EFFECT OF STORAGE UPON BALSAM FIR POLLEN VIABILITY AND SEED DEVELOPMENT[1]

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An important problem with tree breeding is to ensure an adequate pollen supply for controlled pollinations. If pollen storage procedures can be developed, a supply of pollen can be maintained for future use. Studies have been conducted with various tree species to determine storage procedures (Duffield and Snow, 1941, and Duffield, 1954) and guides for pollen extraction and storage have been developed for use with some species (Snyder, 1961). Seed yields with slash and loblolly pine show no consistent differences between fresh and stored pollen (Kraus and Hunt, 1970). Bingham and Wise (1968) report that with western white pine it is safe to use pollen that is three and possibly five years old if it has been deep frozen in storage.

The object of this study was to determine suitable pollen storage techniques for balsam fir (Abies balsamea (L). Mill), and to determine what effect, if any, pollen viability might have upon seed properties.

METHODS

Male strobili were collected in spring 1971, from a single balsam fir tree and they were dried five days at room temperature. The strobili were then sifted to separate the pollen. The extracted pollen was placed in plastic vials. The vials were plugged with cotton and placed in storage.

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The pollen was stored at four temperatures (70, 43, 30, and -6°F), and at three moisture regimes (11 to 17, 23 to 26, and 39 to 48% relative humidity). Moisture regimes were produced by storing the vials in desiccators over super-saturated salt solutions of lithium chloride, potassium acetate, and sodium iodide, respectively. Moisture regimes were duplicated within each temperature. The desiccators were allowed to come to equilibrium relative humidity for one day at the proper temperature before the samples were placed in them.

Pollen viability was determined during the springs of 1972 and 1973 by germination tests. The germinating media consisted of the following: 1) 8% sucrose, 2) 0.85% agar, 3) 0.01% boric acid, 4) 0.03% calcium nitrate, 5) 0.02% magnesium sulfate, and 6) 0.01% potassium nitrate. Media were placed in plastic rings on each end of sterile microscope slides. Pollen was dusted onto the media with a brush and the slides were placed in a desiccator over water at 70° F. One hundred pollen grains in each plastic ring were examined for germination after three days. A grain was counted as germinated when the pollen tube had grown to one-half the width of the pollen grain. Germination in the two rings on each slide was averaged for analysis.

In the spring of 1972, 50 female strobili located on three ramets of one clone (OWST-8) were pollinated in the N.H. State Forest Tree Nursery seed orchard. Pollen from each temperature-moisture combination was used to pollinate two female strobili, and two strobili were used as a control to check bagging technique. Six out of the 48 study cones failed to mature. The remaining cones were collected, dried; the seed was extracted, cleaned, counted and weighed. Average sound seed per cone, and weight to the closest 0.001 grams per 100 seeds were analyzed.

The seed from the controlled pollinated cones were divided into four replications and planted in plastic tubes containing a 3:1, sand: loam mixture. They were then moistened and placed in stratification for 100 days at 41° F. After stratification they were placed under mist in a greenhouse at 75°F days and 60°F nights. Germination was counted twice a week. Some damping off occurred and we attempted to keep count of this to obtain total germination. Germination of the four replications was averaged for analysis.

RESULTS AND DISCUSSION

Pollen storage temperatures were not replicated in this study because of equipment limitations. Duplicate desiccators were used within each temperature to estimate the temperature and to obtain an error sum of squares for temperature. Therefore, the following analysis of variance for temperature effects must be viewed with caution and regarded as an indication instead of representing true differences. The moisture and temperature X moisture interaction effects are valid tests.

Analysis of variance (Table 1) revealed the following: 1) temperature and moisture had highly significant effects upon one year pollen germination ; 2)temperature, moisture, and their interaction had highly significant effects on two year pollen germination; 3) temperature of pollen storage had a highly significant effect upon seed germination. Significant

differences were not found among pollen storage treatments in the production of sound seed or in seed weight.

Table 1.--Mean Squares for One and Two Years Pollen Germination and Seed Germination Data.

Source	df	Mean Squares 1 Yr. Pol. Germ.	2 Yr. Pol. Germ.	Seed Germ.
Temperature	3	1357.97**	3364.76**	19.686**
Error a	4	22.41	88.60	0.836
Whole Plot Total	7			
Moisture	2	1380.80**	2277.01**	15.724
T X M	6	139.54	470.59**	17.875
Error b	8	66.08	21.34	12.033
Split Plot Total	16			

** Significant differences at the 0.01 level

Pollen germination after one year in storage increased as storage temperature decreased and as the relative humidity increased (Table 2 and 3). This is similar to results previously reported in the literature (Duffield and Snow, 1941, and Fechner and Funsch, 1966). The effect of the moisture regimes remained identical after two years (Table 3). However, after two years pollen stored at 30°F had a higher percent germination that that for pollen stored at -6°F, but the difference was not significant (Table 2).

Table 2.--Means for One and Two Year Pollen Germination and Seed Germination By Pollen Storage Temperature.

Temperature °F	Percent Germination[a] 1 Yr. Pol.	2 Yr. Pol.	Seed
70	12 a	14 a	1.5 a
43	20 ab	36 ab	3.6 ab
30	29 bc	70 c	2.7 ab
-6	38 c	54 bc	6.0 b

[a] Means having a small letter in common are not significantly different at the 5% level by Duncan's new multiple range test.

Table 3.--Means for One and Two Year Pollen Germination and Seed Germination by Pollen Storage Moisture.

Chemical	Percent Germination[a]		
	1 Yr. Pol.	2 Yr. Pol.	Seed
LiCl	18	24	2.1 a
$KC_2H_3O_2$	38 a	53 a	4.8 a
NaI	43 a	54 a	4.1 a

[a] Means having a small letter in common are not significantly different at the 5% level by Duncan's new multiple range test.

The pollen had been removed from storage in 1972 for field pollination, and the resulting rapid increase and decrease in temperature may have affected pollen viability. This is supported by Snyder (1961) who reported that pollen deterioration may occur when refrigeration is interrupted for even short time periods, because of water condensation.

Interaction temperature-humidity means for pollen germination were tested for significant differences by Duncan's New Multiple Range Test (Table 4). After one year in storage there was no sharp distinction among the temperature-moisture combination means; however, after two years a distinction among storage treatments was noted. All moisture regimes at 30°F, the middle and high moisture regimes at -6° F, and the high moisture regime at 43°F were significantly different from the remaining treatments.

Pollen viability increased when two year germination is compared to one year germination (Figure 1); both the regression analysis and correlation coefficient (r = .75) are highly significant. Similar variations of results over time have been reported for the southern pines. [1] Also, the increase may be partially attributed to improvements in germinating and counting procedures gained by experience and/or by different people collecting the data.

Storage temperature had an effect upon seed germination (Table 2). Moisture regime during storage did not have an affect upon seed germination even though germination more than doubled between the low and middle moisture regimes (Table 3). This experiment indicates that seed germination may be related to pollen germination (Figure 2).

[1]Bruce Zobel - personal communicat

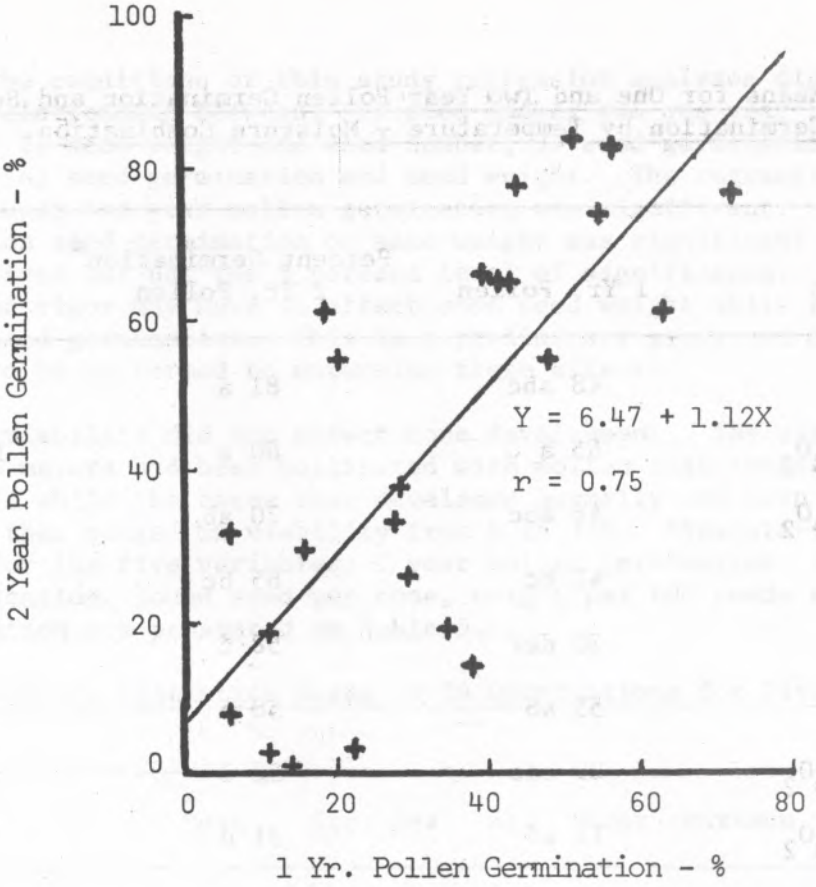

Figure 1. Relationship between one and two year pollen germination.

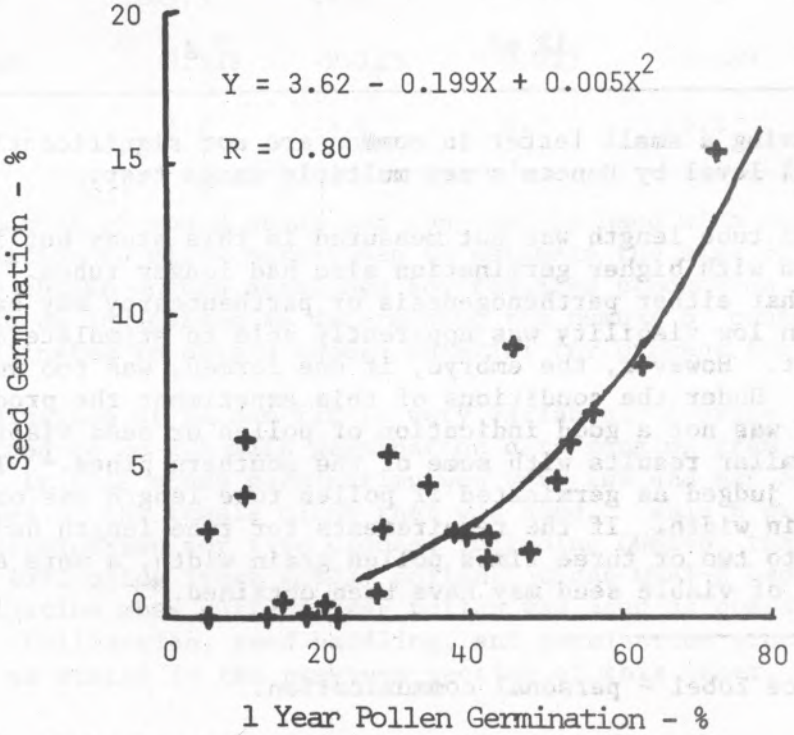

Figure 2. Relationship between seed germination and one year pollen germination.

Table 4.--Means for One and Two Year Pollen Germination and Seed Germination by Temperature - Moisture Combination.

Treatment	Percent Germination[a] 1 Yr. Pollen	2 Yr. Pollen	Seed
30° - NaI	48 abc	81 a	6.8 ab
-6° - $KC_2H_3O_2$	65 a	80 a	10.9 a
30° - $KC_2H_3O_2$	46 abc	70 ab	4.1 ab
43° - NaI	42 bc	65 bc	2.2 ab
30° - LiCl	20 def	58 c	0.2 b
-6° - NaI	55 ab	58 c	5.3 ab
43° - $KC_2H_3O_2$	29 cde	32 d	4.1 ab
70° - $KC_2H_3O_2$	11 ef	31 d	0.2 b
-6° - LiCl	33 cd	24 de	1.8 b
43° - LiCl	8 f	13 ef	4.4 ab
70° - NaI	28 cde	11 fg	2.2 ab
70° - LiCl	12 ef	2 g	2.2 ab

[a] Means having a small letter in common are not significantly different at the 5% level by Duncan's new multiple range test.

Pollen tube length was not measured in this study but it is felt that pollen with higher germination also had longer tubes. This may indicate that either parthenogenesis or parthenocarpy may have occurred. Pollen with low viability was apparently able to stimulate cone and seed development. However, the embryo, if one formed, was too weak to germinate. Under the conditions of this experiment the production of sound seed was not a good indication of pollen or seed viability. Zobel reports similar results with some of the southern pines. [1] In this study, pollen was judged as germinated if pollen tube length was one half the pollen grain width. If the requirements for tube length had been increased to two or three times pollen grain width, a more accurate indication of viable seed may have been obtained.

[1] Bruce Zobel - personal communication.

Under the conditions of this study regression analyses did not indicate a relationship between: 1) seed number and one year pollen germination; 2) seed weight and seed number; 3) seed germination and seed number; 4) seed germination and seed weight. The regression of seed weight upon one year pollen germination was significant. Also, the regression seed germination on seed weight was significant at the 10 percent level but not the 5 percent level of significance. Pollen viability and vigor may have an effect upon seed weight while this factor may affect seed germination. This is a preliminary study and additional work needs to be performed to determine these effects.

Pollen viability did not affect cone development. The six strobili that did not mature had been pollinated with pollen that ranged from 13 to 54% viable while the cones that developed normally had been pollinated with pollen that ranged in viability from 6 to 73%. Elementary statistics for the five variables; 1 year pollen germination, 2 year pollen germination, sound seed per cone, weight per 100 seeds and percent seed germination are presented in Table 5.

Table 5.--Elementary Statistics Based on 24 Observations for Five Characters.

Variable	Mean	Std. Dev.	Std. Error	Maximum	Minimum
1 Yr. Pollen Germ. (%)	33.2	18.8	3.8	72.7	5.5
2 Yr. Pollen Germ. (%)	43.6	28.0	5.7	84.0	1.0
Sound Seeds/Cone	163.3	26.8	5.5	216.0	117.0
Wt/100 Seeds (gms)	0.531	0.113	0.023	0.689	0.120
Seed Germ. (%)	3.7	3.6	0.7	15.1	0.0

Average number of sound seeds per cone may be used with estimates of seed germination to allow nurserymen to estimate the size of the cone crop needed to obtain enough seedlings for required production. It can also be used as an indication in determining the number of cones that need to be pollinated to obtain enough material for progeny tests.

Seed germination of balsam fir is very variable. Average germination has been reported to be 22% with germination data ranging from 1 to 74% (USFS, 1948). In this study germination was very low and may have been partially caused by the female clone that was used. Table 6 presents the average seed germination for five clones, including OWST-8, that were pollinated in 1972 using fresh (1972) pollen. Clone OWST-8 had a very low seed germination when current year pollen was used as compared to other clones. Pollination, seed handling, and germination procedures were the same as stated in the previous section of this paper.

Table 6.--Mean Seed Germination for Five Clones.

Clone	No. of Seeds	% Germ.
OWST-8	1000	3.4
GORA-4	1000	10.4
OWST-7	200	18.5
OWST-5	200	26.0
OWST-3	1000	41.8

SUMMARY

Results of this preliminary study indicate that balsam fir pollen can be stored for at least two years at relative humidity attained with either a super saturated salt solution of potassium acetate or sodium iodide at either 30° F or -6 F temperatures. Pollen viability did not affect sound seed number. There was an indication that pollen viability may have had an effect on seed weight. Pollen storage treatments did have a significant effect upon seed germination. Seed obtained using pollen that was stored under these conditions showed better germination than seed produced from pollen stored at higher temperatures and lower relative humidities.

LITERATURE CITED

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DISCUSSION

Kriebel - I have a couple of questions. One is: How do you define sound seed? I assume that when you weigh the seed you separate the heavier seed.

Lowe - Right, that was our definition of sound seed. More of a mechanical wind separation.

Kriebel - Did you examine the seed by a cutting test?

Lowe - Basically, we did the cutting to get the separator calibrated and then we quit. I was thinking possibly that audioradiographic techniques would work to distinguish between filled and unfilled seeds and could prove to be beneficial for something like this.

Kriebel - I think x-ray analysis could be a good use for this. It is hard for me to see how the pollen viability could affect the filled seed weight. I can see how it can affect filled seed percent, but I can't see how it would affect seed weight.

Ledig - We have some experience in separating balsam fir seed. It was almost impossible to separate viable and non-viable seed because some inviable seed is filled with resin and will not be winnowed out in the aspirator column.

Critchfield - I would like to ask if you checked the moisture content of the pollen. What was the relationship between the relative humidity and the percent viability of your pollen?

Lowe - We didn't check that at all.

Critchfield - Since so much of the pollen literature tells us about storage of pollen at a given moisture level, there is no way to relate that to relative humidity levels.

Hunt - I just want to make sure I heard things right. You used one tree for all the pollen?

Lowe - Yes.

Hunt - And you took it on faith that you would get something to germinate the first time. I mean it could have all been dead. Not viable through some type of accident in production.

Lowe - Yes.

Hunt - Bill asked you--didn't you establish what your beginning moisture content was for each sample bottle?

Lowe - I assumed that to be pretty equal when they were placed in storage because they were dried and sifted in the same room.

Hunt - And then you had no control in which you froze or evacuated the pollen, without any air, without any additional opportunity to grab off moisture. If you could have done this it would have been your control, in each one of your temperature regimes, in each one of your situations and break those open to do your pollinations in the field as a control. If you want to establish what the effect of additional moisture over a long period of time might do. I'm not saying that this is the way to do it, but this is what I think I would have tried to do if...

Critchfield - I don't follow it.

Hunt - You take your sample of pollen and instead of putting a cotton plug on top and leaving it in a desiccator with all the vulgarities of someone pulling a plug and taking it out to clean and so on, water spilling and dripping down when you take it out, you just seal it right up with vacuum, under an evacuated system, as your sample for a control.

Lowe - How are you going to come to equilibrium moisture content?

Hunt - You said it was already at the best possible moisture content when we started.

Lowe - No, I said it was all the same.

Hunt - All right, but isn't that the moisture content that would give you the best results if you took it out in the field and pollinated?

Lowe - That I don't know.

Hunt - Then we should have probably tried to get a particular moisture content when we started--but you still have it in a vial that is subjected to all sorts of other moisture contents at different times during the experiment, and if you took it into the field as you...

Lowe -W e stored it at different moisture contents. It is put in a desiccator and sealed.

Hunt - You have to assume that there is moisture in the air that is moving through the cotton plugs.

Critchfield - You don't have to assume that.

Hunt - Well, I was just saying that you had no control where additional movement back and forth was restricted. I would think that might be considered.

Critchfield - It's at an equilibrium relative humidity. I agree that it would be really nice to know what that moisture content was, but I have no doubt that it was stable during storage.

Kriebel - It is a one-way transfer of moisture out of the seed by keeping the seed in a desiccator with an actively hygroscopic medium.

Hunt - And this has been established? I'm not saying I've done that much pollination, but you have to assume that this is the best policy.

Ledig - Relative humidity stays the same in such a desiccator over salt solutions. I have measured it.

Miller - I have a couple of comments on seed production areas and then one question concerning Kit's paper. In the Lake States area of the Eastern Region, U.S. Forest Service, we have established several seed production areas of red pine and white spruce, and seed collection areas of jack pine. I agree with Kit that the best method for collecting cones in jack pine and red pine is to just cut the tree down and collect the cones. It's the quickest, easiest, and cheapest way, and you get a good supply of seed to fill your needs. With white spruce on the other hand, we have had excellent luck in actually climbing the tree and sawing the top out or by shooting the top out and collecting the cones on the ground. Four to five years later we can go back to the same white spruce tree and make another collection. Just thought that I would introduce that. Now my question: Kit, you mentioned that the seedling seed orchard should be established in the same zone that you are going to use the seed in, and I wondered if you would comment on why.

Yeatman - Because in a seedling seed orchard you are not dealing with selected clones, you are dealing with the progeny of selected trees and the genotype-environment interaction that takes place will be reflected in the trees that survive and produce seed in your orchard. You haven't direct control over your genotypes that are going to be in your orchard. This is dependent on the reaction of your individuals within the progenies to the environment.

Miller - This is a progeny test seed orchard, then?

Yeatman - No. I would not suggest you use the seed orchard as your progeny test. You may get some family information from it, but this would be incidental. You should have a progeny test off to the side as well for a number of reasons, not all of which are directly related to the current seed orchard. For example, efficient progeny tests are needed for advanced generation selection.

Schmitt - Mine is just a general one for Kit and Jerry or anyone else in the group. The question pertains to seed production areas. I have not worked in this area in a good many years, but has anyone had any indication that you get any genetic improvement from seed coming from seed production areas?

Nienstaedt - We have been testing some of the white spruce SPA seed from the Nicolet Forest and have compared the seedlings in a study in the nursery for five years. The comparison was with area collections from the Nicolet Forest; and the SPA seed runs some 10% better than the area collections.

Miller - I would just like to add a little to Hans' comment. Our nurserymen say that this same seed Hans is talking about grows better in the nursery and is easy to handle. People on the forest say that they like the stock from seed production areas much better. Also, there is one other point to consider in favor of establishing seed production areas, especially

in where it is so difficult to get a crop in the first place. We have the area thinned, and we can control the insects to a certain degree. Thus, we are more assured of a seed crop even though the genetic gain might be small. If you are familiar with the red pine seed situation, you know that most states and the Canadian's are very short of red pine seed.

Schmitt - The issue involved is the one that Hans answered. I can appreciate the value of seed production areas because of the short production and inconvenience of selection, but the underlying thought in seed production areas was that you would achieve some genetic gain and I just wondered whether you actually acquired this information.

Critchfield - I have a question for Kit. Did I understand you correctly to say that a ten-year old seedling seed orchard of jack pine is going to be producing very little pollen?

Yeatman - That it would produce relatively little pollen.

Critchfield - A lot of female cones with very little pollen.

Yeatman - The cones at ten years were pollinated when the trees were eight years old and at 10 years, if you went in and collected the cones that were on the trees, they would be from flowers born on those trees say 5, 6, 7, and 8 years and during that period of time there was relatively little pollen produced by jack pine.

Critchfield - That is the assumption that I would like to question, if jack pine is anything like Pinus contorta, because in P. contorta from the very start some individuals produce lots of female cones but other individuals produce quantities, I mean pounds of pollen.

Yeatman - There will be some pollen produced; but at Petawawa, and I must admit my experiences are confined to that jack pine environment, we find that the young jack pine produced relatively more female flowers than male flowers growing on a good site, i.e., growing vigorously. Now, if you put them on a deficient site, you probably will get a different story. I think there is a very large site quality-pollen production interaction.

Critchfield - I just brought it up because it seems like such a wide-spread assumption in forestry that young pine trees are predominately female, and did a study of flowering in Pinus contorta on a small plantation at the age of 6 to 10, and I expected predominately female flowering, but I certainly didn't get it. Some trees were overwhelmingly male.

Yeatman - I think that we must bear in mind the very major differences between the environment which you were working under at your plantation and the one we are used to, which is a moist summer; I think yours is dry, For instance, Mark Holst produced his provenance hybrids on jack pine provenance simply by taking off the few male inflorescence that were on the trees,

Hoist - There were not that few. It took some manpower to do it, but we did it.

Yeatman - However, I would still maintain, Bill, that within a clearing in the middle of a jack pine stand or population, the relatively small amount of the pollen from the young trees would be swamped by the background pollen from surrounding stands.

Critchfield - Okay, I can only say I am pretty sure that wouldn't be true of contorta because an individual tree no taller than 8 feet, that is 7 or 8 years old, can produce as many as 20,000 male strobili--at least 20,000. That is a very conservative figure.

Yeatman - There was a question on the value of seed production areas?

Schmitt - Not the value, whether you achieved any genetical improvement by using seed from production areas.

Yeatman - You will because if you do it properly, you will gain information on population genetics in jack pine, including the relative value of populations sampled. You then have the opportunity to capitalize on it and make in many cases, I am sure, real improvements. As an example, it is particularly true of the Maritimes provenances because we find very large differences between populations sampled within the region unresolved.

Schmitt - From what I hear, I have a positive answer from Hans who has test data which shows more than 10% improvement over area collections. I guess that with the jack pine that the issue still remains.

Nienstaedt - Well, I have one little bit of information on the jack pine. The old jack pine seed source studies that Paul Rudolf put in 1954 was made up of populations from "better than average stands." As a control in all the tests, he used the jack pine seed source commonly sown in the nursery where the material was grown. In 10 out of 11 or 9 out of 10 of the tests, definite improvement resulted from collections of seed from the better than average stand. This, to me, suggests that jack pine SPA seed will be an improvement.

Illingworth - I would like to corroborate Bill Critchfield's remarks about seed production in lodgepole. I don't think it is simply the fact that he is working with southern sources of lodgepole because in north central British Columbia lodgepole starts to flower prolifically at about four years of age and has both male and female cones. I would like to ask Bill or Jerry what their feelings are about clonal seed orchards in jack pine - are these a viable proposition or do they favor seed production areas or seedling seed orchards?

Klein - The clonal seed orchard, I think would be faster. I am assuming you started with selected genotypes, Now once you have selected genotypes whether they be parental clones or seedlings in a family test, you have selected genotypes, you can start either seedling seed orchards or clonal seed orchards. In any case, your seed orchard trees are preselected. Now on this basis, the clonal method of packaging I think gives you a head-start of a couple of years, but grafts are expensive to make--I find grafts are expensive to make--and get established in the seed orchard, so there is a trade of time against resources.

Kriebel - I want to comment on Dan's question because I don't think it was really completely answered about how much you expect to gain from the seed production areas. I think you can't generalize very well; it depends on the genetic uniformity of the stand with regard to the characteristics for which you are selecting. If it is a stand which is uniformly good in the characteristics for which you are selecting, your required selection intensity is low and you can make the thinning that is necessary for seed production and still have a superior stand. But if it is a genetically variable stand, you will have to thin so drastically to achieve the required selection intensity that you will be virtually clear-cutting. If you reduce the selection intensity, the gain is small or nil. So it depends on the variability of your stand, you can't generalize. It may vary with the species. It might be better for jack pine or red pine, for instance, than for white pine. I don't think it would be too effective in white pine.