X-Ray Study of Artificial Crosses in Picea abies (L.) Karst. and Picea glauca (Moench) Voss.

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(Received for publication February 24, 1961)

Seed of high quality is essential to the successful operation of forest nurseries and forestation projects. In addition to standard germination tests an array of viability "quick tests" have been developed to determine seed quality. Almost without exception these testing methods have one thing in common. The seed sample selected for a quick test is not itself germinated but its observable or measurable characteristics after treatment must be compared with the results of a standard germination test employing another sample from the same seed lot. In X-ray seed testing the observed and classified seed is itself germinated affording a direct comparison not otherwise possible.

In forest genetics work compatibility or fertilization could be determined by the development of the embryo. This has been done through cytological studies (8,9) and may be accomplished to some degree by X-ray observations (2).

This paper reports results of a small study indicating some of the possibilities of the X-ray method in determining seed quality and in observing embryo and endosperm development of seed produced by various breeding methods in Norway spruce (Picea abies [L.] KARST.) and White spruce (*Picea glauca* [MOENCH] VOSS.).

Review of Literature

The use of X-ray photography as a tool in seed testing and observation of embryo and endosperm development of forest tree seeds is of rather recent origin.

In 1953 work was initiated employing X-rays as a medium for seed observation by personnel of the State Forest

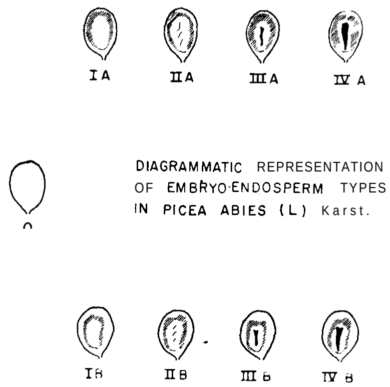

Figure 1. — Embryo-endosperm classification of Norway spruce by features observable on X-ray negatives (GUSTAFSSON and SIMAK). Condition of:

Embryo	Endosperm
I None present II One or more, none filling more than 1/2 of the embryo cavity. III One present, filling from 1/2 to 3/4 of the embryo cavity. IV One fully developed, filling the embryo cavity.	A Fully developed, of good X-ray absorption capacity B Poorly developed or shrunken, of low X-ray absorbing eapacity.

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Research Institute at Stockholm, Sweden. Investigators at this institute; including MÜLLER-OLSEN, GUSTAFSSON, SIMAK, EHRENBERG, and PLYM-FORSHELL (2, 3, 4, 5, 6, 10, 11, 12) have published results of their work which indicate possible applications of X-ray photography in testing forest tree seed. The major species investigated by means of X-ray photography at Stockholm included Picea abies (L.) KARST., *Pinus* sylvestris L. and *Alnus glutinosa* (L.) GAERTN.

SIMAK and GUSTAFSSON (1953) working mainly with Picea abies and *Pinus* sylvestris were able to develop a seed classification system (Figure 1) whereby a numerical rating (I—IV) for degree of embryo development and a letter subscript (A or B) for degree of endosperm development were assigned. Thus seeds were rated from I to IV on the basis of progression from no embryo to one which was fully developed with Roman numerals II and III representing intermediate stages. Letter subscripts of A and B represented well developed endosperrn with good absorption capacity and endosperm in an underdeveloped or shrunkeri condition respectively.

There was also an attempt in their work to determine the behavior of forest tree seed after irradiation including the subsequent plant development, the recovery or death of the growing irradiated individuals and the formation of aberrations from induced mutation. The species selected were: Alnus *glutinosa* and *incana,* Pinus sylvestris, Picea abies, Larix *sibirica* and Thuja plicata. Samples of seed of each of these species were subjected to dosages of 75 to 25,000 roentgens. With dosages of 5,000—25,000 roentgens not a single seed of pine or spruce germinated. In the case of alder 70% germination was obtained after 60 days with a dosage of 5,000 roentgens but the seedlings were weak and few survived. Alder seed subjected to 200 roentgens showed an increase in germination per cent. With dosages as low as 200—300 roentgens there was a decrease in viability of spruce and pine seed while a dosage of 75—150 roentgens gave results similar to the control lots. Of the species tested most exhaustively alder was least sensitive, pine appeared to be intermediate and spruce was the most sensitive.

MÜLLER-OLSEN and SIMAK (1954) working with freshly harvested Scots pine seed obtained a close correlation between actual germination and features of the seed observable on X-ray negatives. Practically all of the seed classified II had two and sometimes more embryos, none of which were fully developed. Most of the germination in this embryo class was abnormal.

SIMAK (1956) developed the X-ray contrast method whereby comparisons were made between observable characteristics and germination of freshly harvested Scots pine seed and seed of the same species which had been stored for varying periods. Both fresh and stored seed were soaked in a 30% $BaCl_2$ solution for two hours and subjected to the X-ray method. The enclosing membranes and endospermous tissue of seed lose some of their semi-permeability as seed vitality decreases through prolonged storage or storage for short periods under unfavorable conditions. Thus there is a greater degree of penetration of $BaCl_2$

solution in seeds of low germinative power. This same principle is applied in quick tests using vital dyes. SIMAK obtained a close correlation between degree of impregnation, ovservable on the X-ray negatives, and loss of viability which now provides a means of testing stored as well as fresh seed of Scots pine.

Some of the first work in X-ray seed testing in the United States was conducted by THORBJORNSEN (1956) using *Pinus ponderosa* LAWS. He obtained little correlation between embryo classes and the results of germination in sand flats. Of four lots of seed the germination capacity of seed classified as IV A ranged from 13 to 69%.

On the basis of the results obtained through the use X-ray photography in Sweden, NAMKOONG (1958) conducted some preliminary studies using X-rays in the investigations of *Pinus resinosa* AIT., *Pinus strobus* L. and *Picea abies*. He obtained a positive correlation between embryo lenght and development of endosperm observed on negatives of X-rayed seed and the response of the same seed when placed in a germinator.

Procedure

In the spring of 1958 various Norway spruce and white spruce trees growing at Pack Demonstration Forest, Warrensburg, New York, were used for artificial hybridization experiments. Five Norway spruce and five white spruce trees were chosen for this purpose. The Norway spruce trees were numbered from 1 to 5 and the white spruce trees from 1 to 5. The trees were intra-crossed, inter-crossed (Norway spruce with a pollen mixture of five white spruce trees and white spruce with a pollen mixture of five Norway spruce trees), selfed, and some flowers were kept unpollinated. All cones were harvested in fall 1958 and open pollinated cones were added. The spruce cones were dried and the seeds extracted and cleaned. Random samples of 100 seed resulting from each of the five treatments indicated were weighed to determine if there was any consistent relationship between treatment and seed weight. Random samples of ten seed each representing one of the treatments of the various trees were placed in rows on X-ray film such that each row consisted of seed obtained from the same lot and the same tree. The equipment employed in the X-ray study was a North American-Philipps X-ray unit used by laboratory personnel for crystal diffraction studies. As such it was not the most suitable type for radiographic work. Despite certain shortcomings of the X-ray unit, quite good results were obtained in securing negatives with sufficient contrast.

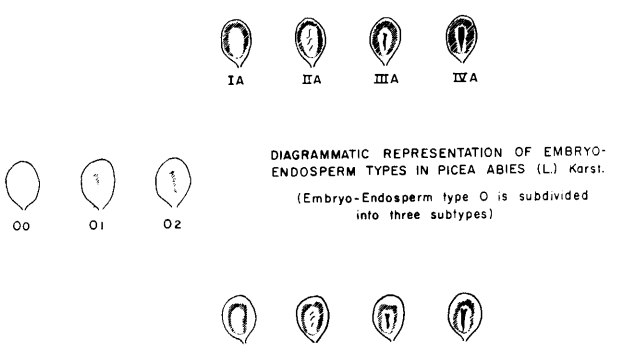

Figure 2. — Embryo-endosperm classification of Norway and white spruce. Embryo-endosperm type 0 is subdivided into three subtypes.

Subtype 0_0	Seed completely empty.
Subtype 0_1	Shadow inside the seed less than 1/3 of total seed length.
Subtype 0_2	Shadow inside the seed longer than 1/3 of total seed length.

Table 1. — Relationship between cross type and weight of *Picea abies* and *Picea glauca* seed.

Species crossed	Cross type (Treatment)	100 gr. wgt. (grams)	Weight in % of open pollinated seed (O.P. = 100%)	Order (by wgt.)
1	2	3	4	5
1. *Picea abies*				
a) No. 1	open-poll.	0.47	100	2
b) No. 1	unpoll.	0.21	47	5
c) No. 1 × *Picea glauca* No. 1—5	inter-cross	0.38	81	4
d) No. 1 × No. 2	intra-cross	0.62	132	1
e) No. 1 × No. 1	selfed	0.40	85	3
2. *Picea abies*				
a) No. 2	open-poll.	0.54	100	1
b) No. 2	unpoll.	—	—	—
c) No. 2 × *Picea glauca* No. 1—5	inter-cross	0.25	46	3
d) No. 2 × No. 1	intra-cross	0.37	68	2
e) No. 2 × No. 2	selfed	0.23	42	4
3. *Picea abies*				
a) No. 3	open-poll.	0.56	100	1
b) No. 3	unpoll.	—	—	—
c) No. 3 × *Picea glauca* No. 1—5	inter-cross	0.38	68	4
d) No. 3 × No. 4	intra-cross	0.47	84	2
e) No. 3 × No. 3	selfed	0.42	75	3
4. *Picea abies*				
a) No. 4	open-poll.	0.75	100	1
b) No. 4	unpoll.	0.19	25	5
c) No. 4 × *Picea glauca* No. 1—5	inter-cross	0.35	47	3
d) No. 4 × No. 3	intra-cross	0.75	100	2
e) No. 4 × No. 4	selfed	0.25	33	4
5. *Picea abies*				
a) No. 5	open-poll.	0.64	100	1
b) No. 5	unpoll.	0.15	23	5
c) No. 5 × *Picea glauca* No. 1—5	inter-cross	0.32	50	4
d) No. 5 × No. 2	intra-cross	0.38	59	2
e) No. 5 × No. 5	selfed	0.33	52	3
6. *Picea glauca*				
a) No. 1	open-poll.	0.27	100	1
b) No. 1	unpoll.	0.045	17	4
c) No. 1 × *Picea abies* No. 1—5	inter-cross	0.08	30	3
d) No. 1 × No. 3	intra-cross	0.15	56	2
e) No. 1 × No. 1	selfed	—	—	—
7. *Picea glauca*				
a) No. 2	open-poll.	0.26	100	2
b) No. 2	unpoll.	—	—	—
c) No. 2 × *Picea abies* No. 1—5	inter-cross	0.11	42	3
d) No. 2 × No. 1	intra-cross	0.31	119	1
e) No. 2 × No. 2	selfed	0.11	42	4
8. *Picea glauca*				
a) No. 3	open-poll.	0.22	100	1
b) No. 3	unpoll.	0.055	25	5
c) No. 3 × *Picea abies* No. 1—5	inter-cross	0.125	57	3
d) No. 3 × No. 2	intra-cross	0.20	91	2
e) No. 3 × No. 3	selfed	0.10	45	4
9. *Picea glauca*				
a) No. 4	open-poll.	0.15	100	1
b) No. 4	unpoll.	—	—	—
c) No. 4 × *Picea abies* No. 1—5	inter-cross	0.075	50	3
d) No. 4 × No. 3	intra-cross	0.10	67	2
e) No. 4 × No. 4	selfed	0.075	50	4
10. *Picea glauca*				
a) No. 5	open-poll.	0.29	100	1
b) No. 5	unpoll.	0.06	21	5
c) No. 5 × *Picea abies* No. 1—5	inter-cross	0.10	34	4
d) No. 5 × 2	intra-cross	0.25	86	2
e) No. 5 × No. 5	selfed	0.13	45	3

The average lenght of time for complete evaluation of a 200 seed sample including attaching the seed to the film, exposing, developing and reading was 1½ hours. This is much less time than would be required to obtain the same information by other methods.

The setting used for exposure of the film was 25 kilovolts and 12 milliamperes for a period of 8—12 seconds (depending upon the species) at a distance of 32 inches (81.3 cm) from the source. At this setting and distance the X-ray dosage for the time indicated was quite small (7—8 roentgens) — much below that indicated by BALDWIN (1942), as being detrimental to seed germination.

The dosage indicated above was also far below the average applied by MÜLLER-OLSEN, SIMAK and GUSTAFSSON (1956) in their work with Scots pine. It was much below that used by THORBJORNSEN who irradiated ponderosa pine with a dosage of approximately 780 roentgens. The limit for Scots pine beyond which there is a pronounced decrease in viability was determined by SIMAK and GUSTAFSSON (1953) to be in the neighborhood of 200—300 roentgens.

Table. 2. — Classification of seed on the basis of the x-ray negatives and their actual germination.

Species and tree number	Cross type (treatment)	Number of seeds in class and germination ( )											Total number of seed
		0_0	0_1	0_2	IA	IB	IIA	IIB	IIIA	IIIB	IVA	IVB	
Picea abies	open-poll.	1	4	4							1 (1)		10
	unpoll.	9	1										10
No. 1	inter-cross		5	5									10
	intra-cross	2	1								7 (7)		10
	selfed	3	3	3							1 (1)		10
	open-poll.	3	3	4									10
	unpoll.	—	—	—	—	—	—	—	—	—		—	—
No. 2	inter-cross	6	2	2									10
	intra-cross	1	2	4							3 (3)		10
	selfed	5	3	2									10
	open-poll.	1	1	7							1 (1)		10
	unpoll.	—	—	—	—	—	—	—	—	—	—	—	—
No. 3	inter-cross	2	2	6									10
	intra-cross	0	3	2							5 (5)		10
	selfed	0	3	7									
	open-poll.	4	1	2							3 (3)		10
	unpoll.	10											10
No. 4	inter-cross	3	3	4									10
	intra-cross	4	1								5 (5)		
	selfed	5	2	3									10
	open-poll.	1	1	1							7 (6)		10
	unpoll.	9	1										10
No. 5	inter-cross	4	3	3									10
	intra-cross	2	3	3							2 (2)		10
	selfed	3	2	5									10
Picea glauca	open-poll.	5	2					1			2 (2)		
	unpoll.	10											
No. 1	inter-cross	7	1	2									
	intra-cross	2	1								7 (7)		
	selfed	—	—	—	—	—	—	—	—	—		—	—
	open-poll.	2	3			1					4 (4)		10
	unpoll.	—	—	—	—	—	—	—	—	—	—	—	—
No. 2	inter-cross	5	3	2									10
	intra-cross	2	1								7 (7)		10
	selfed	5	2	2		1							10
	open-poll.	3	3								4 (4)		10
	unpoll.	10											10
No. 3	inter-cross	9		1									10
	intra-cross	2	2	2							4 (4)		10
	selfed	4	5			1							10
	open-poll.	5	2			1					2 (2)		10
	unpoll.	—	—	—	—	—	—	—	—	—	—	—	—
No. 4	inter-cross	7	1	2									10
	intra-cross	4	1	2							3 (3)		10
	selfed	7	2	1									10
	open-poll.								1		9 (8)		10
	unpoll.	9	1										10
No. 5	inter-cross	4	4	2									10
	intra-cross	0	1								9 (7)		10
	selfed	2	5	3									10
Total		182	90	86		4		1	1		86(82)		450

In former studies using the X-ray technique of evaluating seed of various spruces and fires obtained after controlled crosses it was found rather consistently that shadows of varying length appeared in the seed which would in accordance with the classification system mentioned before, be classified as blind or empty. Small samples of the seed producing these shadows were cut and the interior of each seed checked against the features visible on the X-ray negative. In each instance the length and relative position of the remnant found in the otherwise empty seed corresponded exactly to the characteristics of the shadow appearing on the negative. No cytological study was made of the tissues in these seeds but it is felt that they result from aborted ovules.

To obtain more detailed information on the varying length of the shadows in relation to the cross types the class 0 was subdivided into three classes 0, 01 and 02. *Figure 2* shows the classification system used in this study. Each seed of the ten X-ray negatives was investigated independently by 12 students with magnifying glasses over a strong light source and tabulated according to the corresponding embryo-endosperm class.

The actual germination was obtained by germinating the seeds on filter paper in petri dishes, each seed in the same position it had occupied on the X-ray negative. The seed of white spruce was stratified for two months before the germination test.

Results

The results of the weighing tests are shown in *Table 1.* Column 1 presents the "Species crossed", column 2 "Cross type" (Treatment), 3 "100 grain weight in grams", column 4 "Weight in per cent of open-pollinated seed (o. p. = 100%)" and column 5 "Order by weight".

The weighing data indicate that:

1. A fairly constant relationship is evident between seed weight and the respective cross type. The usual order of seed weight, from heaviest to lightest, is as follows: Open-pollinated, intra-cross, inter-cross, selfed and unpollinated. Seed of unpollinated controls always occupied the last place.

2. The well known incompatibility of Norway spruce × white spruce and the reciprocal cross, was evident (see *Tables 2 and 3*). Seed weight of these crosses is lower than for the corresponding intra-cross, and in a few instances, lower than for the selfeld seed from the same tree.

The X-ray and active germination results are presented in *Tables 2 and 3*. Data from *Tables 2 and 3* indicate that:

1. Unpollinated cones furnished the highest proportion of seed in embryo-endosperm class 0_0 (completely empty) (see *Figures 3 and 5*). Application of any pollen, even from the seed tree in the case of selfing, caused a reduction in the proportion of seeds in this lowest class (see *Figures 3, 4 and 5* and *Table 3*). Thus, selfing has caused a general rise in the embryo-endosperm class of the resulting seeds over that of unpollinated seeds.

2. All selfing procedures resulted in seed of lower embryo-endosperm classes than those from intra-crosses or open pollination. In *Figure 3* only seed of the selfed material is filled (class IVA). (This Norway spruce tree furnished the highest number of selfed seedlings in the nurs-

Table 3. — Summary of X-ray classification and corresponding germination capacity of *Picea abies* and *Picea glauca* in relation to treatment.

Species	Treatment	0_0 %	0_1 %	0_2 %	IA %	IB %	IIA %	IIB %	IIIA %	IIIB %	IVA %	IVB %	Germination seed %	Per Treatment seed %
Picea abies	Open poll.	10 (20)	10 (20)	18 (36)							12 (24)		11 (22)	50 (100)
	unpoll.	28 (93.3)	2 (6.7)											30 (100)
	inter-cross	15 (30)	15 (30)	20 (40)										50 (100)
	intra-cross	9 (18)	10 (20)	9 (18)							22 (44)		22 (44)	50 (100)
	selfed	16 (32)	13 (26)	20 (40)							1 (2)		1 (2)	50 (100)
	Total	78 (33.9)	50 (21.7)	67 (29.1)							35 (15.2)		34 (14.8)	230 (100)
Picea glauca	open poll.	15 (30)	10 (20)			2 (4)		1 (2)	1 (2)		21 (42)		21 (42)	50 (100)
	unpoll.	29 (96.7)	1 (3.3)										—	30 (100)
	inter-cross	32 (64)	9 (18)	9 (18)									—	50 (100)
	intra-cross	10 (20)	6 (12)	4 (8)							30 (60)		27	50 (100)
	selfed	18 (45)	14 (35)	6 (15)		2 (5)							—	40 (100)
	Total	104 (47.3)	40 (18.2)	19 (8.6)		4 (1.8)		1 (0.45)	1 (0.45)		51 (23.2)		48 (21.8)	220 (100)
Total		182 (40.5)	90 (20)	86 (19.1)		4 (0.9)		1 (0.2)	1 (0.2)		86 (19.1)		82 (18.2)	450 (100)

% of Class IVA germinated (Total) 97.6

ery bed. Out of 285 seeds sown in spring 1959, 5.3% germinated and a year later most of the seedlings showed very clearly selfing characteristics.)

3. There was a very close agreement between X-ray results and actual germination in the experiment. 97.6% of the seed of embryo-endosperm class IVA germinated while seed of embryo-endosperm class 0_0 to IIIA and IIIB did not. The smaller size of all the unpollinated control seed lots and in most instances uniformly large size of the lots obtained from all the other treatments (see *Figure 3 and 5*) is noteworthy.

Discussion

The results of the X-ray photographs and seed weight pose a question of particular interest. This question concerns the effect of pollen on the development of the egg in crosses where production of viable seed is low or completely lacking. The weighing tests have shown that pollinations using pollen from the seed tree or from a tree of another species which is incompatible will produce seed weight much less than that of filled seed but greater than that of seed from unpollinated cones. The appearance of aborted ovules in the X-ray negatives (see *Figures 3, 4 and*

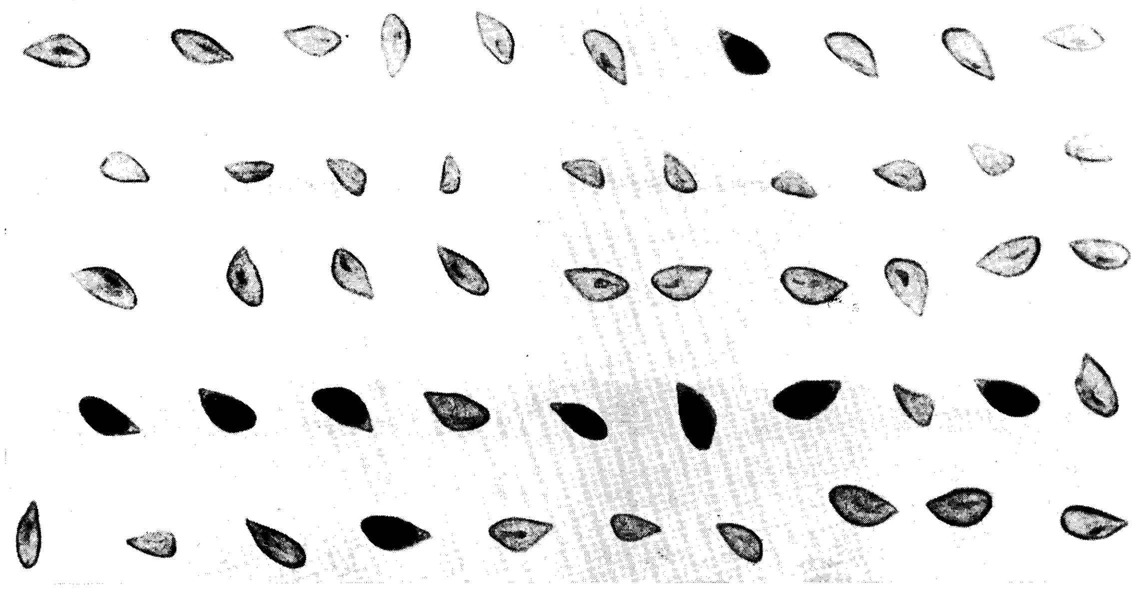

Figure 3. — *Picea abies* Tree No. 1:

Row 1	Open-pollinated.
Row 2	Unpollinated control.
Row 3	Inter-crossed (obtained after crossing with mixed pollen of five *Picea glauca* trees).
Row 4	Intra-crossed (*Picea abies* × *Picea abies*).
Row 5	Selfed.

Note the small size of unpollinated control and uniformly larger size of inter-crossed empty seed.

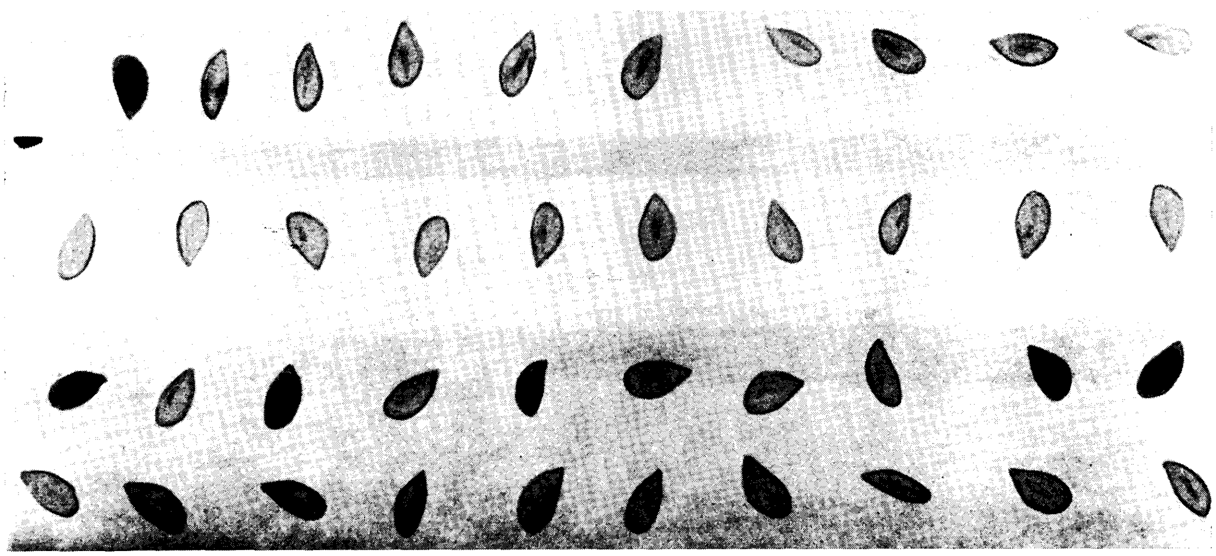

Figure 4. — *Picea abies* Tree No. 3:

Row 1	Open-pollinated.
Row 2	Inter-crossed (obtained after crossing with mixed pollen of five *Picea glauca* trees).
Row 3	Intra-crossed (*Picea abies* × *Picea abies*).
Row 4	Selfed.

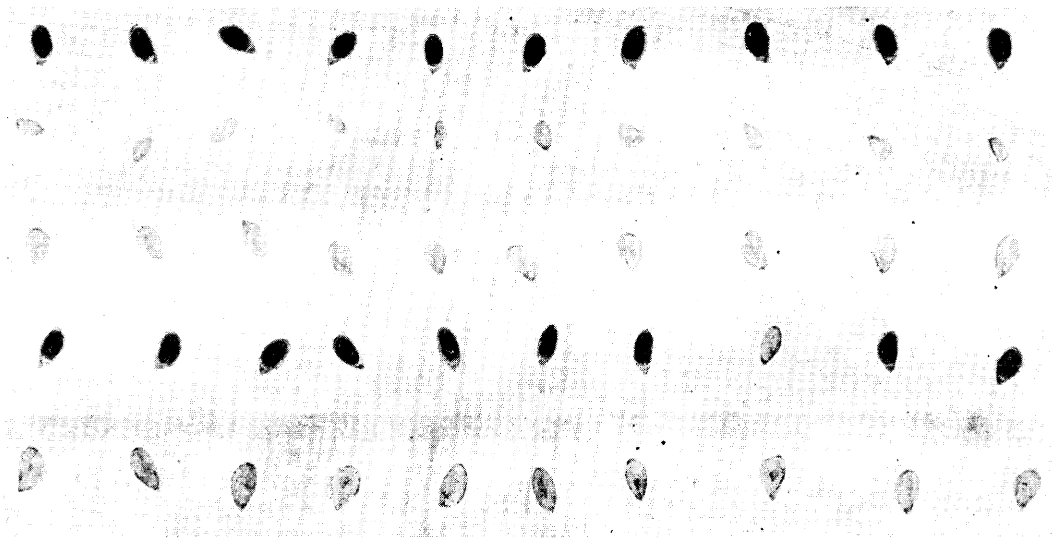

Figure 5. — *Picea glauca* Tree No. 5:

Row 1	Open-pollinated.
Row 2	Unpollinated control.
Row 3	Inter-crossed (obtained after crossing with mixed pollen of five *Picea abies* trees).
Row 4	Intra-crossed (*Picea glauca* × *Picea glauca*).
Row 5	Selfed.

Note the small size of unpollinated control and uniformly larger size of the inter-crossed and selfed seed which are empty.

5) indicated that selfed as well as inter-crossed seed are not completely empty. Ninety-five per cent of the seed from all the unpollinated cones was completely empty. In contrast the number of completely empty seed resulting from all the other treatments ranged from 20 to 64% (see *Table 3*). On the average, however, only 33.1% were completely empty. The remaining seeds were filled or at least contained the remains of an aborted ovule.

Referring to work done by ORR-EWING (1957) on the effect of self-pollination of *Pseudotsuga menziesii* (MIRB.) FRANCO it is possible that the tissues detected in most of the selfed and inter-bred *Picea abies* and *Picea glauca* seed result from collapsed embryos and remnants of the female gametophyte. Such collapsed embryos were found quite frequently by ORR-EWING in his cytological study of inbred ovules. Since he found a variation in the developmental stage at which collapse of the embryo occurred, usually followed by breakdown of the cells of the female gametophyte, it is probably not surprising that the amount of tissue and corresponding observable features on the X-ray negatives exhibit a like variation. In some instances it is possible that an embryo may develop as a result of selfing or inter-crossing between two normally incompatible species which would not collapse but would develop fully to produce a seed of Class IVA or IVB (*Figure 3* — Row 5).

If the described shadows in the X-ray negatives are the result of collapsed embryos, this would mean:

1. That selfing procedures at least in *Picea abies* and *Picea glauca* and the above mentioned inter-crosses between these two species lead in many instances to fertilization of the egg and that after a fairly normal pro-embryo formation the embryo collapses at a rather early stage. This stage is expressed by the length of the shadow.

2. That whether or not fertilization has taken place and the stage of development at which the collapse occurs may be detected by X-ray techniques.

Summary

X-ray technique developed in Sweden for evaluating seed has been tested for four years at the State University College of Forestry at Syracuse University. The use of the techniques for estimating seed quality and germination offers interesting research opportunities. This study shows possible application of the method used in forest genetics studies to evaluate seed resulting from cross and self-pollinations with reference to the degree of embryo and endosperm development even if the seed is incapable of germination.

It is felt in accordance with work done by ORR-EWING that selfing procedures and crosses of normally incompatible species of spruces lead in many instances to fertilization of the egg and that it depends upon the "degree of incompatibility" as to whether a viable seed occurs or not. This degree can be determined by X-ray photography also on seed which normally might be classified as belonging to Class 0.

Zusammenfassung

Titel der Arbeit: *Röntgen-Untersuchung bei künstlichen Kreuzungen von Picea abies und Picea glauca.*

Die Röntgenstrahlen-Technik, eine Saatgut-Testmethode, die in Schweden entwickelt wurde, ist in den letzten vier Jahren einer Prüfung am State University College of Forestry an der Syracuse University unterzogen worden. Die Methode, die Qualitäts- und Keimfähigkeits-Schätzungen möglich macht, erlaubt die Durchführung interessanter Forschungsvorhaben. Diese Arbeit zeigt einen Weg der Anwendung auf forstgenetischem Gebiet und behandelt die Auswertung von Kreuzungs- und Selbstungssaatgut und dessen Embryo- und Endospermausbildung auch in Fällen, in denen das Saatgut selbst nicht keimfähig ist.

Es wird unter Berücksichtigung von Arbeiten von ORR-EWING angenommen, daß Selbstungen und Kreuzungen von normalerweise nicht-kreuzbaren Fichtenarten in vielen

Fällen zur Befruchtung der Eizellen führen und daß es von dem Grad der „Unverträglichkeit" abhängt, ob lebensfähige Samen erzeugt werden oder nicht. Dieser Grad der „Unverträglichkeit" kann, auch an Samen, die normalerweise zur Klasse 0 zu rechnen sind, mit Hilfe der Röntgenphotographie bestimmt werden.

Résumé

Titre de l'article: *Etude par radiographie des croisements artificiels entre Picea abies (L.) Karst. et Picea glauca (Moench) Voss.*

La technique de radiographie, mise au point en Suède pour l'évaluation de la qualité des graines a été essayée depuis 4 ans au »State University College of Forestry« à l'université de Syracuse. L'emploi des techniques d'évaluation de la qualité des graines et de la faculté germinative présente des voies intéressantes pour la recherche. Cette étude montre une application possible de cette méthode en génétique forestière pour l'examen des graines résultant des croisements et des autofécondations en se basant sur le développement de l'embryon et de l'endosperme, cela même si la graine n'est pas capable de germer.

On estime, d'accord avec le travail réalisé par ORR-EWING que l'autopollinisation et les croisements entre espèces d'épicéa normalement incompatibles conduisent dans beaucoup de cas à la fertilisation de l'oeuf; c'est le »degré d'incompatibilité« qui détermine s'il se produit ou non une graine viable. Ce degré peut être évalué par radiographie également sur les graines qui seraient normalement réunies dans la classe 0.

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A Karyotypic Analysis of Selected Species of Pinus

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(Received for publication March 14, 1961)

Introduction

Genetics and breeding have received considerable emphasis in recent years as means of improving commercial pine species. Although the value of such programs has been amply demonstrated, their progress is often hindered by the lack of basic information from such disciplines as physiology, cytology, plant breeding, and genetics.

Lack of cytogenetic information in the genus *Pinus* can probably be attributed to at least two factors. First, it is likely that the results of early karyological investigations discouraged additional studies in this field. These findings indicated that the genus is homoploid ($2n = 24$)[2]) with very little if any interspecific variation in karyotype. As a consequence it has been assumed that evolution has occurred almost exclusively at the genic level without involving detectable chromosomal alterations. To date no major evidence has been found to contradict this opinion, even though it is based on information from less than one-third of the species. Secondly, attempts to make detailed studies of chromosome morphology have been hampered because the numerous and long chromosomes, characteristic of this genus, frequently overlap and become obscured. Before comprehensive cytological investigations can be undertaken, therefore, techniques must be perfected that enable a thorough examination of the chromosomes. Then, if structural rearrangements of the chromosomes have occurred during the course of evolution of this genus, cytogenetic studies may prove useful in elucidating species relationships.

The present study is part of a continuing cytogenetic investigation of the entire genus *Pinus*. This paper describes: (1) new or modified techniques for obtaining critical data on the chromosomes of this genus; (2) results obtained from a karyotypic analysis of a selected group of species.

Review of Literature

The few investigators who have examined the genus *Pinus* for karyotypic variation have stressed the uniformity of chromosomes within karyotypes, as well as the general similarity of the karyotypes among the different species. SAX and SAX (1933) contributed the first significant information concerning this genus. These authors concluded: "The chromosomes of all species of *Pinus* seem to be very similar. One of the 12 chromosomes is somewhat heterobrachial, and the others have approximately median

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Contribution from the Department of Genetics and the Department of Forest Management, North Carolina Agricultural Experiment Station as Journal Paper No. 1289.

The author is indebted to Dr. BEN W. SMITH and Dr. BRUCE J. ZOBEL for their helpful advice during the course of this investigation.

[2]) Polyploid and mixoploid seedlings occasionally occur naturally (KHOSHOO, 1959; MERGEN, 1958), although these seedlings rarely if ever reach maturity because of defective growth.