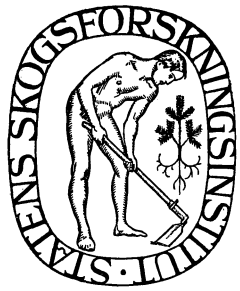

The X-ray contrast method for seed testing

Scots Pine—Pinus silvestris

Grobarhetstestning av tallfrö med röntgenkontrastmetod

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CONTENTS

Introduction

The estimation of the germination value and vitality of forest tree seeds by means of the Copenhagen tank germinator is usually a slow process. The analysis of Scots pine and Norway spruce seeds requires a 28-day germination period, and for fir, ash, and Weymouth pine the time is even longer; 48, 60, and 90 days, respectively (Holmes 1954). These extensive times make such analyses difficult to perform satisfactorily, especially because of the tendency of mould to develop on the seeds and thereby disturb the uniformity of the testing conditions. Within the scope of seed control it is therefore desirable to explore the possibilities of finding quicker ways of seed analysis to replace or at least to supplement the old method. The majority of the existing quick methods are based upon the response of living seed tissue to various treatments, physical or chemical. A number of such methods are described by Wach (1942), Mac Key (1949), Lakon (1953) etc.

The radiographic method (Simak and Gustafsson 1953 a, b, c, 1954) is based on the anatomical characteristics of the seed. The different parts (seed-coat, endosperm, and embryo) absorb the X-rays to varying degrees, so it is possible on the radiograph to recognise the more or less developed embryo and endosperm, and also to detect mechanical injuries as well as injuries caused by insects. Investigations by Simak and Gustafsson (1954), Müller-Olsen and Simak (1954), Müller-Olsen, Simak, and Gustafsson (1956), have divided Scots pine and Norway spruce seed into 5 embryo-classes (0—IV), and 2 endosperm-classes (A & B). As the very mild X-ray dose, sufficient for obtaining good radiographs, does not injure the seed, it was possible, subsequently, to determine the germinative qualities of the seed-classes in the germinator. A seed test can thus be made by ascertaining the frequencies of the seed-classes—the so-called embryo/endosperm-spectrum—in the germinator. A seed test can thus be made by ascertaining the specific conversion factors (table 1). For further information on the aspects of the method, see Plym Forshell 1953, Ehrenberg et. al. 1955, Simak 1955 a & b, Gustafsson and Simak 1956, Simak, Gustafsson, and Granström 1956, and Ehrenberg and Simak 1956 and the above mentioned papers.

It should be pointed out, however, that these conversion factors are applicable to physiologically sound, *e.g.* freshly collected, seed only, a condition which has been emphazied in previous works. A sample consisting entirely of class IV-A seed which, according to the empiric germination value characteristic of its E-spectrum, should germinate with 99 per cent may in actual fact possess a very low vitality, or none at all, if the seed have been injured;

Table 1. Conversion factors.

Species	Endosperm class	Embryo class				
		O	I	II	III	IV
Scots pine	A	—	—	(50)	88	99
	B	—	—	(5)	(43)	(68)
Norway spruce	A	—	—	36	82	97
	B	—	—	15	71	92

Key: The conversion factors are defined as the germination percentage of the various embryo and endosperm classes as found in the Copenhagen tank germinator after 30 days under the following conditions. Temperature 23° C (constantly). Lighting: 3×40 watt daylight tubes, placed 50 cm above the seed, for 8 hours daily. The figures in parentheses rest on insufficient material.

Embryo class:

O: 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 or more embryos, the longest 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. Diminutive embryos rarely occur.

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.

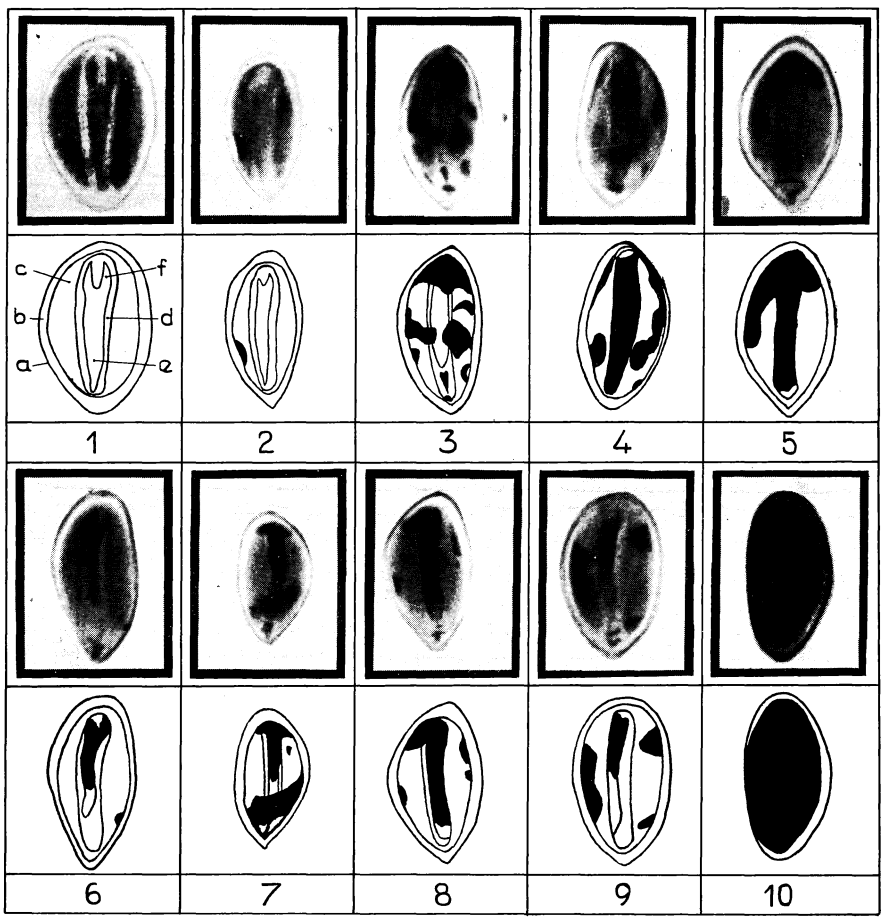
for instance through prolonged storage. This appears clearly from table 2 if one compares the actual germination percentage in the germinator (the G %) with the calculated one (Ap). It would be misleading to employ the E-spectrum alone in testing seed of this inferior quality. Physiological changes in embryo and endosperm are but seldom revealed on the radiograph.

In the following will be described a method—the X-ray contrast method —by means of which even physiologically injured seed can be tested. It is founded on the principle of semipermeability, a quality implicit in living cells only. Thus seed of a low vitality which is not occasioned exclusively by an inferior E-spectrum only display this characteristic to a small degree, and in dead seed no semipermeability is found at all. Chemical agents unable to penetrate into the living tissues of the seed diffuse freely through the nectrotic parts, thereby rendering the physiologically different tissues clearly distinguishable on the radiograph by a pronounced contrast of density.

As the scope of the X-ray seed-testing method has now been enlarged so as to include stored seed, it has been necessary to make a few alterations in and additions to the technical terms concerning the test:

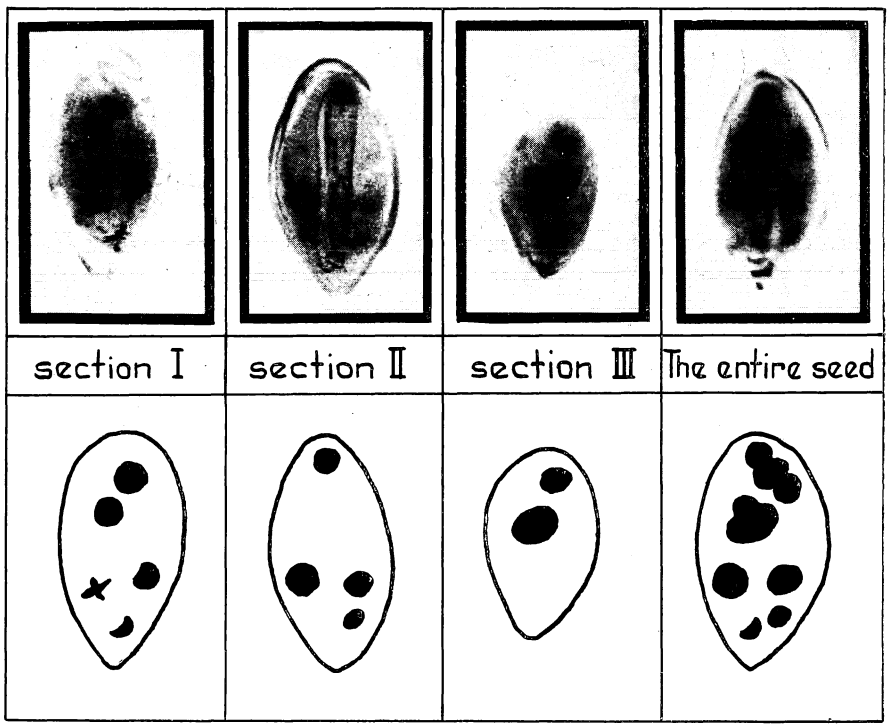
Plate I.

Photographic enlargements of radiographs are difficult to reproduce faithfully; they do not display the same clarity of detail as the original films.



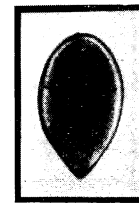
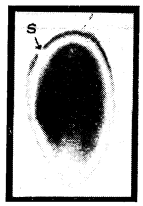
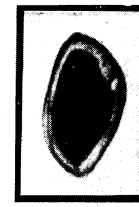
Various impregnation patterns.

For each radiograph is drawn a sketch on which the impregnated areas are marked in black. 1. Non-impregnated seed: a. Seed-coat, b. Cavity between seed-coat and endosperm, c. Endosperm, d. Embryo cavity, e. Embryo, f. Cotyledons. 2&3. Only endosperm impregnations. 3. Note the small impregnated embryo near the micropyle. 4. Thin layer of impregnation in endosperm. 4&5. Spurious impregnation of embryo through influx of $BaCl_2$ into embryo cavity. 6. Impregnation of half the embryo. 7. Half of embryo impregnated. The unimpregnated radicle is "covered" by an endosperm necrosis. 8&9. Various degrees of embryo and endosperm impregnation. 10. Wholly impregnated seed.



Radiographs of an impregnated seed and its 3 sections (I—III). The impregnated areas from sections I—III and from the seed shown as black patches. They will be seen to agree with the corresponding radiograph (Experiment 2).

Plate III.

Kind of injury	Croacked seed-coat		Pierced endosperm	
Impregnation / Sample	without soaking	with soaking	without soaking	with soaking
No. 7 high vitality	 No impregnation.	 Impregnation is centred, with blurred outline. The embryo contours are often emphasized.	 Intensive impregnation of the wound: with sharp contours	 Intensive impregnation of the wound with strong contrast to the other more grainily impregnated, parts. The embryo contours emphasized.
No. 11 low vitality	Always small impregnation areas, but impregnation does rarely occur. 	Ordinary impregnation of necrosis. 	Intensive impregnation of the wound: with blurred contours 	Ordinary impregnation of necrosis. 

Mechanical injuries revealed by the X-ray contrast method.

The examined injuries are not always easily distinguishable on the unimpregnated seed. Impregnation causes these injuries to appear more clearly and helps to identify the kind of injury. s: fissures in the seed-coat (Experiment 4).

The calculated germination percentage, termed *CG %,* will hereafter represent a value for the computation of which the anatomical development as well as the physiological condition of the seed are taken into account. Practically, it should be an approximation to *the actual germination percentage,* (G %), as found in the Copenhagen tank germinator.

The anatomical potential, A-potential, or Ap is a new term denoting the germinative capacity under the standardized conditions of the germinator, in per cent, of a seed-sample or one seed, calculated in accordance with its E-spectrum by means of the conversion factors of table 1, *i.e.* irrespective of any possible physiological disturbances of the seed. For physiologically sound seed the A-potential is identical with the calculated germination percentage as employed in previous articles on the X-ray method.

Material

The investigation was made with Scots pine seed only. Facts about the seed are given in table 2. Samples of reduced germinative power, primarily due to long-time storage, are identified by the discrepancies between their A-potential and G %.

Table 2. Data of the seed-material.

Sample No.	Locality	Latitude	Altitude metres	Collection year	Ap	G %
1	Gävleborgs län, Sweden.....	61°50′	225	1946	99	8
2	Norrbottens län, Sweden.....	65°36′	390	1946	96	74
3	Västerbottens län, Sweden.....	63°20′	150	1946	93	8
4	Västernorrlands län, Sweden....	63°30′	450	1946	92	72
5	Skaraborgs län, Sweden.....	58°30′	80	1948	99	44
6	Jämtlands län, Sweden.....	62°12′	370	1946	99	56
7	Västernorrlands län, Sweden....	63°30′	225	1946	99	98
8	Västerbottens län, Sweden.....	64°25′	270	1950	79	74
9	Östergötlands län, Sweden.....	58°09′	160	1954	99	96
10	Västernorrlands län, Sweden....	62°00′	450	1946	97	28
11	Västerbottens län, Sweden.....	65°30′	550	1946	84	0
12	Jämtlands län, Sweden.....	63°30′	280	1945	82	14
13	Norrbottens län, Sweden	65°30′	150	1940	98	30
14	Västerbottens län, Sweden.....	64°05′	240	1956	97	94
15	Västerbottens län, Sweden.....	65°15′	350	1956	74	60
16	Västerbottens län, Sweden.....	64°11′	200	1932	68	0
17	Brandenburg, Germany.....	52°53′	50	1929	88	0
18	Zala, Hungary.....	46°40′	150	1928	95	0
19	Östergötlands län, Sweden.....	58°10′	150	1937	99	0
20	Värmlands län, Sweden	59°02′	80	1954	95	96
21	Gotlands län, Sweden.....	57°27′	40	1953	99	98
22	Östergötlands län, Sweden.....	58°37′	100	1928	95	0
23	Liptov, Slovakia.....	49°00′	1 100	1955	97	92
24	Liptov, Slovakia.....	49°00′	1 100	1955	97	10
25	Västerbottens län, Sweden.....	64°10′	340	1957	8	6

Method

The following questions were examined:

1. The choice of an impregnating agent,
2. The process of impregnation,
3. The connexion between the impregnation pattern and the germinative quality of the seed.

1. The choice of an impregnating agent

An impregnating agent should fulfil two requirements:

a. it should be in harmony with the specific qualities of the seed so as to penetrate unchecked, in its dissolved form, into the necrotic tissues, and

b. the cation of the agent—an organic or inorganic salt—should possess a high atomic weight in order to secure a high degree of contrast on the radiograph betwen the impregnated and the non-impregnated parts of the seed.

After some preliminary experiments with various salts ($AgNO_3$, $Ba(NO_3)_2$, $(CH_3COO)_2Pb$, a.o.), $BaCl_2$, (Ba = 133) turned out to meet the above requirements best of all. $BaCl_2$ is cheap and its toxic effect on the living seed-tissue is negligible; an attractive quality from a methodical point of view.

In order to accelerate the process of impregnation the seed were first soaked in water for 16 hours at room-temperature and after that transferred directly to the $BaCl_2$-solution.

2. The process of impregnation

The impregnation process was tested on:
mechanically uninjured seed,
the different embryo-classes,
mechanically injured seed.

The impregnation of mechanically uninjured seed. Experiment 1. The procedure was tested for various concentrations of aqueous solutions of $BaCl_2$ and treatment periods on seed of good and inferior vitality.

Material: Samples No. 4 and 9.
$BaCl_2$ concentrations: 5 %, 10 %, 20 %, and 30 % (saturated solution).
Treatment periods: 15, 30, 60, and 120 minutes.
Temperature: 35° C.
No. of treatments per seed sample: 18 (incl. 2 controls).
No. of seeds per treatment: 50.

Table 3. The impregnation pattern in relation to the $BaCl_2$-treatmens (Experiment 1 c).

$BaCl_2$-concentrations	Time of treatment	Number of seeds with impregnated			
		No impregnation	Embryo alone	Endosperm alone	Embryo and endosperm
5 %	15′	19	—	6	—
	30′	12	1	12	—
	60′	13	1	10	1
	120′	2	—	20	3
10 %	15′	7	—	15	3
	30′	7	—	15	3
	60′	4	1	16	4
	120′	3	1	15	6
20 %	15′	7	—	11	7
	30′	3	—	13	9
	60′	6	—	10	9
	120′	6	1	9	9
30 %	15′	7	—	12	6
	30′	4	—	15	6
	60′	4	—	10	11
	120′	3	—	6	16

After the treatment the seeds were rinsed in water (to remove any surplus $BaCl_2$ which might have blurred the radiograph), dried at room-temperature, and radiographed. 25 seeds per treatment were picked at random from photographic enlargements of the radiographs and submitted to further examination as to the density and relative area of the impregnated parts.

The results were as follows:

a. The high vitality seed (sample No. 9) remained insusceptible of treatment with $BaCl_2$, even in the strongest solution (30 %) after the longest period (2 hours).

b. The low vitality seed (sample No. 4) showed a distinct permeation by $BaCl_2$ on the radiograph where they were clearly distinguishable from the controls.

c. The impregnation of the No. 4 seeds showed great variation. It either affected the whole seed or only part of it; it was limited to the embryo or to the endosperm; or it included both (Plate I). Table 3 shows that the impregnation patterns are fairly constant for all treatments except for the extreme concentrations and times. In the weakest $BaCl_2$-solution many seeds do not absorb the impregnating agent during the shortest period, but only after prolonged treatment (the 5 % series, table 3). The 10 % and 20 % treatments result in almost similar impregnation patterns for all times; even the 30 % treatment is not much different from this standard, at least for the shorter times. There is, however, a general

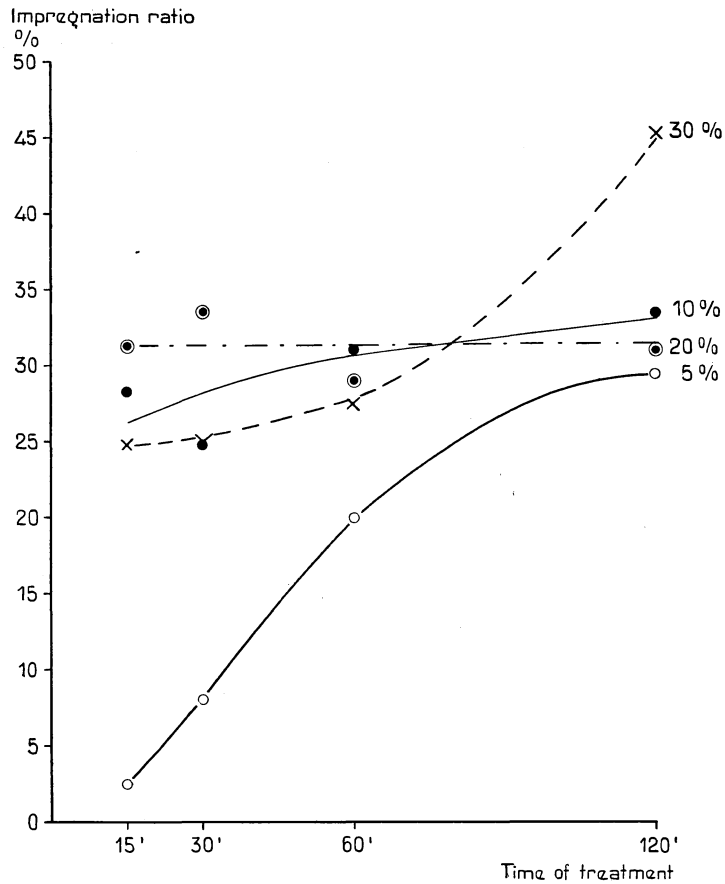


Fig. 1. The response, expressed by the impregnation ratio, of low vitality seed to various concentrations of $BaCl_2$ and times of treatment (Experiment 1 d).

increase of the frequency of seeds with impregnated embryo and endosperm by an increase of concentration and treatment time. The most extreme treatment (30 % and 2 hours) causes a considerable increase of the number of such seeds.

d. Quantitatively the impregnation process was examined by planimetry of the seeds on the enlarged radiographs as mentioned above. For each treatment the ratio of the impregnated area to the total area of the 25 seeds was determined (fig. 1). For the 5 % series this collective *impregnation ratio* increased by prolonged treatment and reached at 120 minutes a maximum value of 25—30 per cent. All the other series and treatment times were found to lie within the same limits, except the 30 %/120 min. the collective impregnation ratio of which was 45 %.

e. A contemplation of the results mentioned under c and d discloses an interesting feature of those series whose collective impregnation ratio lies between 25 and 30 per cent: An increase of concentration and time caused a higher frequency of seeds with impregnated embryo and endosperm without appreciably increasing the impregnation ratio. A more intensive treatment increases the volume of impregnated tissue—probably by way of the embryo cavity as well as from the surface of the endosperm but this does not necessarily increase the impregnation ratio, which only involves areas.

Summing up it can be said that a constancy of conditions for the $BaCl_2$-treatment is of some importance for the attainment of comparable results, and especially so for seed of low vitality.

As the radiograph presents a two-dimensional projection of the seed it was often difficult, in the beginning, to decide the depth of the impregnation. In order to examine this question the following experiment was made.

Experiment 2. Material: Sample No. 4.
$BaCl_2$ concentration: 10 %.
Treatment period: 1 hour.

The treated seed were radiographed and afterwards sliced into 3 sections parallel to the plane of projection. The sections were radiographed, too. Through a study of the radiographs of the seeds and their respective sections it was possible to attain an ability of considering the radiographs three-dimensionally. (Plate II).

The impregnation process in relation to the embryo-classes. The following experiment serves to elucidate whether the inferior germination of the lower embryo-classes (II & III) is caused by their incomplete anatomical development alone or has its root in other conditions which may be ascertained by the X-ray contrast method.

Experiment 3. Two samples were chosen: No. 25 (E-spectrum: 0: 6 %; I: 6 %; II: 78 %; III: 10 %; IV: 0 %; B-endosperm; Ap = 8 %; G % = 10 %), and No. 16 (E-spectrum: 0: 0 %; I: 2 %; II: 40 %; III: 36 %; IV: 22 %; A-endosperm; Ap = 73 %; G % = 0 %). No. 25 was perfectly fresh; No. 16 25 years old. All seed were soaked in water for 16 hours and treated in 10 % $BaCl_2$ for 1 hour. The freshly collected seed were not, and the stored seed completely impregnated which suggests that the low germination value of No. 25 is due wholly to the anatomical development of the seed (Ap = 8 %), conditional, of course, on the extent to which the physiological constitution is altogether ascertainable by the $BaCl_2$-treatment.

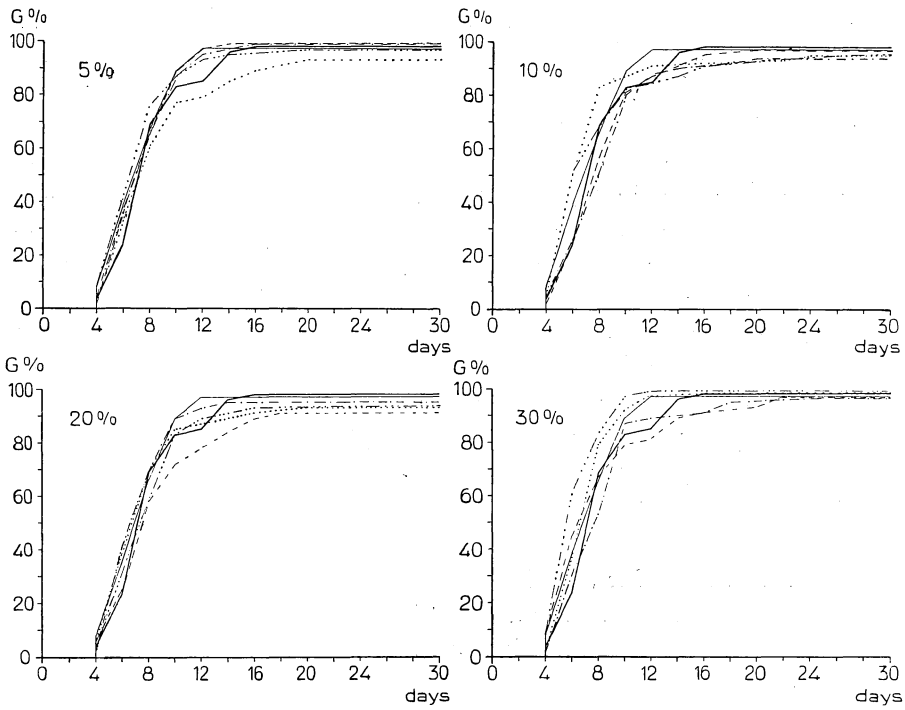


Fig. 2 a. The course of germination of high vitality seed for various $BaCl_2$-concentrations and times of treatment: ▬▬ Control C_1, ——— Control C_2, — — — 15′, —·—·— 30′, ... 60′, —···—···— 120′. (Experiment 5 b).

The impregnation of mechanically injured seed. It is of practical interest to establish the extent of mechanical injury to a seed sample. Most of the injuries are caused by the process of de-winging and may considerable reduce the germinative value of the sample (Huss 1950). The injuries are often—but not invariably—visible on the radiographs as fine fissures in the seed-coat. To what extent such injuries can be made more conspicuous was examined in experiment 4.

Experiment 4. Two different types of mechanical injury — broken seedcoat and pierced endosperm — were studied on both high and low vitality seed, the material being samples No. 7 and No. 11, respectively. With the thumb the seed were pressed against a hard surface until the coat cracked; the piercing was done with a thin needle. Half of the seeds were soaked in water for 16 hours prior to the $BaCl_2$-treatment (30 %—2 hours); the other half was treated without soaking. The number of seeds for each of the 8 treatments was 20. The ensuing radiographs (Plate III) prove that it is feasible to tell high and low vitality seed from each other and to determine the exact extent of mechanical injury.

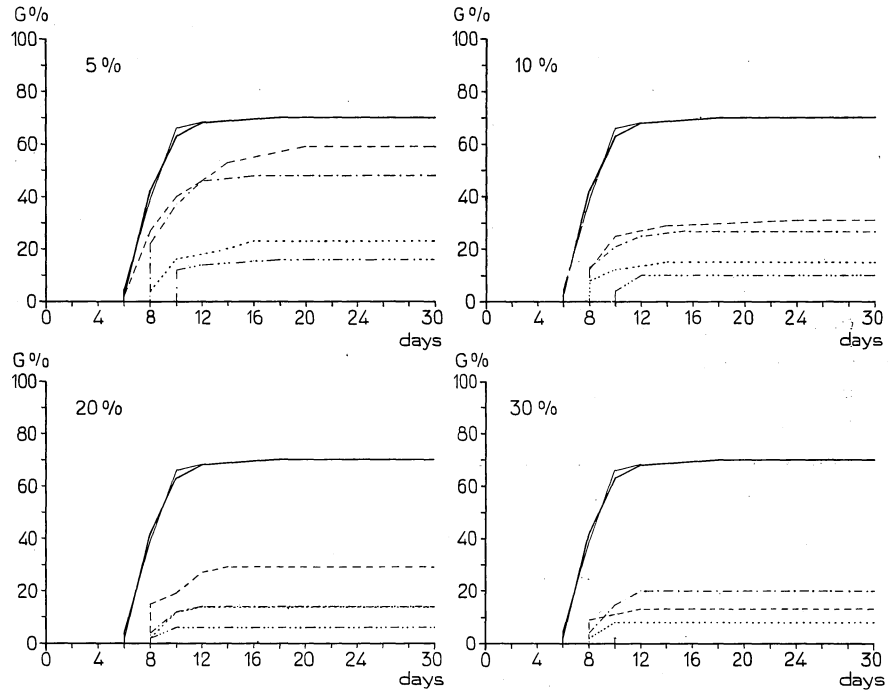


Fig. 2 b. The course of germination of low vitality seed for various $BaCl_2$-concentrations and times of treatment: ▬▬ Control C_1, ——— Control C_2, — — — 15′, — · — · — 30′, ... 60′, — ··· — ··· — 120′. (Experiment 5 c).

3. The connexion between the impregnation pattern and the germinative quality of the seed

The object of *experiment 5* was to study how seed, impregnated with $BaCl_2$, germinated in the Copenhagen tank germinator. Material and treatments were identical with those of experiment 1. (Sample No. 4 and 9; 4 concentrations of $BaCl_2$; 4 treatment times; 50 seeds per treatment.)

During the 30-day germination period a constant temperature of 23° C was maintained, and the lighting—8 hours daily—was provided by 3×40 Watt daylight tubes, placed 50 cm above the germinator. As the 50 seeds of each treatment were laid out in the germinator in exactly the same order as in the radiograph, it was easy to compare the germination of each single seed with its anatomical structure and degree of impregnation. Assessment was made every 2 days of seeds having developed radicles of 5 mm length.

Two kinds of non-impregnated controls were used, C_1 and C_2. C_1-seeds were laid out directly in the germinator while the C_2-seeds were first soaked in water for 16 hours. This distinction was done in order to ascertain any

possible effect of soaking on the germination. All $BaCl_2$-treated seeds were soaked. The course of germination of the different treatments will appear from fig. 2 a and 2 b.

The results may be interpreted as follows:

a. The two controls did not differ from one another. This refers to both samples.
b. Sample No. 9 (good quality): There was no significant difference in germination between the treatments and the controls. Even the strongest concentration at the longest period had no greater influence on the vitality of the seed (fig. 2 a).
c. Sample No. 4 (poor quality): Differences were found both in the ultimate germination percentage (G %) and in the course of germination. Fig. 2 b proves beyond doubt that the impregnation checks the germination of this kind of seed, and it seems as if the time treatment exercises a greater restrictive effect than the concentration.

The connexion between the various impregnation patterns and their corresponding germination values was more thoroughly examined on further 15 samples. According to their Ap/G % ratio they were grouped as follows:

A. Ap approximately equal to G %.
B. Ap much higher than G %, (G % > 0).
C. G % = 0, (Ap > 0).

Experiment 6. Material: Group A: sample No. 14, 20, 21, 23, and 25.
Group B: » » 1, 6, 12, 13, and 15.
Group C: » » 16, 17, 18, 19, and 22.

From each sample 2 × 50 seeds were radiographed and their Ap calculated (table 4). One portion was used as a control for the determination of G %. The other portion was soaked in water for 16 hours, treated with 10 % $BaCl_2$ for 1 hour, radiographed again, and laid out in the germinator together with the control.

Every treated seed was classified with regard to what part of the seed had absorbed the impregnating agent (embryo, endosperm, or both), and to the ratio of impregnation (table 4).

The individual control of every single seed made it possible also in this experiment to compare the impregnation pattern with the germination value of the treated seeds; it was, however, necessary first to institute a safer basis for comparison than that presented by the controls alone.

A reduction of germinative power was to be expected for the treated seed (experiment 5 c). An exact estimation of that reduction was reached through comparing their G % with a corrected germination percentage, termed corr. G %, of the same seeds, had they not been treated. In addition, the corr. G %

Table 4. Relation between impregnation ratio and germination percentage (corr. G %) (Experiment 6).

Group	Controls			Impregnated seeds																	
				E-spectrum								Number of seeds with the impregnation ratio									
	Sample No.	Ap	G %	O	I	II	III	IV	Ap	G %	corr G %	o	Endosperm alone				Embryo alone	Embryo and endosperm			
													¼	½	¾	total		¼	½	¾	total
A	14	97	94	—	—	4	2	94	97	100	94	50^{50}	—	—	—	—	—	—	—	—	—
	20	95	96	—	—	—	—	100	99	100	100	50^{50}	—	—	—	—	—	—	—	—	—
G % ≈ Ap	21	99	98	—	—	—	10	90	98	92	97	49^{46}	1^{0}	—	—	—	—	—	—	—	—
	23	97	92	—	—	4	6	90	96	98	91	50^{49}	—	—	—	—	—	—	—	—	—
	25*	8	6	6	6	78	10	—	8	10	6	50^{5}	—	—	—	—	—	—	—	—	—
B	1	99	8	—	—	2	6	92	97	2	8	—	2^{0}	10^{1}	4^{0}	2^{0}	—	—	6^{0}	14^{0}	12^{0}
	6	99	56	—	—	—	8	92	98	38	55	6^{6}	21^{13}	5^{0}	2^{0}	—	1^{0}	—	—	5^{0}	10^{0}
G % ≪ Ap	12	85	14	—	—	12	20	68	91	6	16	2^{2}	6^{1}	2^{0}	1^{0}	—	1^{0}	2^{0}	7^{0}	13^{0}	16^{0}
G % > o	13	98	30	—	4	—	2	94	95	24	29	8^{6}	10^{4}	3^{1}	1^{0}	—	—	3^{1}	8^{0}	7^{0}	10^{0}
	15	74	60	12	2	26	30	30	69	50	56	32^{17}	8^{6}	2^{2}	1^{0}	—	—	—	—	1^{0}	—
C	16	68	0	—	2	40	36	22	73	0	0	—	—	—	—	—	—	—	—	—	50^{0}
	17	88	0	—	—	6	20	74	94	0	0	—	—	—	—	—	—	—	—	—	50^{0}
G % = o	18	95	0	—	—	—	6	94	98	0	0	—	—	—	—	2^{0}	—	—	—	—	48^{0}
	19	99	0	—	—	—	2	98	99	0	0	—	2^{0}	5^{0}	9^{0}	3^{0}	—	—	2^{0}	10^{0}	19^{0}
	22	95	0	2	—	—	—	98	97	0	0	—	—	—	—	1^{0}	—	—	—	7^{0}	41^{0}

* Endosperm B.

The exponent indicates the number of germinated seeds.

was defined so as to equal the G % of the control, provided the respective Ap's were equal, too. The following identity meets both requirements:

$$\text{corr. G\%} = \frac{\text{G\%}}{\text{Ap}}\,(\text{control}) \times \text{Ap}\ (\text{impr.})$$

A comparison between the corr. G % and the G % of the treated seed led to the following conclusions:

a. All the high vitality seed of *group A,* whose G % and Ap were more or less in agreement with one another, remained unimpregnated except for one seed (from sample No. 21), the impregnation ratio of which was, however, only 1/4. For the treated seeds the G % was slightly higher than the corr. G % in three instances (100:94; 98:91; 10:6), in one case they were identical (100:100), and in one case the G % was lower (92:97). This category of seed thus does not suffer any reduction of the germination capacity through the $BaCl_2$-treatment.

b. The low vitality seed of *Group B* (G % much less than Ap) were all more or less impregnated. The G % of all five samples was inferior to the expected corr. G % (2:8; 38:55; 6:16; 24:29; 50:56) in good agreement with the results of experiment 5 c, fig. 2 b: $BaCl_2$ distinctly inhibits the collective germination of low vitality seed. From table 4 it appears, though, that for all 5 samples, the $BaCl_2$-treated, but non-impregnated seed germinated normaly (according to Ap); even impregnated seed with an impregnation ratio about 1/4 germinated. An interesting case is the one germinated seed (sample No. 13) with impregnated endosperm *and* embryo. The impregnation included the endosperm to 1/4 and some of the cotlyledons. Apparently, seeds with a partial necrosis of the embryo are capable of germinating.

c. *Group C,* the dead seeds. The Ap is high, but the G % = 0. Almost all seeds were wholly impregnated; only sample No. 19 showed a more varied impregnation pattern (table 4).

The conclusions of this experiment are that a *direct* correllation exists between the A-potential, modified by the impregnation pattern, on one side and the G % on the other for seed of high vitality (Ap = G %) and for dead seed (G % = 0). For the intermediate group of low vitality seed (Ap > G % > 0) such a correlation does not exist, as the impregnation influences the germination to an unknown degree. Probably the seeds which are inhibited in their germination through impregnation are such as possess small necroses of the endosperm. Further experiments are required to determine an exact quantitative interpretation of the impregnation patterns.

In order to find out whether a minor necrosis incapacitates a seed for germination a small preliminary experiment was made with low vitality seed from group B. The seeds were first laid out for germination in the germinator, and when the radicles had just appeared the seeds were treated with $BaCl_2$ and radiographed. Apart from a spurious impregnation of the embryos, due to the solution's having diffused into the embryo cavity through the aperture made by the radicle, there were few necroses greater than 1/2 of the endosperm. Because of the disturbing influx of $BaCl_2$ it was impossible to demonstrate the existence and, consequently, the significance of necroses of the embryo. As regards the endosperm necroses one may hypothesize from this experiment and from experiment 6 b that non-impregnated seeds and seeds whose impregnation ratio is less than 1/4 are practically viable. All other impregnated seeds can be considered incapable of germinating. This assumption was tried in experiment 7.

Experiment 7. All 25 samples from table 2 were used and represented by 2 × 50 seeds each. One set of each sample served as a control, the other was treated with a 10 % $BaCl_2$-solution for 1 hour after a preceding 16-hour

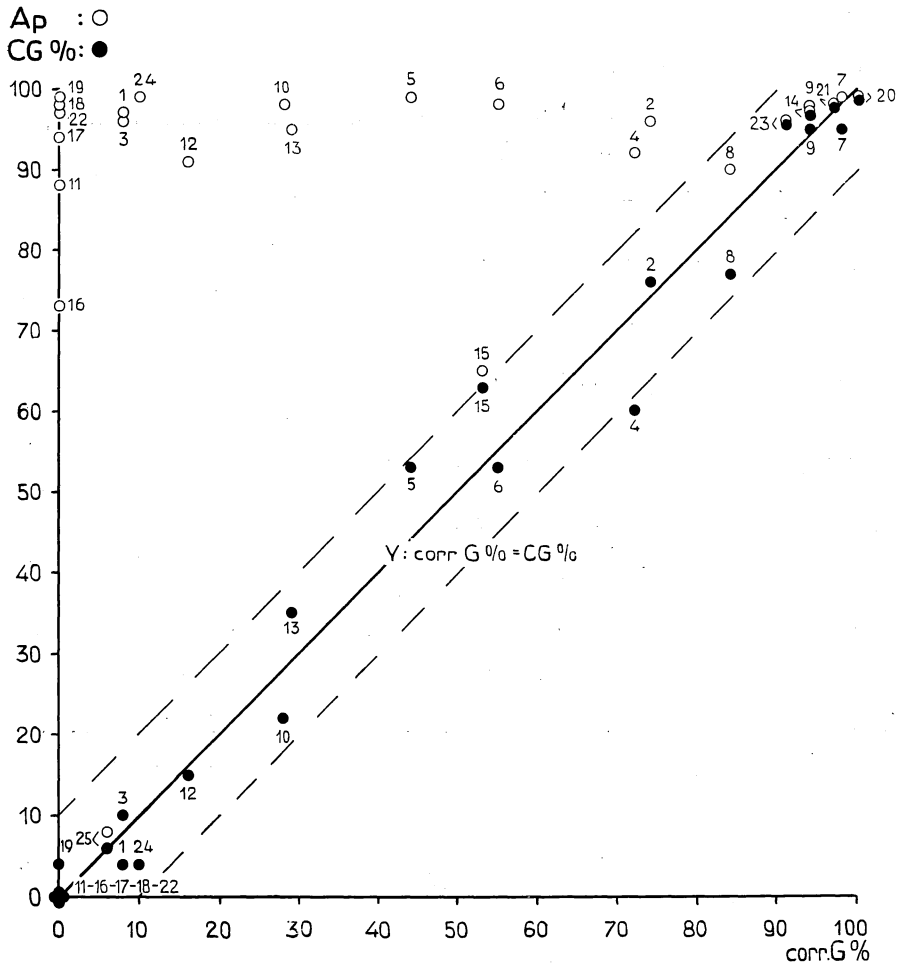


Fig. 3. A comparison between the calculated germination (CG %), according to the X-ray contrast method and the corrected germination percentage (corr. G %), based on the test in the Copenhagen tank germinator. The corresponding anatomical potentials (Ap) are also given. Material: The 25 samples of table 1. (Experiment 7).

soaking in water. All sets were analysed as to Ap and G %. The G % was determined after 30 days' germination under the same conditions as prescribed for experiment 5 and used in its corrected form (corr. G %, pg. 13). For the treated seed a calculated germination percentage (CG % see p. 5) was made in accordance with the working hypothesis of this experiment *i.e.* the CG % was considered as being equivalent to the Ap of those seeds which remained unimpregnated or whose impregnation ratio was less than 1/4:

$$CG\% = \frac{\sum_{1}^{n} Ap}{N} \cdot 100$$

n = number of seeds with an impregnation ratio between 0 and $^1/_4$

N = total number of examined seeds

Example: sample No. 6, table 4.

$N = 50$

$n = 27$ (25 seeds of class IV A and 2 seeds of class III A)

$$\sum_{1}^{n} Ap = \frac{(25 \times 99 + 2 \times 88)}{100} = 26.51$$

$$CG\% = \frac{26.51}{50} \cdot 100 = 53.02\ \%.$$

A comparison between the CG % and the corresponding corr. G % is given in fig. 3. The Ap of the long-stored seed is considerably higher than the respective corr. G %. Only for the new-harvested seed and for seed, stored but a relatively short time, the Ap and the corr. G % agree. The agreement between the CG % and the corr. G % is reasonably good. For all 25 samples — excepting No. 4 — the divergences are within the ± 10 % limit.

The two samples, No. 23 and 24, both originated from the same compound lot of high vitality seed, but whereas No. 23 was analysed *in statu quo*. No. 24 was first killed by 20 minutes' steaming above boiling water. The subsequent $BaCl_2$-treatment rendered the seed from No. 23 unimpregnated while the No. 24-seed were almost totally impregnated and germinated with 10 %. Even such provoked injuries can be discovered by the X-ray contrast method.

The investigation has thus resulted in proving the practicability of distinguishing between viable and dead Scots pine seed by radiographs of $BaCl_2$-treated material. Although it must be admitted that the distinction is somewhat rough, the established correlation between the germination percentage, calculated after the X-ray contrast method (CG %), and the corrected actual germination percentage in the Copenhagen tank germinator (corr. G %) satisfies the demands of practice.

Discussion

The results of this investigation are supported by a series of experiments. For the sake of greater clarity in presenting the X-ray contrast method only such aspects of the experiments have been mentioned as have a direct bearing on the problem at issue. Further commentaries as well as a series of experiments which will test the method at greater detail are to be published later.

The separate control of all seeds made it difficult to handle a material more extensive than the present; on the other hand, the limited number of samples and seeds facilitated the control of more factors and thereby added to the reliability of the results.

The method differs from most other quick methods of germination analysis by the latter's actively utilizing the reactions of living tissue, while the former demonstrates the presence and extent of dead matter in the seed. In this respect it is similar to Neljubow's indigo-carmine method (Neljubow 1925).

Most of the quick methods, which are based upon reactions in embryo or endosperm, presuppose the removal of the seed-coat and often the additional removal and preparation of the embryo. This slow and laborious affair is avoided by the X-ray contrast method which may be slow on account of the necessary treatment period, but does not involve much labour. $BaCl_2$ in hydrous solution penetrates the seed-coat and permeates the dead seed tissue without difficulty, and the germination analysis proper is made on the radiograph on which the necrotic, impregnated parts of the seed are observed in striking contrast to the living, unimpregnated parts. Neljubow's indigo-carmine method, in spite of its other points of similarity with the X-ray contrast method also demands that the seed-coat is peeled off to permit the aniline compound to enter the seed; the processes of diffusion and permeation are controlled by many factors, and the dissimilarity between the two methods is probably due to divergences of one or some of these factors.

The advantage of the X-ray contrast method is that it offers a combined analysis of the anatomical and physiological condition of the seed; anatomically by ascertaining the embryo/endosperm-spectrum; physiologically by the $BaCl_2$-treatment. Most quick methods consider the physiological side only, and pass over the anatomical more lightly; this may result in misleading analyses, especially for Scandinavian or other northern seed. Here, so close to the boundaries of arboreal vegetation, the climate highly influences the embryo and endosperm development of conifer seed, and may be the critical factor for the seed quality, (Hagem 1917, Wibeck 1920, Kujala 1927, Simak and Gustafsson 1954). Seed with incompletely developed embryos (class II & III) germinate poorly, primarily due to this anatomical deficiency, *e.g.* sample No. 25 (experiment 3) consisting of recently collected seed with an inferior E-spectrum and a low germination value; the $BaCl_2$-treatment caused no impregnation: the seed were physiologically sound. Even the excellent tetrazolium seed-test falls short on this point; it registers the living embryos irrespective of their anatomical constitution which may render them incapable of germinating.

The X-ray contrast method introduces the term "the anatomical potential" or "Ap", denoting the germinative potential, in per cent, of a seed sample

on a purely anatomical basis. It replaces "the calculated germination percentage", used in previous papers on the X-ray technique for which the anatomical condition of the seed was the sole determinative factor, as those investigations dealt with freshly collected and thus physiologically sound seed only. Now, as the analyses include seed with a variety of physiological defects it is necessary to dissociate the two terms. The anatomical potential is still immediately applicable to seed of fairly recent crops, provided they have been properly stored, and is therefore of great practical value. In such cases a $BaCl_2$-treatment is usually dispensable.

While storage does not alter the Ap it may cause biochemical changes in the seed and reduce their germination value; the Ap is consequently useless as a sole criterion for analysis. Niethammer (1929) has shown that storage, *i.e.* the aging of seed causes a partial loss of semipermeability and a reduction of vitality. This peculiarity has been turned to practical ends by the X-ray contrast method.

Also other defects can be adequately detected by the method. Mechanical injuries which often result from extraction and de-winging of the seed become clearly visible on the radiographs of $BaCl_2$-treated material.

Of rather more theoretical importance is the effect of $BaCl_2$ on the vitality of the seed. Notwithstanding the toxic influence of heavy-metal compounds on most organisms, seed of high vitality (experiment 5 b, fig. 2 a) germinated normally even though they had been immersed in a 30 % $BaCl_2$-solution for the considerable period of 2 hours. The slight stimulation, or retardation, in germination, observed with many high vitality seed, treated with $BaCl_2$, may be explained as the effect of the diminutive deposits of the salt left on the surface of the seed in spite of careful rinsing.

More attention should be paid and more detailed investigation devoted to the conditions which control the $BaCl_2$-treatment: concentration; time; temperature, etc. A 20 % $BaCl_2$-solution could possibly have been substituted, with profit, for the 10 % solution used in experiment 6, which would have improved the contrast of the radiographs without essentially altering the impregnation pattern, (fig. 1).

The chosen distinction between viable and not viable seed, marked at an impregnation ratio of 1/4, is admittedly pretty rough. The severest objection to this dividing value—or to any other, for that matter—arises from the fact that a three-dimensional impregnated necrosis in a seed cannot be exactly determined on a two-dimensional radiograph. Thus a thin necrotic layer parallel to the film will put the seed in the "fully-impregnated" class, whereas the same seed radiographed at right angles to the first position may come out at an impregnation ratio of 1/4. It ought, however, to be possible to make the X-ray contrast method more sensitive on this point. Improve-

ments of dependability should result from correlating the germinative value with the impregnation pattern of all embryo and endosperm classes, taken separately, for a series of seed samples of different ages.

The single seed of sample No. 13, which germinated in spite of impregnated endosperm and cotyledons, indicates the admissibility of employing the—slightly modified—topographic method developed by Lakon (1950) and Bulat (1956) for the Tetrazolium test. Possibly this test and the X-ray contrast method which, as mentioned, are complementary in their principles, can be applied to one and the same seed and thereby evolve a topographic classification for the X-ray contrast method.

It is thus probable that the X-ray contrast method can be developed to a higher degree of sensitivity. The impregnation ratio of 1/4 which has been defined to mark the border between viable and dead seed seems already, however, to meet the practical demands of a reasonably exact, quick analysis, (fig. 3).

Finally I wish to point out that $BaCl_2$ is no universal impregnation agent for an X-ray contrast analysis. Seed of different species react differently to the same chemical substance. The results of this investigation pertain to Scots pine seed only.

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Sammanfattning

Grobarhetstestning av tallfrö med röntgenkontrastmetod

Föreliggande arbete utgör en länk i en serie av uppsatser om den röntgenografiska metoden, som användes vid grobarhetstestning av skogsfrö (Å. Gustafsson, C. Müller-Olsen [C. Bang] och M. Simak).

Frönas grobarhetsförmåga kan fastställas med ledning av följande fröegenskaper:

1. fröets anatomiska utveckling,
2. fröets fysiologiska tillstånd.

Fröets anatomiska utveckling: Vid röntgengenomlysning av frö absorberas röntgenstrålarna av de olika frödelarna på olika sätt, varigenom man på röntgenbilden kan urskilja embryo- och endospermutvecklingen. Undersökningarna av ovannämnda auktorer har bevisat att mellan embryo- och endospermutvecklingen å ena sidan och frönas grobarhetsförmåga å andra sidan föreligger ett starkt samband, som kan utnyttjas för beräkningen av grobarhetsprocenten hos ett fröprov. Den på grundval av embryots och endospermets utvecklingsgrad *beräknade* groningsprocenten eller den anatomiska potensen (Ap), som den här kallas, överensstämmer med den *aktuella* grobarhetsprocenten endast under förutsättning att fröna ej är fysiologiskt skadade. Beräkningen av Ap har stor praktisk användning vid snabba kvalitetsundersökningar av nyskördat frö eller vid bestämning av frömognaden etc.

Fröets fysiologiska tillstånd: Med den i detta arbete beskrivna röntgenkontrastmetoden kan även gammalt eller på annat sätt fysiologiskt skadat frö med framgång testas. En speciell förbehandling av fröet är därvid nödvändig. I princip bygger denna metod på den semipermeabilitet, som kännetecknar levande frö. Döda frön saknar helt denna egenskap. Lågvitala frön besitter den endast delvis. Följaktligen diffunderar vissa kemiska ämnen — i detta fall vattenlösning av $BaCl_2$ — som ej kan penetrera de levande frödelarna utan svårighet in i nekrotiska frövävnader. Frö, som impregnerats med saltet, kan sedan på röntgenografisk väg identifieras. Röntgenstrålarna absorberas nämligen mycket starkt av saltets tunga kationer, varigenom levande och döda fröpartier på så sätt lätt åtskiljas.

Metodproblemen behandlades i experiment 1—7 av detta arbete. Arbetsgången vid röntgenkontrastmetoden kan sammanfattas på följande sätt:

1. fröet stöpes över natten (16 timmar) i vanligt vatten;
2. från vatten överföres fröet direkt i 20 % $BaCl_2$, där impregneringen pågår 1 timme;
3. efter behandlingen med $BaCl_2$ tvättas fröet ytligt i vatten, varigenom den på fröskalet befintliga $BaCl_2$ bortspolas;
4. fröet torkas i termostaten;
5. röntgenbild av fröet utföres;
6. röntgenbilden klassificeras.

Eftersom röntgenkontrastmetoden tar hänsyn till såväl den anatomiska utvecklingen av fröet som till fröets fysiologiska tillstånd, kan klassificeringen genomföras enligt följande principer:

a. endast frö, som ej är impregnerat eller som på röntgenbilden har endosperm impregnerat till mindre än $^1/_4$ av endospermytan, är grobart;
b. grobarheten hos dessa frön (a) är ekvivalent med frönas anatomiska potens. Anatomiska potensen beräknas enligt reduktionsfaktorerna i tab. 1.

Metoden, som vidare kommer att utvecklas, torde uppfylla praktikens krav på noggrannhet.

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