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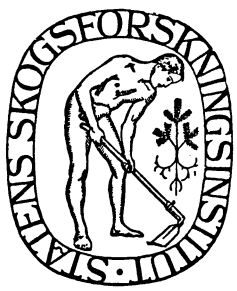

Germination analyses by the X-ray method: Picea Abies (L.) Karst.

Röntgenfotografering vid grobarhetsanalyser av gran

by

CARL MÜLLER-OLSEN,
MILAN SIMAK and ÅKE GUSTAFSSON

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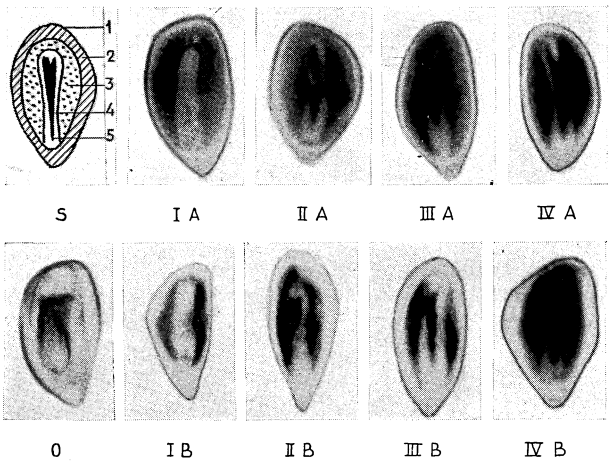
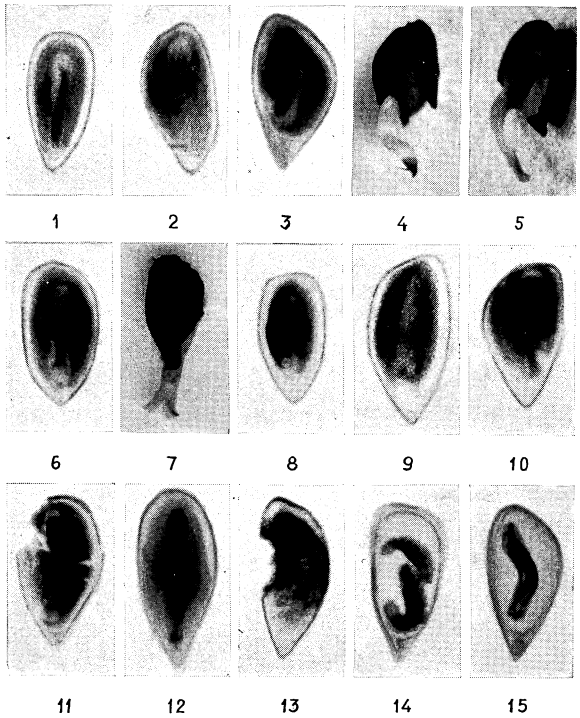
Plate I.



The Norway spruce seed-types. Key to the diagram "S": 1. seed-coat, 2. empty space between seed-coat and endosperm, 3. endosperm, 4. embryo cavity, 5. embryo.

Plate II.



1 and 2: deviating types of class IV A. 3, 4, and 5: seed with abnormal development of embryo cavity. 6 and 7: reversed embryo. 8 and 9: polyembryony. 10: seed with double embryo cavity and embryos. 11: mechanically damaged seed. 12: "luminous" embryo. 13, 14, and 15: examples of insect damage.

Germination analyses by the X-ray method: Norway spruce

The present study is a continuation of a series of experiments which have been carried out at the Genetics Department of the Swedish Forest Research Institute, Stockholm, with the object of developing a method of germination analysis of conifer seeds, based on X-ray photography. The following publications, employing or mentioning this method, have previously been issued from the Department: SIMAK & GUSTAFSSON, 1953 a, b, & c; PLYM FORSHELL, 1954; SIMAK & GUSTAFSSON, 1954; MÜLLER-OLSEN & SIMAK, 1954; EHRENBERG, GUSTAFSSON, PLYM FORSHELL, & SIMAK, 1955; and SIMAK, 1955.

The method of predicting the germinative properties of a seed from its X-ray photograph is based on the close correlation between said properties and the morphological characteristics of the seed's embryo and endosperm. In the report on X-ray analysis of Scots pine seed (MÜLLER-OLSEN & SIMAK, 1954) we have defined a range of 9 embryo-endosperm classes, and these have been employed in this experiment, too.

The investigation was divided in two parts:

1) Determination of the relation between embryo-endosperm characteristics and seed-quality, *i. e.* germination percentages and germinative vigour.
2) Assessment of the results for practical seed-testing purposes.

The seed material for the experiment was harvested in the autumn of 1954, the output from each tree being kept separate. To obtain the widest possible range of seed classes within one tree the stress was laid on collections from northern localities where the less developed seed types are found more frequently than in the south. Seed of South-Swedish origin was, however, also used. The ideal seed-lot, comprising all embryo-endosperm classes in sufficient quantities was, unfortunately, not to be found. We wish to emphasize the fact that the analysis refers to freshly collected, physiologically uninjured seeds only.

The X-ray plant as well as the technique of photography have previously been described in detail, for which reason only a summary of the procedure for this experiment shall be given here.

Perforated cardboard frames, each perforation holding one seed (to make practicable a control of each individual seed) are laid on the film-container and exposed to radiation. The X-ray dosage for a satisfactory exposure is very small (f = 25 cm, 12 kV, 25 mAs, without the use of intensifying screens) and is absolutely harmless to the germinative qualities of the seed, Fig. 1 (cf. BALDWIN, 1936, and SIMAK & GUSTAFSSON, 1953 a, b). When the film is

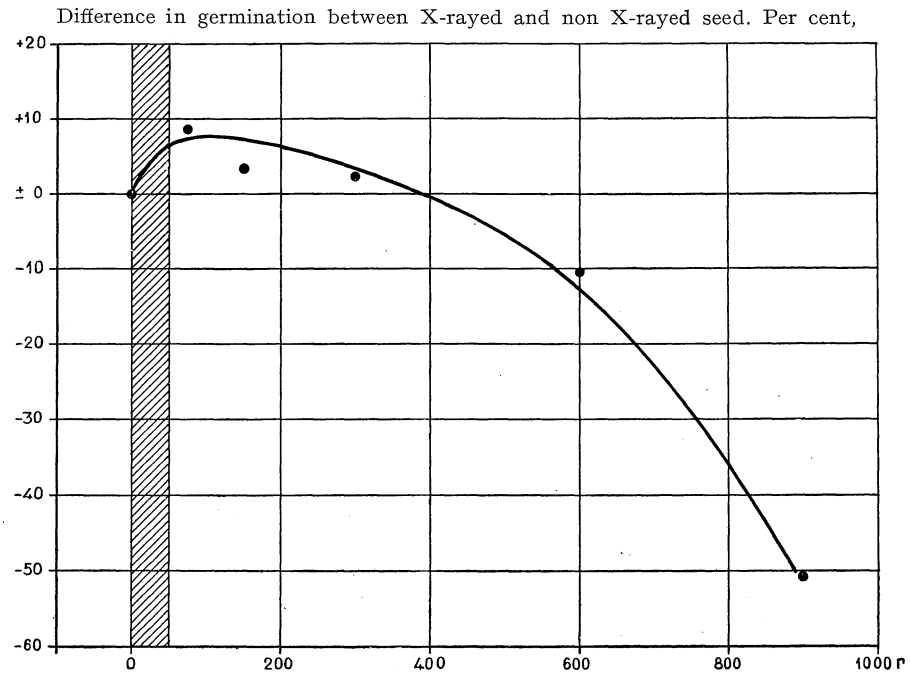


Fig. 1. The effect of X-radiation (r) on the germinative capacity (per cent) of Norway spruce seeds. The hatched column indicates the dosage (exaggerated) for photography.

processed and dry it can be studied in a simple ground-glass-viewer, if convenient with the aid of a magnifying lens.

After the usual extraction and de-winging, the seeds were photographed as described above and sorted out to seed classes in accordance with the photographs. The quantity of seeds thus examined has been determined only by the expediency of having at least 500 seeds of each class available for the experiment.

To establish a consistency in seed classification throughout we have, as mentioned, used the definitions for Scots pine seed types as a pattern, (SIMAK & GUSTAFSSON, 1954, and MÜLLER-OLSEN & SIMAK, 1954), namely:

Embryo class
- 0: Neither embryo nor endosperm (= empty seed).
- I: Endosperm, but no embryo.
- II: Endosperm, and one or several embryos, none of which longer than half of the embryo cavity.
- III: Endosperm, and one, not wholly developed, embryo, the length of which measures between half and three quarters of the embryo cavity.
- IV: Endosperm with one fully developed embryo, completely or almost completely occupying the embryo cavity.

Each of the embryo classes I to IV are divided into two endosperm classes, defined as follows:

Endosperm class A: The endosperm almost fills the seed coat to capacity and absorbs the X-radiation well.

B: The endosperm only fills the seed coat incompletely and is often shrunken or otherwise deformed. The X-ray absorption is inferior to that of class A.

In addition to these, the following seed types have been observed:

Abnormal seeds: Seeds with embryo in reversed position, (the cotyledons towards the micropylar end). Plate II, 6 and 7.

"Luminous" embryos: The embryo is very clearly outlined against the endosperm and possesses a higher capacity for X-ray absorption (shows up lighter on the X-ray negative) than the endosperm. Plate II, 12. Seeds of this category are not viable.

Insect damage: May be observed in the seeds by the presence of caterpillars or frass. Plate II, 13, 14 and 15 (cf. SIMAK, 1955).

After classification and sorting-out to embryo-endosperm classes the seeds were laid out in portions of 50, for germination in the Jacobsen germinator for 50 days from December 29th, 1954 to February 17th, 1955. Temperature was kept constant at 23° C and the seeds were irradiated for 8 hours daily by artificial light from three 40 Watt daylight-tubes, placed 50 cm above the germinator (Fig. 2).

Fig. 2. The Jacobsen germinator of the experiment.

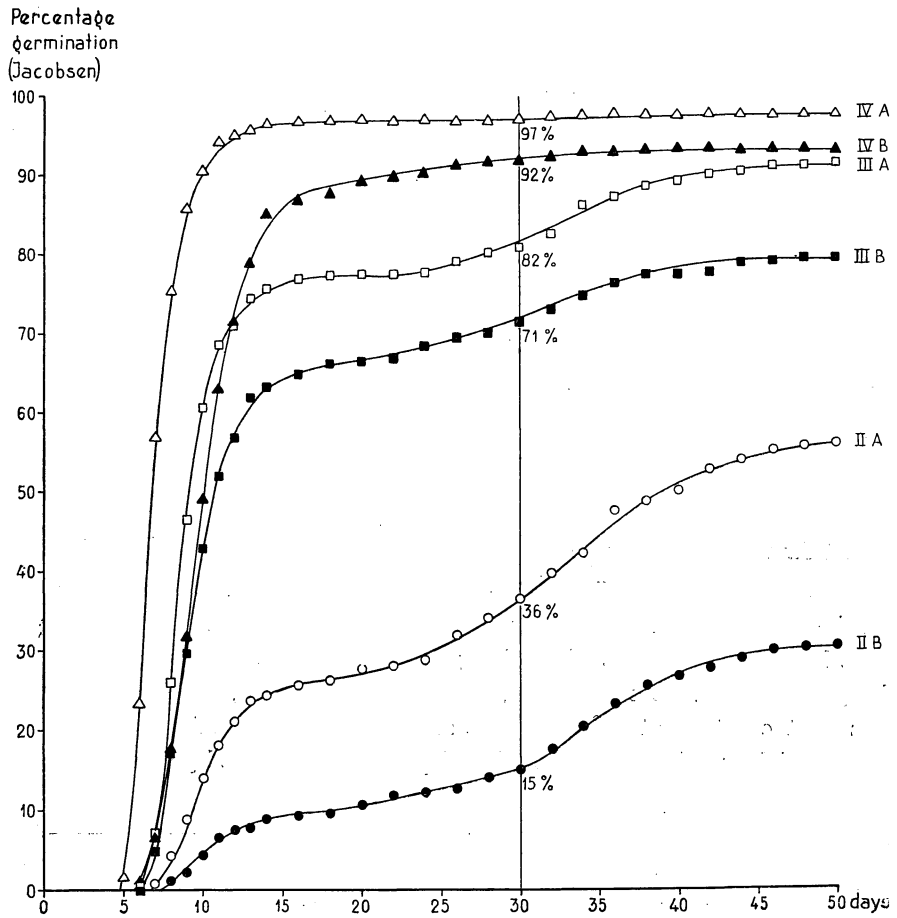


Fig. 3. Progress of germination of embryo-containing seed-types of Norway spruce during a 50-day period in the Jacobsen germinator. Values of germination in per cent.
The 30-day stage provides the reduction-factors of Table 1.

Table 1. Reduction-factors, *i. e.* germination percentages after 30 days in the Jacobsen germinator, of Norway spruce seed-types.

Endosperm class	Embryo class				
	0	I	II	III	IV
A.....	0	0	36	82	97
B.....	0	0	15	71	92

Assessments were made daily. The seeds were counted as having germinated when the roots of the seedlings had reached a length of 5 mm. The seedlings were left for further growth on the filter-papers till the end of the first 30 days, after which time the remaining, non-germinated seeds were transferred to fresh filter-papers for continued germination.

The germination results are shown in Fig. 3 and Table 1.

To put these values to the test, another series of seed samples were analysed. This seed material was very kindly placed at our disposal by E. Huss, Master of Forestry, and consisted of collections, also made in the autumn of 1954,

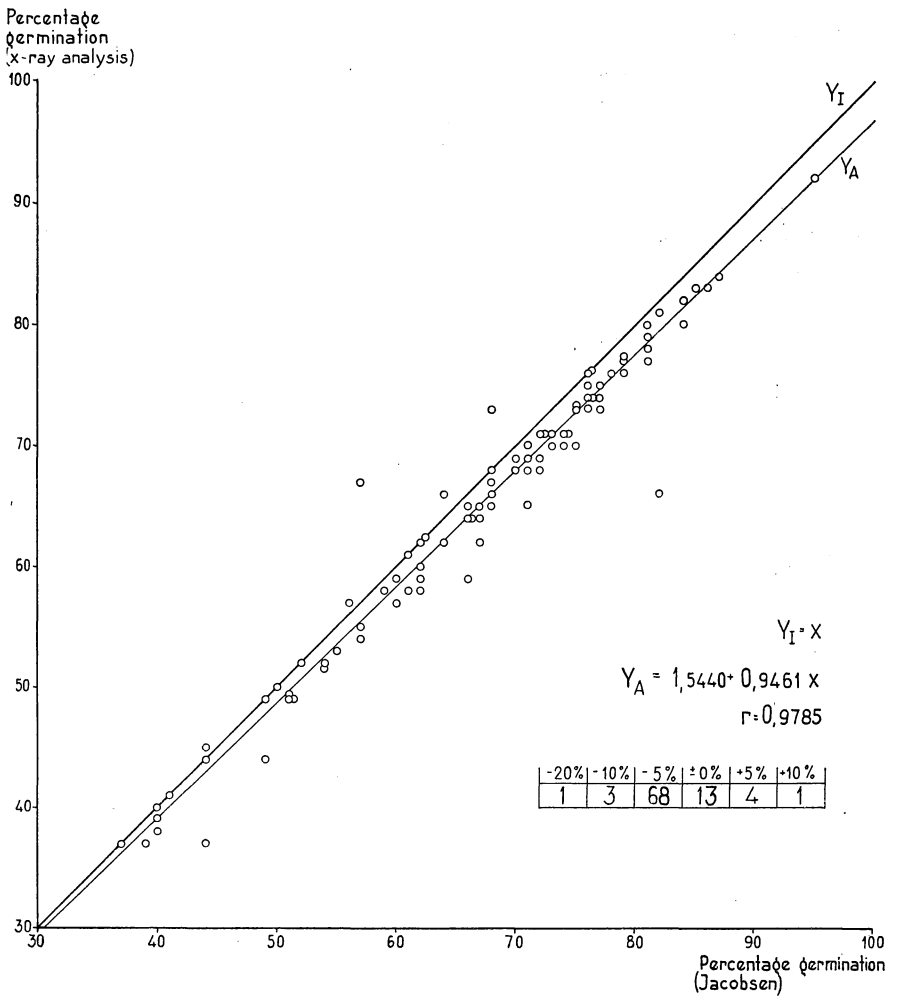


Fig. 4. The regression line (Y_A) of the X-ray analyses of 90 test samples of Norway spruce seeds in relation to the complete agreement (Y_I) between X-ray and Jacobsen analyses.

covering most of Sweden. 100 seeds from each of these 90 samples were photographed—spread directly on the film-container without the use of perforated frames—and subsequently grown in the Jacobsen germinator for 30 days from March 18th to April 17th, 1955. The seeds were controlled daily and seedlings with roots over 5 mm counted and removed. After that period the non-germinated seeds, including empty seeds, were photographed again. The germination results are shown in Fig. 4.

To ascertain to what degree of accuracy classifications from the photographs can be done, the seeds from the 90 test-samples were classed by two persons. The two sets of results are shown in Fig. 5. The thick line, Y_I, represents the ideal agreement between the X-ray (Y-axis), and the Jacobsen analyses, the X-ray figures being based upon the reduction-factors from the first experiment. Y_A and Y_B represent the two classifications. As will be

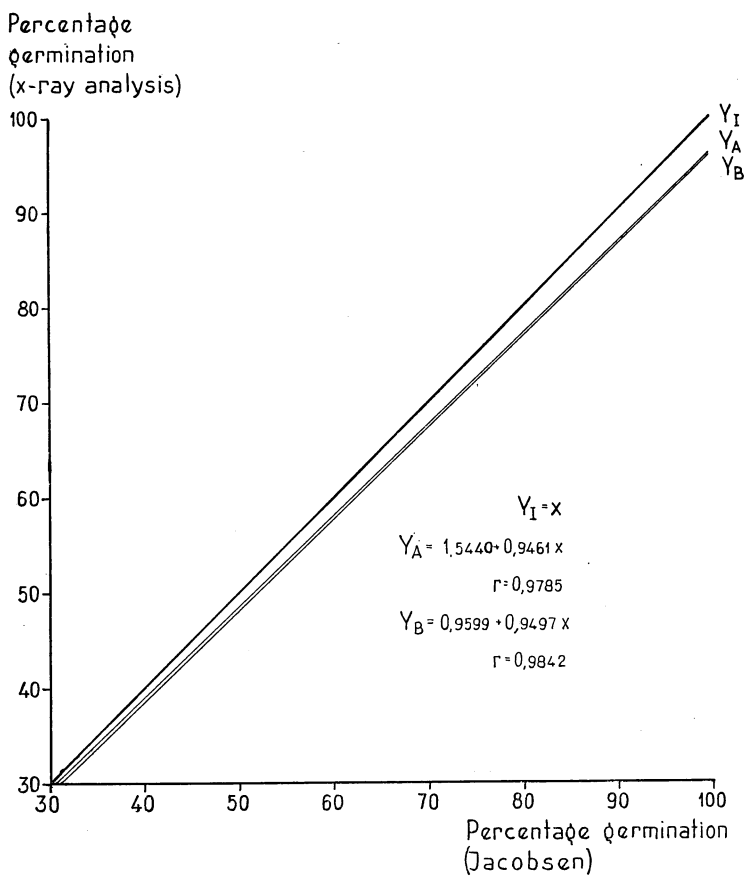


Fig. 5. In essence the same as Fig. 3, with the addition of a second regression line (Y_B), representing another person's set of classifications of the 90 test samples.

seen, Y_A and Y_B agree quite satisfactorily with each other and show that the classification of seeds from X-ray photographs can be done with good accuracy by sufficiently trained persons.

Considering the slight but significant discrepancy between Y_I and Y_A/Y_B, it must be borne in mind that the second test analysis was carried out during the months of March-April, whereas the reduction-factors, used for Y_A and Y_B, were based on tests made in January-February. SCHMIDT (1930) and REHACKOVA (1954) have proved that the seeds of several conifers, among them Norway spruce, germinate with varying vigour, dependent on the time of sowing with a minimum of germinative vigour for seeds sown in December-January and a maximum in April-May. The difference between the two series of analyses is presumably due to this circumstance but may also, in part, be explained by slight alterations in the external conditions for the germination tests, in spite of all care to keep them identical; the inferior seed-classes are especially susceptible even to minor changes in temperature and humidity. However, these fluctuations affect X-ray analyses in the same measure as the Jacobsen germinator analyses, and for most practical purposes they are without importance (cf. Fig. 4).

It is a common practice with seed testing laboratories, using the Jacobsen method or related methods, to assess the number of seedlings after a duration of 7 or 10 days in the germinator, thus to get an idea of the germinative vigour of the samples. Assessments at these early stages of the tests will be seen (Fig. 3) to be of questionable comparative value, as even small deviations in the germinative conditions—temperature, light, humidity—as well as the actual time of assessing, may give highly different results, because of the rapid growth of the better-class seeds. Thus a deviation of one day at the 10-day stage will cause a difference in germination percentage from 43 to 52 % for class III B, and from 49 to 63 % for class IV B; at earlier stages the difference will be even greater.

From Fig. 3 will also be seen that for all classes, except IV A and B, a comparative stagnation sets in between the 13th and the 25th day, approximately. This peculiarity is not sufficiently explained by the transferring of non-germinated seeds to fresh filter-papers after the first 30 days, although this may have had a stimulating effect on the germination, especially of the inferior seed classes. It may also be due to a development of the embryos of the inferior seed classes until the germination proper begins. To support this serves Table 2 which shows four seed samples from the control test, photographed and classed before (a), and after (b) the germination. As there was not kept account on the individual seeds, the evidence can only be circumstantial, but the samples No. 130 and 136 show an unmistakable increase of the number of class IV seeds which must have developed from class III and perhaps even from class II seeds.

Results from the first experiment, showing comparatively early germination of even class II A and B seeds, leads one to the assumption that an embryo need not develop to the full length of a class IV seed before germination can begin, and further—as a confirmation of WIBECK's suspicions (WIBECK, 1928)—that the proximity of the embryo to the micropylar end of the seed influences the germination speed.

Table 2. Examples of changes, effected during a 30-day germination period, in the embryonic composition in Norway spruce seeds.

No	X-ray analysis a. before b. after germination	Seed—types										Seeds germinated after 30 days	Total of seeds
		0	I		II		III		IV		Insect damaged seeds	Luminous embryos	
			A	B	A	B	A	B	A	B			
102	a	28	—	4	7	17	10	24	1	6	2	1	—	100
	b	35	—	8	—	8	—	2	5	—	2	1	39	100
130	a	5	1	15	10	14	36	12	4	2	—	1	—	100
	b	20	—	8	—	3	6	2	8	1	—	1	51	100
136	a	28	—	6	17	13	24	8	3	1	—	—	—	100
	b	44	—	2	—	1	—	1	7	—	—	1	44	100
074	a	17	—	—	—	—	—	—	71	2	10	—	—	100
	b	17	1	—	—	—	—	—	—	—	10	—	72	100
Nr	Röntgen-analys a. före b. efter groningen	0	A	B	A	B	A	B	A	B	Insekt-skadat frö	"lysande" embryoner	Antal grodda frön efter 30 dygn	Total antal frön
			I		II		III		IV					
		Frötyper												

Likewise, Table 2 proves that seeds may deteriorate during the germination. Thus in sample No. 074, one class IV-seed has changed into a class I, *i.e.* the embryo has disappeared, and in the other three samples, the number of empty seeds is considerably higher after the germination period than before it. The common practice of slitting the non-germinated seeds, in order to ascertain the quantities of empty, insect-damaged, and viable seeds, must be deemed insufficient where accurate analyses are concerned, because of the impossibility of distinguishing between "genuine" empty seeds, seeds damaged by certain insects, and previously filled seeds.

It must be admitted that these details are of small consequence to the majority of practical analyses which have the determination of viable seeds of a special definition as their sole object, but for analyses, accounting for a seed sample in greater detail, the X-ray method provides these characteristics as well as the "practical" facts.

Sammanfattning

Röntgenfotografering vid grobarhetsanalyser av gran

Föreliggande arbete ingår i en serie experiment med syfte att bestämma skogsfröets grobarhet med hjälp av röntgenfotografering. Metoden har utarbetats vid genetiska avdelningen (SIMAK och GUSTAFSSON 1953 a, b och c), och dess mångsidiga användbarhet i genetisk forskning har beskrivits i uppsatsen »Seed quality and the principles of forest genetics» (C. EHRENBERG, Å. GUSTAFSSON, C. PLYM FORSHELL and M. SIMAK 1955). Som tidigare visats för tall föreligger det ett starkt samband mellan frögrobarheten och den med röntgenfotografisk metod bestämda embryo- och endospermkvaliteten (MÜLLER-OLSEN och SIMAK 1954). Detta samband har nu även undersökts för granfrö och resultaten kan sammanfattas som följer.

1. Hos granfrö kan urskiljas samma embryoklasser (0—IV) och endospermklasser (A—B) som hos tallfrö (Plate I). Fröklassernas groningsprocent efter 30 dygn i Jacobsens apparat är följande:

	Endosperm-klass	Embryoklass 0	I	II	III	IV
gran	A	—	—	36	82	97
	B	—	—	15	71	92

Med hjälp av dessa procenter — s. k. reduktionsfaktorer — kan groningsprocenten för varje fröprov snabbt bestämmas om man känner till provets embryo- och endospermklassfördelning.

2. På röntgenfotografierna kan även iakttagas insektskadade frön och andra abnormiteter (Plate II).
3. Vid undersökningen har en del intressanta iakttagelser gjorts beträffande groningsförloppet hos frö med olika embryo- och endospermklasser (Fig. 3), embryots tillväxt samt embryots och endospermets destruktion under groningstiden (Tab. 2).
4. Metodens lämplighet har prövats på 90 olika fröprov från hela landet med tillfredställande resultat (Fig. 4).

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