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The x-ray contrast method for testing germinability of *Picea abies* (L.) Karst. seed

Die Röntgenkontrastmethode für Keimfähigkeitsbestimmung von Fichtensamen

Röntgenkontrastmetoden för grobarhetsbestämning av granfrö

by

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ABSTRACT

In this investigation the working out and the standardisation of the x-ray contrast method for determination of the germinability of *Picea abies* (L.) Karst. seed is described. Eighty-four samples from different countries and with various germination values were used for the study. Treatment of a sample with a 40 per cent solution of sodium iodide for 15 minutes at room temperature was found to be suitable for calculating its germinability. Seeds with both embryo and endosperm free of impregnation or those with embryo unimpregnated and the endosperm impregnated in not more than 25 per cent of its projected area on the x-ray film were considered as germinable. The development of the embryo and the endosperm was also taken into consideration for determining the germinability of a seed. The results of the germinability of the samples calculated by the x-ray contrast method were found to agree well with their germination percentages on Jacobsen apparatus, which were used as the standard in this study.

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1. Introduction

The x-ray contrast method for the rapid determination of the germinability of Norway spruce seed is of great practical importance in forestry both for routine seed testing and for research. The method is based on the principle of semi-permeability. Thus when seeds are treated with a suitable contrast agent, their living tissues are able to prevent it from entering them due to their semi-permeability, whereas the dead tissues being permeable are penetrated by it. Consequently, the dead tissues of a seed become impregnated with the contrast agent, whereas the living ones remain free of impregnation. On the basis of the location and the area of impregnation, etc., in a seed, it is possible to estimate its germinability.

As regards a suitable contrast agent for *Picea abies* seed, the author in an earlier paper (Kamra, 1963 *a*) showed that several of the organic contrast agents used in human radiography, could be utilized for determining the germinability of the seed of this species. However, since these chemicals are rather expensive and moreover in most countries can only be bought on a doctor's prescription, the search was continued for other contrast agents which could be used for Norway spruce seed. The present paper deals with these investigations and describes the working out and the standardisation of the x-ray contrast method for testing the germinability of *Picea abies* seed.

2. Material and methods

Eighty-four samples of Norway spruce (*Picea abies* (L.) Karst.) seed from different countries and with various germination values were used for the investigation. The details of the locality, the country of origin, the latitude, the altitude and the year of collection of the samples, as far as known, are given in Table 1.

Before describing the methods, the paper will be divided into two parts: Part A: dealing with the working out of the x-ray contrast method for Norway spruce seed, and Part B: with a comparison of the results of this method with those of the germination on Jacobsen apparatus (JA), which were used as the standard.

2.1. Part A: The x-ray contrast method

In order to work out the x-ray contrast method for Norway spruce seed, a number of experiments were performed. These will be described under the Results. The proceduce for the x-ray contrast method in general was as follows.

Representative portions of each sample (about 300 seeds) were soaked in water for 16 h at room temperature. After draining off the water, the seeds were dried superficially with a filter paper. They were then treated with a solution of the contrast agent (s) for a definite time (for details, see the Results). The treated seeds were washed with slowly running tap water for about 2 min after which the extra water from their surfaces was removed with a filter paper. They were then allowed to dry in a thermostatically controlled oven at 70°C for 1.5 h. However, when the seeds were to be germinated after the treatment, they were dried by spreading them on a filter paper overnight at room temperature. In order that the individual seeds could be studied and compared with their radiographs, they were placed in special plastic patterns, one seed in each hole. However, in those cases where the study of the individual seeds was not necessary, they were spread directly on the envelope containing the film for radiographing. The radiographs were taken with soft x-rays under the following conditions: kV=14, mA=5, focus—film distance= 50 cm, time of exposure=5 sec (for seeds in a plastic pattern), 3 sec (for seeds spread directly on the envelope containing the film). The x-ray industrial films type "L" ("Low speed"), manufactured by CEA Works, Strängnäs, Sweden were used. They were developed in the X-ray Rapid Developer and fixed in the X-ray Express Fixative, manufactured by Tetenal Photo Works, Hamburg, West Germany.

For determining the germinability of Norway spruce seed by the x-ray contrast method, it is necessary to take the development of the embryo and the endosperm into consideration. It has been shown by Müller-Olsen, Simak & Gustafsson (1956) that there is a correlation between the development of the embryo and the endosperm in a seed and its germination capacity. On the basis of the development, they have divided the seeds into the following embryo and endosperm classes: (Definitions according to the above authors):

"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."

Müller-Olsen, Simak & Gustafsson (1956) gave the following reduction factors, i.e. germination percentages after 30 days on Jacobsen apparatus for the above classes of Norway spruce seed: (Table I of the above authors):

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

These reduction factors have been used in the present investigation for determining the germinability of Norway spruce seed by the x-ray contrast method and for the sake of completeness have been reproduced here.

In order to calculate the germinability of a sample by the x-ray contrast method, the following procedure was used. The number of empty and insect-attacked seeds was counted first on the x-ray film of the sample and deducted from the total number of seeds. Thus the number of filled seeds only was used as the basis for the calculations. The number of impregnated seeds was then counted on the x-ray film of the sample, using the standards of impregnation described under the Results. Thereafter, the number of unimpregnated seeds was counted and the embryo and endosperm class of each seed was determined. The number of seeds belonging to each embryo and endosperm class was then multiplied with the corresponding reduction factor given by Müller-Olsen, Simak & Gustafsson (1956) in their

Table I (see above), and thus the number of germinable seeds in the sample was obtained. The germination percentage of the sample was then calculated on the basis of the total number of filled seeds (i.e. the total number of seeds on the x-ray film minus the number of empty and insect-attacked seeds). As will be seen in Part B of this paper, the germination percentages of the samples on Jacobsen apparatus were also calculated on the basis of the number of filled seeds and thus the results of the germination and those of the germinability by the x-ray contrast method were made comparable for each sample.

2.2. Part B: Germination on Jacobsen apparatus

For the germination tests, pure seed (4×100) of each sample was used. In order to determine the number of empty and insect-attacked seeds in the material to be germinated, each lot was radiographed using soft x-rays. The conditions for radiography and the procedure for processing the films were the same as described above.

The germination tests were in general carried out according to the recommendations of the International Seed Testing Association (ISTA, 1966). Thus the light was given from the day-light tubes for 8 h daily (intensity=about 1,000 lx), and the germination period was 21 days. The constant temperature of 20°C was used for the tests. This temperature has been shown to be as good as the alternating temperature of 20—30°C for the germination of Norway spruce seed (Simak & Kamra, 1968). Thus it has been proposed to the International Seed Testing Association that the constant temperature of 20°C may also be included in the International Rules for Seed Testing along with the alternating temperature of 20—30°C for the germination of *Picea abies* seed (cf. Simak & Kamra, 1968).

The germinated seeds were counted from the day the germination started. This counting was done every day during the first ten days and every other day thereafter. The counted seeds were removed from the tests. A seed was considered as germinated when the length of the root was at least equal to that of the seed itself. This criterion has been found to be dependable in experiments where the germinated seeds are checked daily (cf. Simancik *et al.*, 1966; Kamra, 1967). The advantages of this criterion have been discussed earlier by the author (Kamra, 1969). The distance between the water level and the seed bed was kept constant at 13 cm.

The investigation was carried out under strictly controlled conditions. Each seed lot was radiographed before it was laid out for

germination. In this way, the number of empty and insect-attacked seeds in the lot could be determined and the calculations of the germination percentage and of the germination rate made on the basis of the number of filled seeds only. For the germination tests two Jacobsen apparatuses of stainless steel of exactly the same model were used, and the type and the level of water were the same in both of them. The light was switched on and off automatically by an electrical clock at the fixed hours simultaneously for both the apparatuses. The Jacobsen apparatuses were placed in a climate chamber, the temperature and the relative humidity of which (20°C and 60 per cent, respectively) were controlled automatically by special devices. The maximum variation of the temperature was 1°C.

3. Results

3.1. Part A: X-ray contrast method

3.1.1. Expt. 1. To select a suitable contrast agent for Norway spruce seed

For this purpose, the following chemicals were tested: sodium iodide, potassium iodide, sodium bromide, potassium bromide and lithium bromide. About 300 seeds of four samples (Nos. 2, 6, 21 and 61) with different germination values were treated with 20, 30 and 40 per cent solutions of the above salts for one hour at room temperature. It was observed that the iodides gave a better contrast than the bromides for the corresponding concentration. From the three concentrations tested, 40 per cent solutions showed a higher contrast than 20 and 30 per cent solutions of the same salt. These results indicated that 40 per cent solutions of sodium and potassium iodides could be used as contrast agents for Norway spruce seed. However, in order to see if higher concentrations of sodium and potassium iodides could give still better contrast than 40 per cent solutions, seeds of the same four samples were treated with 50 and 60 per cent solutions of these salts for one hour. The impregnation of the seeds was stronger with these concentrations than with 20, 30 and 40 per cent solutions, but the distinction between the impregnated and the unimpregnated seeds was more difficult to make than in the case of samples treated with 40 per cent solutions. Consequently, 40 per cent solutions of sodium and potassium iodides were considered suitable for the impregnation of Norway spruce seed. However, in view of the fact that the impregnation behaviour of sodium and

potassium iodides was similar, further studies were carried out with only one of them, namely sodium iodide, in order to avoid duplication of work.

The selection of a particular contrast agent and a certain concentration of it, however, only solves a part of the problem of the impregnation of Norway spruce seed. The other important part was to choose such a time of treatment that it, in addition to showing a satisfactory contrast on the x-ray film between the impregnated and the unimpregnated seeds, also showed a good correlation between the impregnation behaviour and the germination behaviour of a sample. In order to find out the suitable time of treatment, the following experiment was performed.

3.1.2. Expt. 2. To study the relationship between the time of treatment with sodium iodide and the amount of impregnation observed in a sample

For this purpose, about 300 seeds of each of the six samples (Nos. 1, 6, 17, 53, 71 and 83) representing fresh and old seed of different years of harvest and with different germination values, were treated with 40 per cent solution of sodium iodide for 10, 15, 20, 30, 45 and 60 min. The results are shown in Fig. 1. It may be observed that in sample No. 83 (fresh with high germinability), the impregnation percentage remains practically constant up to 45 min of treatment and rises after that. In the case of samples 53 and 71 (of different ages and with high germinability), the impregnation percentage remains practically constant up to 15 and 20 min respectively and increases thereafter. For the remaining three samples with reduced viability, the impregnation percentage rises continuously with the increase in the time of treatment. These differences in the impregnation behaviour of the samples will be explained in the Discussion.

From the above experiment it was also observed that in each of the six samples, the percentage of the seeds which remained unimpregnated after 15 min of treatment with sodium iodide, agreed well with the germination percentage of the corresponding sample. Thus 15 min treatment with 40 per cent sodium iodide seemed suitable for the determination of the germinability of the Norway spruce samples.

3.1.3. Expt. 3. To study the germination behaviour of seeds treated with sodium iodide

About three hundred seeds of seven samples (Nos. 2, 4, 20, 44, 48 52 and 64) of different years of harvest and with different germination

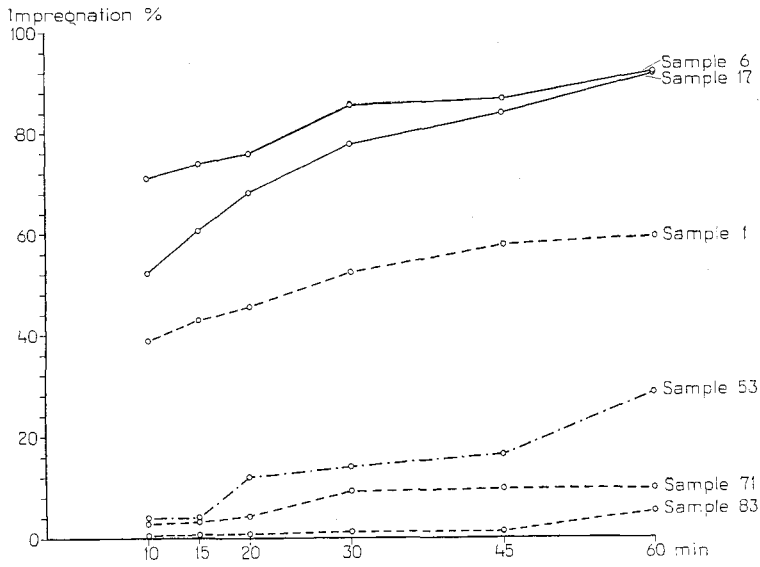


Fig. 1. Rates of impregnation with sodium iodide of the fresh and the old samples of Norway spruce seed.

values were used for the experiment. They were treated with 40 per cent solution of sodium iodide for 15 min, washed with running tap water for 2 min to remove traces of the chemical from their surfaces, and dried overnight at room temperature. The seeds were then radiographed and the impregnated ones were separated from the unimpregnated ones. The unimpregnated seeds of each sample were counted and separately placed for germination on Jacobsen apparatus for three weeks. Simultaneously, the seeds showing different degrees of impregnation on the radiographs were picked out individually, numbered and allowed to germinate on Jacobsen apparatus, separately for each sample, for the same period. It was observed that the unimpregnated seeds were in general able to germinate. The germination percentage was either as good as that of the controls or only slightly less in the case of well-developed seed with high viability. For seed with reduced viability, the germination percentage was less and varied from sample to sample. This showed that the treatment with sodium iodide does not kill the seeds.

Seeds showing impregnation (partial or complete) in the embryo alone or both in the embryo and in the endosperm, did not germinate. Among the seeds with embryo free of impregnation and the endosperm impregnated to different degrees, there were differences in the ger-

mination. Thus in samples of fresh seed with high viability and with well-developed embryo and endosperm, a few seeds with as much as 30—40 per cent of the projected area of the endosperm on the x-ray film impregnated (embryo free of impregnation) were able to germinate. On the other hand, in samples with reduced viability, some seeds with about 25 per cent of the projected area of the endosperm on the x-ray film impregnated, in others, a few with only about 15—10 per cent or less of the projected area of the endosperm on the x-ray film impregnated (embryo free of impregnation in all these cases) could germinate, often somewhat in direct relationship with the germination percentage of the corresponding control sample.

From the results just described, it will be appreciated that it is very difficult to lay down a hard and fast limit of impregnation of the endosperm (embryo free of impregnation) up to which a seed will germinate and beyond which it will not. The limit will vary from sample to sample, somewhat in relation to the germination capacity of the sample, which in its turn is influenced by the development of the embryo and the endosperm in the seed, the age of the sample, the conditions of storage, etc. Thus one can only arrive at an approximate limit of the partial impregnation of the endosperm in a seed, and the ability of the latter to germinate.

Keeping the above considerations and the results of Expt. 3 in view, it seemed reasonable to conclude that seeds with embryo free of impregnation and with impregnation in the endosperm not exceeding 25 per cent of its total projected area on the x-ray film, may be considered as germinable. Conversely, seeds with impregnation in the endosperm alone (embryo not impregnated) in more than 25 per cent of the total projected area of the endosperm on the x-ray film, or with partial or complete impregnation in the embryo alone, or with partial or complete impregnation in both the embryo and the endosperm, should be considered as non-germinable. However, as shown by Expt. 3, seeds in which both the embryo and the endosperm remain free of impregnation, should be considered as germinable. These criteria were adopted as the standards for the evaluation of the impregnation of Norway spruce seed with sodium iodide by the x-ray contrast method.

To determine the germinability of a sample by the x-ray contrast method, in addition to studying the impregnation behaviour of a sample, it is also necessary to take the development of the embryo and the endosperm into consideration. However, since the germinable seeds alone contribute to the germinability of a sample, the evaluation of the development of the embryo and the endosperm may be confined

to such seeds only, which according to the standards of impregnation mentioned above, are to be considered as germinable. As stated in the Methods, for studying the development of the embryo and the endosperm, the classification of Müller-Olsen, Simak & Gustafsson (1956) for *Picea abies* seed was followed, and the reduction factors given by them were used for the calculation of the germinability of the samples.

3.2. Patterns of impregnation with sodium iodide

The seeds of Norway spruce showed different patterns of impregnation with sodium iodide. Some of the common patterns of impregnation, an unimpregnated fully developed seed, some empty and insect-attacked seeds are shown in Fig. 2.

3.3. Part B: Comparison of the results of the x-ray contrast method with those of germination on Jacobsen apparatus

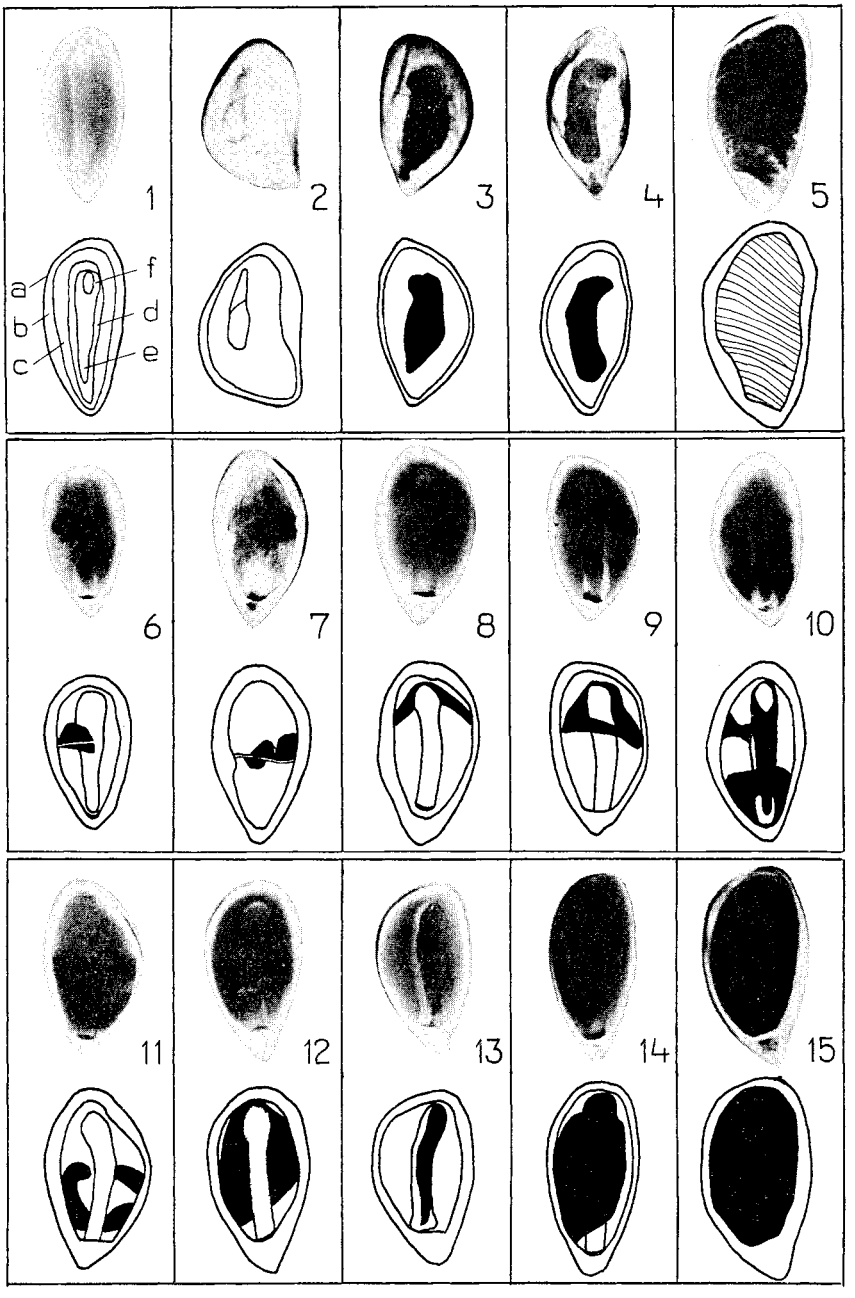
In Table 1 the germinability percentages of the samples of Norway spruce studied by the x-ray contrast method are compared with their germination percentages on Jacobsen apparatus under the conditions described in the Methods. In both cases, the percentages are based on the number of filled seeds only in each sample.

As may be seen from Table 1, the values of germinability according to the x-ray contrast method agree well with those of germination on Jacobsen apparatus. The differences in the values of a sample by the two methods in most cases lie within the tolerance limits allowed by the International Rules for Seed Testing (ISTA, 1966).

3.3.1. Rates of germination

The samples used in this investigation showed different rates of germination on Jacobsen apparatus. In Fig. 3 the germination rates of the samples are shown.

It may be observed from Fig. 3, that the fresh seed samples with high viability germinate rapidly and attain high germination percentages in a relatively short time. The old samples with reduced viability germinate relatively slowly and need a longer time to attain their final germination values. Thus the rate of germination of a sample gives an impression of the vitality of a sample. This information cannot be obtained by simply knowing the germination percentage or the year of collection of a sample. Thus, for instance, samples with practically the same germination values can have different rates of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germination (compare germination rates of samples 1, 3 and 13; 29, 38 and 44; and 7, 67 and 80 as examples of samples with reduced viability, and samples 8, 52, 63; and 25, 26 and 28; as examples of samples with relatively high germination values). Also samples with different germination percentages can show similarities in the rates of germination (cf. samples 18 and 20). Thus the germination rates give useful information about the samples. Consequently, they have been studied carefully in this investigation and shown in Fig. 3.

Fig. 2. *Pictures 1—5*: Fully developed, empty and insect-attacked seeds.

1. A fully developed seed (class IV A):
   a. seed coat. *b.* cavity between seed coat and endosperm. *c.* endosperm. *d.* embryo cavity. *e.* radicle. *f.* cotyledons.
2. An empty seed. Note the endosperm remains (not impregnated).
3. An empty seed. Note the impregnated endosperm remains.
4. Seed showing a caterpillar.
5. Seed with insect excrement.

Pictures 6—15: Seeds showing different degrees of impregnation with sodium iodide.

6. Seed showing a small crack and impregnation around it.
7. Seed with a long transverse crack and with some impregnation patches around it.
8. Partial impregnation of the endosperm alone.
9. Partial impregnation of both the embryo and the endosperm.
10. Endosperm partially and embryo almost completely impregnated.
11. Patched impregnation in the endosperm.
12. Impregnation in most of the endosperm.
13. Impregnation in the embryo, endosperm not impregnated.
14. Most of the embryo and the endosperm impregnated.
15. Complete impregnation of the embryo and the endosperm.

Table 1. Locality and country of origin, latitude, altitude, year of collection, germinability by x-ray contrast (XC) method, and germination on Jacobsen apparatus (JA) of the samples investigated.

Sample No.	Locality and country of origin	Latitude, °N	Altitude, m	Year of collection	Germinability by XC-method, %	Germination on JA, %
1	Kristianstads län, Sweden	55°00′	110	1954	55	56
2	Södermanlands län, Sweden	59°19′	40	1954	36	36
3	Kalmar län, Sweden	57°30′	260	1958	54	57
4	Södermanlands län, Sweden	59°10′	65	1950	27	32
5	Kalmar län, Sweden	56°00′	60	1964	87	90
6	Norrbottens län, Sweden	66°30′	150	1955	24	24
7	Skaraborgs län, Sweden	58°00′	130	1954	11	13
8	Litschau, Austria	48°56′	400—700	1954	81	81
9	Kristianstads län, Sweden	56°00′	150	1964	76	76
10	Bohus län, Sweden	Not known	Not known	1952	16	15
11	Malmöhus län, Sweden	56°00′	75	1964	83	86
12	St. Martin, Austria	48°20′	400—600	1954	62	68
13	Landskammer Vorau, Austria	47°24′	Under 900	1954	53	55
14	Kobernhausser Wald, Salzburg, Austria	48°05′	500—700	1954	3	3
15	Kronobergs län, Sweden	56°58′	265	1951	46	49
16	Eisenkappel, Austria	46°30′	Under 900	1954	67	72
17	Gosau, Austria	47°35′	600—800	1954	38	38
18	Norrbottens län, Sweden	66°00′	100	1951	35	34
19	Aflenz, Austria	47°33′	1000	1954	30	31
20	Eisenkappel, Austria	46°30′	Under 900	1954	21	21
21	Not known	Not known	Not known	1951	0	0
22	Not known	Not known	Not known	1943	0	0
23	Čierny Váh, Slovakia	49°00′	800	1965	80	85
24	Čierny Váh, Slovakia	49°00′	800	1965	80	85
25	Habovka, Slovakia	49°35′	800	1965	84	91
26	Liptovský Hrádok, Slovakia	49°00′	900	1966	85	90

Table 1. *(continued)*

Sample No.	Locality and country of origin	Latitude, °N	Altitude, m	Year of collection	Germinability by XC-method, %	Germination on JA, %
27	Brenzo, Slovakia	48°75′	680	1965	83	88
28	Beňuš, Slovakia	48°50′	1000	1965	86	90
29	Žilina, Slovakia	49°30′	600	1965	50	51
30	Predajná, Slovakia	48°50′	900	1965	92	96
31	Kriváň, Slovakia	48°45′	750	1966	92	94
32	Liptovský Mikuláš, Slovakia	49°10′	800	1965	80	86
33	Betliar, Slovakia	48°10′	1000—1100	1965	80	84
34	Oravský Podzámok Slovakia	49°35′	750	1965	86	90
35	Poprad, Slovakia	49°05′	920—950	1965	83	83
36	Liptovský Hrádok, Slovakia	49°00′	800	1965	66	67
37	Hnušta, Slovakia	48°45′	900	1965	92	93
38	Červená Skala, Slovakia	48°45′	1000	1965	49	50
39	Fichtelberg Revier, E. Germany	50°26′	800	1964	85	90
40	Klingenthal, E. Germany	50°24′	500—800	1959	85	87
41	Sosa-Torfhaus Revier, E. Germany	50°35′	800	1958	16	15
42	Bad Schandau, E. Germany	50°56′	300—425	1964	93	94
43	Burgk Revier, E. Germany	50°34′	475	1965	96	97
44	Schwäbisch-Frankischer Wald, W. Germany	49°20′	Not known	1961	50	50
45	Oberharz, W. Germany	51°35′	300—600	1964	97	98
46	Oberharz, W. Germany	51°40′	300—600	1967	93	97
47	Harzvorland Westerhof, W. Germany	51°45′	Below 300	1967	96	99
48	Westdeutsches Bergland, W. Germany	50°50′	300—600	1967	95	97
49	Bodenseegebiet Oberschnaben, W. Germany	48°05′	Below 700	1958	91	95
50	Östergötlands län, Sweden	58°20′	122	1966	95	96
51	Kalmar län, Sweden	57°36′	115	1966	96	100
52	Älvsborgs län, Sweden	58°00′	75	1964	81	80
53	Skaraborgs län, Sweden	59°00′	170	1964	93	93
54	Södermanlands län, Sweden	58°45′	60	1967	96	99

Table 1. *(continued)*

Sample No.	Locality and country of origin	Latitude, °N	Altitude, m	Year of collection	Germinability by XC-method, %	Germination on JA, %
55	Jämtlands län, Sweden	62°03′	430	1967	95	98
56	Bohus län, Sweden	57°45′	75	1967	95	99
57	Älvsborgs län, Sweden	58°29′	100	1967	94	98
58	Örebro län, Sweden	59°07′	112	1967	90	94
59	Gävleborgs län, Sweden	61°00′	280	1967	95	97
60	Gävleborgs län, Sweden	61°30′	180	1967	94	98
61	Jönköpings län, Sweden	57°18′	210	1967	95	98
62	Kronobergs län, Sweden	56°49′	232	1967	93	98
63	Hallands län, Sweden	56°51′	105	1967	80	79
64	Västmanlands län, Sweden	59°00′	140	1967	94	94
65	Värmlands län, Sweden	59°26′	55	1967	89	91
66	Västerbottens län, Sweden	65°00′	200—300	1943	8	7
67	Jämtlands län, Sweden	63°00′	200	1955	14	12
68	Kopparbergs län, Sweden	60°00′	150	1967	90	90
69	Västmanlands län, Sweden	60°00′	50	1967	95	97
70	Westdeutsches Bergland, W. Germany	50°50′	Above 600	1968	93	93
71	Westdeutsches Bergland, W. Germany	50°50′	Above 600	1968	94	95
72	Westdeutsches Bergland, W. Germany	50°50′	Above 600	1968	92	94
73	Neuwilen, Switzerland	47°38′	560	1966	96	97
74	Muotathal, Switzerland	46°59′	1450	1966	95	97
75	Küblis, Switzerland	46°54′	1000	1966	97	98
76	Murg, Switzerland	47°06′	1000	1966	97	99
77	Bex, Switzerland	46°15′	800	1958	94	98
78	Blekinge län, Sweden	56°00′	150	1964	89	88
79	Jämtlands län, Sweden	63°00′	Not known	1952	0	0
80	Värmlands län, Sweden	59°55′	100	1951	11	13
81	Westerhof, Westharz W. Germany	51°57′	250	1962	89	94

Table 1. *(continued)*

Sample No.	Locality and country of origin	Latitude, °N	Altitude, m	Year of collection	Germinability by XC-method,%	Germination on JA,%
82	Istebna, Poland	49°35′	400—600	1954	11	10
83	Värmlands län, Sweden	60°00′	190	1969	94	96
84	Gävleborgs län, Sweden	61°00′	340	1967	94	96

Note: The samples have been numbered and arranged in the order in which they arrived at the laboratory for investigation.

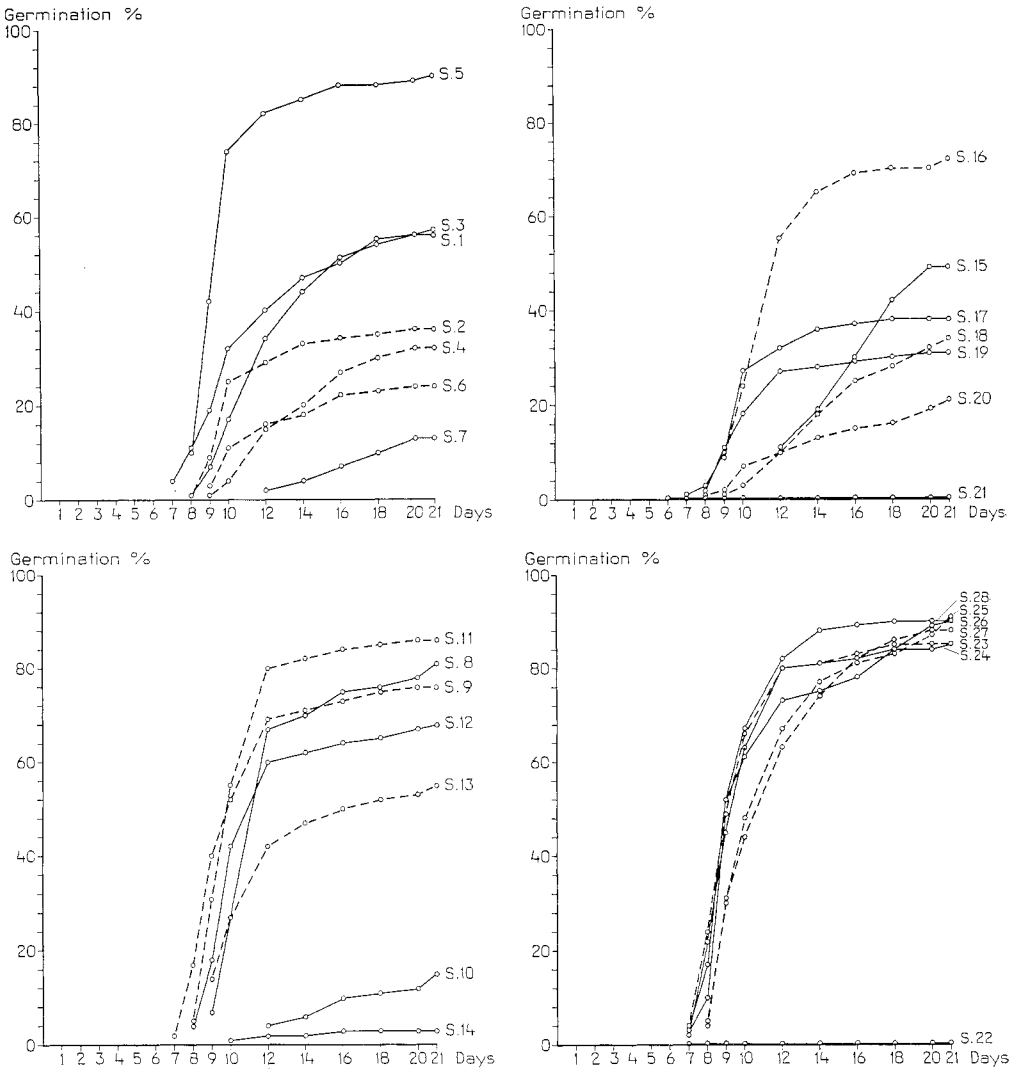

Fig. 3. Rates of germination of the 84 samples investigated (s = sample).

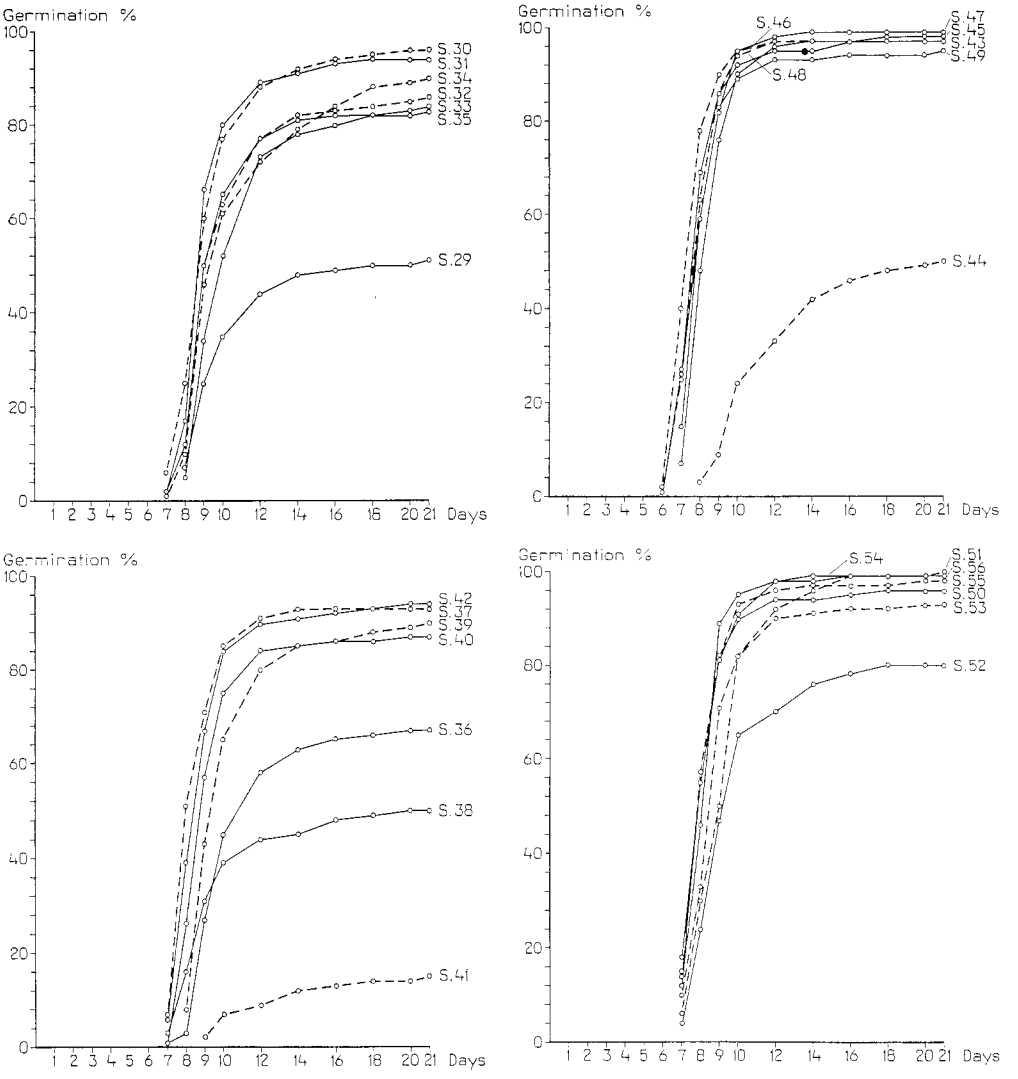

Fig. 3. (*continued*) Rates of germination of the 84 samples investigated (s = sample).

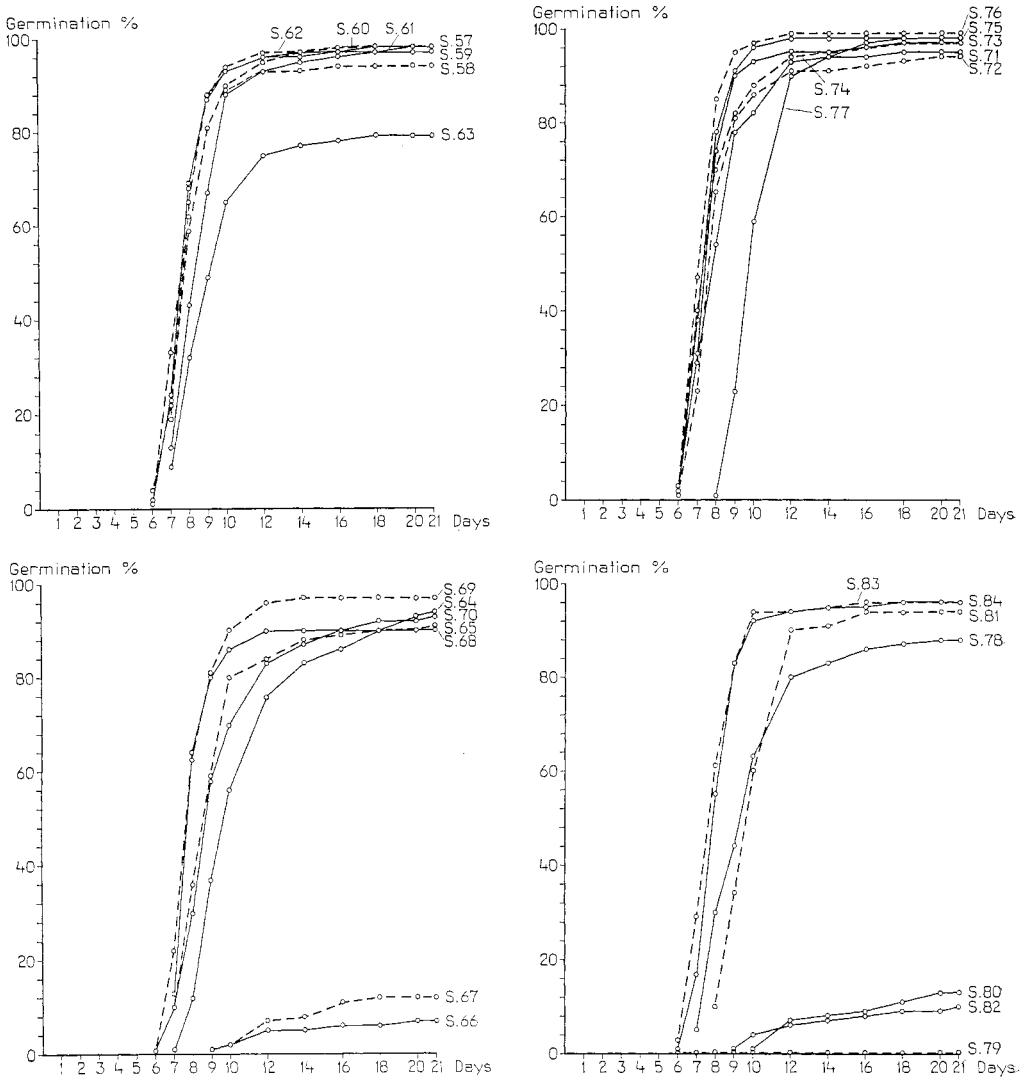

Fig. 3. (*continued*) Rates of germination of the 84 samples investigated (s = sample).

4. Discussion

When one compares the values of germinability of the samples investigated by the x-ray contrast method with their germination values on Jacobsen apparatus (Table 1), one finds that the results of the two methods agree very well with each other. This shows that the x-ray contrast method gives reliable results of the germinability of Norway spruce samples. Consequently, the criteria described under the Results for the judgement of the germinability of *Picea abies* seed by the x-ray contrast method on the basis of impregnation with sodium iodide are dependable. Of special interest in this connection are seeds with partially impregnated endosperm, and embryo free of impregnation. On the basis of Expt. 3, described under the Results, the standard was adopted that seeds in which the embryo remains unimpregnated and the endosperm does not become impregnated in more than 25 per cent of its projected area on the x-ray film, may be considered as germinable. That this standard is reliable is shown by the close agreement between the values of the germinability of the samples by the x-ray contrast method and their germination percentages on Jacobsen apparatus (Table 1). It is interesting to mention here that also Simak (1957), on the basis of his experiments, reached the same standard of impregnation for partially impregnated seeds of Scots pine, using barium chloride as the contrast agent. This indicates that there is some similarity in the behaviour of Scots pine and Norway spruce seeds with respect to their impregnation with barium chloride and sodium iodide respectively.

In Expt. 2 in which samples 1, 6, 17, 53, 71 and 83 were treated with 40 per cent solution of sodium iodide for 10, 15, 20, 30, 45 and 60 min, it was observed (see Fig. 1), that there were differences in the impregnation behaviour of the samples. Thus in the fresh sample with high viability (No. 83), the impregnation remained practically constant up to 45 min of treatment and increased thereafter. In sample No. 71, which was about two years old and with high viability, the impregnation remained practically constant up to 20 min of treatment, rose thereafter up to 30 min, and became almost constant again up to 60 min of treatment. In sample 53, which was six years old but with high viability, the impregnation remained constant up to 15 min of treatment and increased thereafter, first slowly

up to 45 min and then rapidly. In the other three samples (Nos. 1, 6 and 17), the impregnation percentages increased with time. The above-mentioned behaviour of the samples of Norway spruce seed treated with sodium iodide, is similar to the impregnation behaviour observed in the case of Scots pine (Simak & Kamra, 1963; Kamra, 1963 *b*), cucumber (Kamra, 1964), and melon (Kamra, 1966) seed on treatment with barium chloride. The explanation given for the above-mentioned impregnation behaviour of the seed of Scots pine, cucumber and melon in the investigations just cited was, that the seeds with weak vitality (e.g. old seed) or with mechanical damage may gradually be killed with the prolongation of the time of treatment with barium chloride and as a result be impregnated. Consequently, the percentage of impregnated seeds increases in such samples with the prolongation of the time of treatment with barium chloride. The similarity in the impregnation behaviour of the samples of Norway spruce treated with sodium iodide to that of the samples of Scots pine, cucumber and melon treated with barium chloride, would suggest that the explanation given above for these species could also apply to Norway spruce seed.

For calculating the germinability of the samples by the x-ray contrast method, the reduction factors for Norway spruce seed worked out by Müller-Olsen, Simak & Gustafsson (1956) were used. Although these reduction factors have been worked out on Swedish material, they were used both for Swedish and for foreign material in the present investigation. This has been done for the sake of uniformity, so that one could calculate the germinability of a sample without having to know its country of origin. However, the difference between the maximum potential germinability which a sample with well-developed seed can attain (100 per cent), and the average value of 97 per cent on the basis of the reduction factors, is so small that it can be overlooked in the interest of uniformity in the calculation of the germinability by the x-ray contrast method. Thus the reduction factors were used for all the samples in the present investigation.

Another point in connection with the reduction factors is that they have been calculated by Müller-Olsen, Simak & Gustafsson (1956) on the basis of a germination period of 30 days. However, in the present investigation, following the International Rules for Seed Testing (ISTA, 1966), the germination period of 21 days was used. Referring to Fig. 3 of the above authors, in which the rates of germination of the material studied by them are shown, it may be observed that there is no difference in the germination percentages

of the well-developed seeds (class IV A) after the germination periods of 21 and 30 days. Since the material in the present investigation mostly consisted of samples with well-developed seeds (class IV A), the germination period of 21 or 30 days would not make any difference in the results. Thus the reduction factors given by the above authors after 30 days of germination in their Table I could be used in the present investigation.

The impregnation percentages of the six samples shown in Fig. 1 after 15 min of treatment with sodium iodide (non-germinable seed), when added to their germinability percentages (Table 1), do not together attain the value of 100 per cent for each sample. The reason for this difference is the use of the reduction factors for Norway spruce seed for calculating the germinability of unimpregnated seeds, which lowers the germinability percentage of the sample depending upon the embryo and the endosperm development in the seeds.

As with the other rapid methods for determining the germinability of seed, so also with the x-ray contrast method, a certain amount of experience is needed in order to judge the seeds correctly on the radiographs. However, a few suggestions in this connection may be made here.

It is a common experience that there are some natural differences in the absorption of x-rays by individual seeds of a sample. As a result, some seeds appear dark and the others bright on an x-ray film, even though no treatment with a contrast agent has been given to the sample. It is therefore necessary to become acquainted with such differences in the intensity of brightness of the seeds on a radiograph so as not to confuse the bright, unimpregnated seeds with the impregnated ones where both kinds occur on an x-ray film. The proper way of doing this is to use a few seeds (about 50) as the control. They are soaked in water overnight along with the seeds which are to be treated with the contrast agent, but are separated before treatment and are kept soaked in water for the same period for which the others are treated with the contrast agent. Thereafter, these two portions of a sample are dried in separate petri dishes but simultaneously and under similar conditions. While taking the radiograph of the sample, the control seeds should be placed separately in a corner of the film and marked as "control", and the treated ones placed on the rest of the film. In this way, the control and the treated seeds of a sample on a radiograph receive the same exposure and the same processing treatment. The control seeds thus serve as a reliable reference for distinguishing the unimpregnated and the impregnated seeds on the

x-ray film, and consequently reduce the chances of confusing the bright unimpregnated seeds with the impregnated ones on a radiograph.

Regarding the processing of the x-ray films, it is important to remember that the films should not become too light or too dark, as in both cases the distinction between the impregnated and the unimpregnated seeds is difficult to make. The films processed to a medium darkness are usually satisfactory for evaluating the impregnated and the unimpregnated seeds.

If the seeds after treatment with a contrast agent are not washed thoroughly with water, the remaining chemical on the surface of the seeds may cause misleading impregnation artefacts (cf. Simak & Kamra, 1963, for Scots pine seed). It is therefore necessary to wash the seeds well with water. However, a too long washing of the seeds is not desirable, as in this case the contrast agent from the impregnated areas inside the seed coat can be washed off, if the testa for some reason (e.g. due to mechanical damage, etc.) permits easy entry of water into the seed.

In some cases, the contrast agent with which the seeds are treated, is able to enter the seed coat for some reason (e.g. mechanically damaged testa), but stops just under the testa and does not impregnate the endosperm or the embryo. Such seeds should not be confused with the impregnated ones, as here the endosperm and the embryo remain free of impregnation. In a few instances, the contrast agent was found in the tip of the seed between the radicle and the testa.

As may be seen from Fig. 2: 6 and 2: 7, it is possible to observe mechanical damage to seeds on a radiograph. While determining the germinability of a sample by the x-ray contrast method, it is important to know which part of a seed is damaged and to what extent. It stands to reason, for example, that if the root or the shoot forming regions of the embryo are damaged, the seed may not be able to germinate. On the other hand, a seed with a small crack in the testa or minor damage to the endosperm, may eventually sprout. Therefore, it is desirable that mechanical damage is given consideration for determining the germinability of a sample by the x-ray contrast method.

Summary

1. The present paper deals with the working out and the standardisation of the x-ray contrast method for the determination of the germinability of Norway spruce seed. The results of the germinability of the samples obtained by the x-ray contrast method were compared with those of their germination on Jacobsen apparatus, which were used as the standard.

2. Eighty-four samples of Norway spruce seed (*Picea abies* (L.) Karst.) from different countries and with different germination values were used for the investigation (Table 1).

3. From the contrast agents tested (sodium iodide, potassium iodide, sodium bromide, potassium bromide and lithium bromide), the iodides gave a better contrast on the x-ray films than the bromides for the corresponding concentration. Out of the different concentrations of the iodides tried (20, 30, 40, 50 and 60 per cent), 40 per cent was found to be suitable.

4. Regarding the time of treatment, the tests carried out with 40 per cent solutions of sodium iodide for 10, 15, 20, 30, 45 and 60 min (Fig. 1) showed, that the results of the germinability calculated on the basis of 15 min of treatment agreed well with those of the germination of the samples on Jacobsen apparatus.

5. Experiments carried out to study the relationship between the different degress of impregnation of the seeds and their germination, showed that seeds could then be considered as germinable, when both the embryo and the endosperm remained free of impregnation; or when the embryo was free of impregnation and the endosperm was impregnated in not more than 25 per cent of its projected area on the x-ray film. These criteria were adopted as the standard for the determination of the germinability of Norway spruce seed by the x-ray contrast method.

6. The anatomical development of the embryo and the endosperm in a seed was taken into consideration for calculating its germinability by the x-ray contrast method. For this pourpose, the reduction factors worked out by Müller-Olsen, Simak & Gustafsson (1956) were used.

7. The comparison of the results of the germinability of the samples by the x-ray contrast method with their germination values on Jacobsen apparatus (Table 1), showed that the x-ray contrast method can be reliably used for the determination of the germinability of Norway spruce seed.

8. A few common patterns of impregnation of the seeds of Norway

spruce with sodium iodide are shown in Fig. 2. In the Discussion, some suggestions for the interpretation of the germinability of the samples from the radiographs are made.

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Zusammenfassung

Die Röntgenkontrastmethode für die Keimfähigkeitsbestimmung von Fichtensamen

1. Die vorliegende Arbeit behandelt die Ausarbeitung und Standardisierung der Röntgenkontrastmethode für die Bestimmung der Keimfähigkeit von Fichtensamen. Die mit der Röntgenkontrastmethode gewonnenen Ergebnisse für die Keimfähigkeit der Proben wurden mit ihren Keimwerten auf dem Jacobsen-Apparat verglichen, die als Standard dienten.

2. Vierundachtzig Samenproben der Fichte (*Picea abies* (L.) Karst.) aus verschiedenen Ländern und mit unterschiedlicher Keimfähigkeit wurden für die Versuche verwendet (Tabelle 1).

3. Von den geprüften Kontrastmitteln (Natriumjodid, Kaliumjodid, Natriumbromid, Kaliumbromid und Lithiumbromid) zeigten die Jodide einen besseren Kontrast als die Bromide für die gleiche Konzentration. Aus den verschiedenen Jodidkonzentrationen, die geprüft wurden (20, 30, 40, 50 und 60 %), erwies sich 40 % als geeignet.

4. Was die Behandlungszeit betrifft, so zeigten die Versuche, die mit einer 40 prozentigen Lösung von Natriumjodid für 10, 15, 20, 30, 45 und 60 min durchgeführt wurden (Fig. 1), dass die Ergebnisse der Keimfähigkeit, die nach einer 15 minutigen Behandlung berechnet wurden, gut übereinstimmten mit den Keimwerten derselben Proben auf dem Jacobsen-Apparat.

5. Versuche, die durchgeführt wurden, um die Beziehung zwischen verschiedenen Graden der Imprägnierung und der Keimfähigkeit der Samen zu studieren, zeigten, dass die Samen dann als keimfähig betrachtet werden konnten, wenn sowohl Embryo als auch Endosperm unimprägniert blieben, oder wenn der Embryo unimprägniert und das Endosperm auf höchstens 25 % seiner auf den Röntgenfilm projizierten Fläche imprägniert war. Diese Kriterien wurden als Standard angenommen für die Bestimmung der Keimfähigkeit von Fichtensamen mit der Röntgenkontrastmethode.

6. Für die Berechnung der Keimfähigkeit wurde die anatomische Entwicklung des Embryos und des Endosperms in den Samen in Betracht gezogen. Dabei wurden die Reduktionsfaktoren von Müller-Olsen, Simak & Gustafsson (1956) angewandt.

7. Der Vergleich der Ergebnisse der Keimfähigkeit der Proben nach der Röntgenkontrastmethode mit den Keimwerten derselben Proben auf dem Jacobsen-Apparat (Tabelle 1) zeigte, dass die Röntgenkontrastmethode für die Bestimmung der Keimfähigkeit der Fichtensamen mit Zuverlässigkeit verwendet werden kann.

8. Fig. 2 zeigt einige gewöhnliche Imprägnationsmuster der Fichtensamen nach Behandlung mit Natriumjodid. Die Diskussion enthält u.a. einige Vorschläge für die Beurteilung der Keimfähigkeit der Proben auf Grund von Röntgenaufnahmen.

Sammanfattning

Röntgenkontrastmetoden för grobarhetsbestämning av granfrö

1. Detta arbete behandlar utarbetandet och standardiseringen av röntgenkontrastmetoden för grobarhetsbestämning av granfrö. Grobarhetsresultat av de undersökta proven erhållna med röntgenkontrastmetoden jämfördes med samma provers groningsvärden på Jacobsensapparat. De sistnämnda värdena användes som standard i denna undersökning.

2. Åttiofyra prov av granfrö (*Picea abies* (L.) Karst.) från olika länder och med olika groningsvärden användes för undersökningen (Tabell 1).

3. Av de kontrastmedel som testades (natriumjodid, kaliumjodid, natriumbromid, kaliumbromid och lithiumbromid), visade jodiderna vid samma koncentration bättre kontrast på röntgenfilm än bromiderna. Från de olika koncentrationerna av jodid som provades (20, 30, 40, 50 och 60 %) visade sig 40 % vara lämpligast.

4. Angående behandlingstiden, visade försök gjorda med 40 % lösning av natriumjodid under 10, 15, 20, 30, 45 och 60 min (Fig. 1), att grobarhetsvärden beräknade på grundval av experiment med 15 min behandlingstid väl stämde överens med groningsprocenten för samma prov på Jacobsensapparat.

5. De försök som gjordes för att studera sambandet mellan olika grader av impregnering av frö och deras groningsförmåga visade att frö med embryot och endospermet oimpregnerade, eller frö med embryot oimpregnerat och endospermet impregnerat på högst 25 % av dess på röntgenfilm projicerade yta, kunde anses vara grobara. Detta antogs som standard för bestämning av grobarhet av granfrö med röntgenkontrastmetoden.

6. Den anatomiska utvecklingen av embryot och endospermet i fröet togs i betraktande vid beräkning av dess grobarhet vid röntgenkontrastmetoden. För detta ändamål användes de reduktionsfaktorer som utarbetats av Müller-Olsen, Simak & Gustafsson (1956).

7. Jämförelsen mellan resultaten av grobarheten av de undersökta proven med röntgenkontrastmetoden och deras groningsvärden på Jacobsensapparat (Tabell 1), visade att röntgenkontrastmetoden är pålitlig vid bestämning av grobarheten hos granfrö.

8. Några vanliga impregneringsmönster hos granfrö med natriumjodid visas i Fig. 2. I diskussionen finnes några förslag för bedömningen av grobarheten hos granprov från röntgenbilder.