds stored in their cones doubled their dry weight, but those stored in moist vermiculite failed to increase in dry weight. Differences in cone

specific gravity did not influence changes in seed weight after seed storage.

Seeds stored in moist vermiculite increased significantly in embryo length and in their ability to germinate-despite their failure to increase in total dry weight. This difference suggests a shift in the available nutrients from the soluble or the storage form in the female gametophyte to the embryo.

By the end of August, the embryos had reached 95 percent of their final size. Any additional enlargement of the embryos during storage appears to be due to lengthening of their cotyledons; mean length of the embryo axis showed no increase. After this time no significant changes in the mean embryo length or dry weight of the female gametophyte and embryo could be related to cone specific gravity or storage condition.

Table 4.--*Dry weight of the female gametophyte and embryo from seeds collected August 14 before and after 50 days of storage.*

Cone specific gravity	Weight of gametophyte plus embryo ¹		
	At time of collection	After storage in ² ...	
		Cone	Vermiculite
		<i>Milligrams</i>	
1.00 - 0.96	77.2	155.3	(3/)
.95 - .91	78.3	157.6	78.1
.90 - .86	76.5	156.3	74.3

¹A difference of 21.2 mg. was significant at the 0.95 level.

²Cones and seeds stored at 10°C.

³Female gametophyte plus embryo disintegrated during storage.

Discussion

This study showed that a proportion of immature sugar pine seeds harvested after a certain date could be brought to maturity in storage. For these artificial ripening treatments to succeed, the immature seeds must reach a stage of physiological and anatomical maturity at which the seeds are no longer dependent directly on the tree for nutrition. In the 1959 study, sugar pine seeds had reached this stage of development by August 14. By then some seeds were no longer dependent for further maturation on either the tree or the cone, and were able to mature in vermiculite. Others were independent of the tree, but still dependent on the cone; they failed to mature in vermiculite.

Whether storage of immature seeds collected during mid-August is at all practical is questionable. Problems in artificial ripening were encountered with the August 14 collections. First, a high percentage of artificially ripened seeds germinated abnormally. Perhaps a longer storage period would have reduced this abnormal germination. Second, cones of all specific gravities collected on August 14 failed to open normally when dried after storage. Third, mold on the seeds was a serious problem in many of the cones, although mold formation was not excessive on the cone surface. This mold formation greatly reduced the number of seeds available for testing and added to the variation in final germination. The presence of mold definitely lowered seed quality.

Cones collected during the last week of August for all 3 years of the study contained mostly mature seeds. Additional ripening of cones collected then eliminated the few immature seeds initially present, and made the cones easier to open by hand. Cones collected during the last week of August and artificially ripened would open naturally if slowly air-dried at temperatures of 25°C.

The artificial ripening of seeds in immature cones harvested after the middle of August appears possible in a commercial operation. But if this

method is to be used successfully, the forest manager must first recognize that the rate of seed maturation can vary from tree to tree, locality to locality, and year to year.

Thus, past collection dates are of limited value as the sole measure of when harvesting and artificially ripening of immature sugar pine cones would be possible. However, the use of cone specific gravity can be helpful and can be used as a guide for seed maturity. In the 3 years of this study, cones with a specific gravity of 0.85 to 0.81 always held seeds advanced enough in their maturation to be artificially ripened in the cone. Fowells¹³ and Schubert¹⁴ both recommended that cones having a specific gravity of 0.80 or less be collected to obtain mature seeds; their recommendations were confirmed in this study.

Cones of specific gravities greater than 0.80 also can produce mostly mature seeds. But they can not be depended upon to yield only mature seeds consistently if they are opened shortly after harvesting. Additional moist storage of cones from the specific gravity class 0.85 to 0.81 or lower would make earlier collection to obtain mature seeds possible. This procedure would add several additional weeks to the cone collecting season. In addition, should immature cones be collected, it would be possible to ripen further the enclosed seeds.

The method of storage used in this study—cones in individual bags—would not be suitable for a commercial operation. Silen¹⁵ used a method of storage for immature Douglas-fir cones that could also be used with pines. He obtained full germination from Douglas-fir seeds collected in the first week of August by storing the cones at 63°F. in damp peat moss. Such a method may be applicable to bulk sugar pine cone samples, and should be studied further.

¹³*Op. cit.*

¹⁴*Op. cit.*

¹⁵*Op. cit.*