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# Comparative studies on germinability of *Pinus silvestris* and *Picea abies* seed by the indigo carmine and x-ray contrast methods

*Jämförande studier över grobarhet hos tall- och granfrö med indigokarmin- och röntgenkontrastmetoderna*

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# Abstract

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*In this investigation the results of germinability of ten samples each of Pinus silvestris and Picea abies seed by the indigo carmine and the x-ray contrast methods are compared with their germination values on Jacobsen apparatus. Two different criteria, namely those of Hao (1939) and Krzeszkiewicz (1939) were used for judging the germinability by the indigo carmine method. In general, the values of germinability of the samples by the indigo carmine method agreed better with the germination values in the case of samples with high viability than in those with reduced viability. On an average, the germinability values after Krzeszkiewicz showed relatively larger variation from the germination values than those after Hao. The results of germinability by the x-ray contrast method agreed well with the germination percentages in all the samples of both the species. The investigation has shown that the x-ray contrast method is more reliable than the indigo carmine method for rapid determination of the germinability of Scots pine and Norway spruce seed.*

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# 1 Introduction

The method of staining embryos with indigo carmine to determine the germinability of seeds was introduced by Neljubov (1925). He found that indigo carmine stains dead or dying tissues of the embryo readily but leaves the living tissues unstained. From the degree of staining of the embryo, the germination capacity of the seed is estimated. The method has been used on seed of agricultural plants and of forest trees and many publications are available (Grimm *et al.* 1928, Neljubov and Issatschenko 1929, Issatschenko 1931, Doroshenko 1933, Shefer-Safonova *et al.* 1934, Tskoidze 1936, Piskarew 1937, Hao 1939, and Krzeszkiewicz 1939, etc). Interesting reviews of the rapid methods for testing viability, including the indigo carmine technique, have been given by Baldwin 1942, Gadd 1950 and Barton 1961, etc. Moore (1966 and 1968) describes the historical highlights of the biochemical methods and Ovcharov *et al.* (1971) explain their development and use in the U.S.S.R.

It is of interest to point out here that the indigo carmine and the x-ray contrast methods are similar in the sense that they stain or impregnate respectively only the dead tissues of an embryo or a seed, leaving the living tissues unaffected. In the x-ray contrast method, when the 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 which have more or less lost this property, are penetrated by it. Consequently, the dead tissues of the 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 of the embryo and the endosperm etc, the germinability of the seed is determined. In this respect, the x-ray contrast method differs from the indigo carmine method in which the germinability of Scots pine and Norway spruce seed is judged from the degree of staining of the embryo alone.

Since both the indigo carmine and the x-ray contrast methods are rapid methods for determining the germinability of seed, it is of interest to compare their results on a common material. The present investigation was undertaken for this purpose.

# 2 Material and Methods

Ten samples each of Scots pine (*Pinus silvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) from various countries and of different years of harvest were used for the investigation. The specifications of the samples are given in Table 1.

## 2.1 Indigo carmine method (IC)

Representative portions of each sample (1×100) seeds were soaked in water for 16 hours at room temperature after which the embryos were dissected out of the seeds. The embryos were treated with 100 ml of 1 : 1000 solution of indigo carmine for one hour at room temperature. The indigo carmine used for the tests (manufactured by E. Merck, Darmstadt, W. Germany) was dissolved in tap water at about 30° C, no heating of the solution being necessary. After treatment, the embryos were washed with running tap water in order to remove the extra dye from their surfaces. They were then transferred to a glazed glass plate with the help of a small brush and the staining studied.

Two different criteria for judgement were used, namely after Hao (1939) and Krzeszkiewicz (1939). Hao (1939) considers those embryos as germinable which are

Table 1. Locality and country of origin, latitude, altitude and year of collection of the samples investigated.

| Sample No. | Locality and country of origin | Latitude °N | Altitude in metres | Year of collection |
|---|---|---|---|---|
| *Pinus silvestris* | | | | |
| 1 | Ceriana, Italy | 43° 52′ | 850 | 1970 |
| 2 | Granada, Spain | 37° 04′ | 1 750 | 1970 |
| 3 | Hessisches Bergland, W. Germany | 50° 40′ | 300—600 | 1970 |
| 4 | Flims, Switzerland | 46° 47′ | 1 050 | 1969 |
| 5 | Södermanland, Sweden | 59° 03′ | 55 | 1964 |
| 6 | Reigoldswil, Switzerland | 47° 20′ | 830 | 1963 |
| 7 | Kalmar län, Sweden | 57° 45′ | 110 | 1963 |
| 8 | Schlattingen, Switzerland | 47° 35′ | 500 | 1963 |
| 9 | Odenwald Spessart, W. Germany | 50° 15′ | 300—600 | 1961 |
| 10 | Gotland, Sweden | 57° 27′ | 40 | 1952 |
| *Picea abies* | | | | |
| 11 | Brajes, Italy | 46° 41′ | 1 350—1 500 | 1970 |
| 12 | Westdeutsches Bergland, W. Germany | 50° 50′ | 300—600 | 1968 |
| 13 | Kalmar län, Sweden | 57° 36′ | 115 | 1966 |
| 14 | Liptovský Hradok, Slovakia | 49° 00′ | 800 | 1965 |
| 15 | Habovka, Slovakia | 49° 35′ | 800 | 1965 |
| 16 | Klingenthal, E. Germany | 50° 24′ | 500—800 | 1959 |
| 17 | Les Breuleux, Switzerland | 47° 08′ | 1 030 | 1958 |
| 18 | Istebna, Poland | 49° 35′ | 400—600 | 1954 |
| 19 | Aflenz, Austria | 47° 33′ | 1 000 | 1954 |
| 20 | Kristianstads län, Sweden | 55° 00′ | 110 | 1954 |

Note: The samples have been arranged in the descending order of the year of collection.

completely unstained (white) or those which are white but possess a very small blue spot at the radicle end. According to Krzeszkiewicz (1939) completely white embryos as also those which are stained (blue) from the radicle end to less than 1/4 th of their total size, or those with only parts of the cotyledons stained, are considered germinable.

### 2.2 **X-ray contrast method (XC)**

A representative portion of each sample (about 300 seeds) was soaked in water for 16 hours at room temperature. After draining off the water, the seeds were dried superficially with a filter paper. They were then treated with a 40 per cent solution of sodium iodide for 15 minutes in the case of Norway spruce and with a concentrated solution (about 30 per cent) of barium chloride for one hour in the case of Scots pine. After treatment, the seeds were washed with slowly running tap water for two minutes. The extra water was wiped off the seeds with a filter paper and they were allowed to dry in a thermostatically controlled oven at 70° C for 1 1/2 hours. After drying, the seeds were put in plastic patterns containing 300 holes each, one seed in every hole. They were then radiographed with soft x-rays under the following conditions: kV = 14, mA = 5, focus-film distance = 50 cm, time of exposure = 5 seconds. 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, W. Germany.

For determining the germinability of Scots pine and 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 and Gustafsson (1954 and 1956) that in these species there is a direct 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 seed of Scots pine and Norway spruce into five embryo classes (0—IV) and two endosperm classes (A and B), and have worked out the average germination percentage of each class on Jacobsen apparatus (the so-called "reduction factors"). The embryo and the endosperm classes and the reduction factors of the above authors were used in this investigation.

### 2.3 **Germination on Jacobsen apparatus (JA)**

The germination tests were carried out on pure seed (4×100) of each sample. In order to determine the number of empty and insect-attacked seeds in the material to be put for germination, each lot was radiographed with soft x-rays. No contrast agent was used. The conditions for radiography were the same which have been described above under the x-ray contrast method, except that the time of exposure was 3 seconds, as the seeds were spread directly on the envelope containing the film. The procedure for processing the films was the same as described above.

The seeds were germinated on Jacobsen apparatus of stainless steel under the following conditions: Temperature = 20° C (constant); this temperature has been found to be as good as the alternating temperature of 20—30° C for the germination of Scots pine and Norway spruce seed (cf. Kamra and Simak 1968, Simak and Kamra 1968). Light = 1000 lux for 8 hours daily; distance between water level and seed bed = 13 cm; period of germination = 21 days (cf. Kamra 1969).

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. The advantages of this criterion have been discussed earlier by

the author (Kamra 1969).

In the germination tests as also in the indigo carmine and the x-ray contrast methods, the percentage of the germinable seeds of each sample was calculated on the basis of the number of filled seeds only (i.e. the total number of seeds minus the number of empty and insect-attacked seeds in each seed lot). Thus the results of the three methods were comparable with each other.

# 3 Results

## 3.1 Germinability and germination percentages

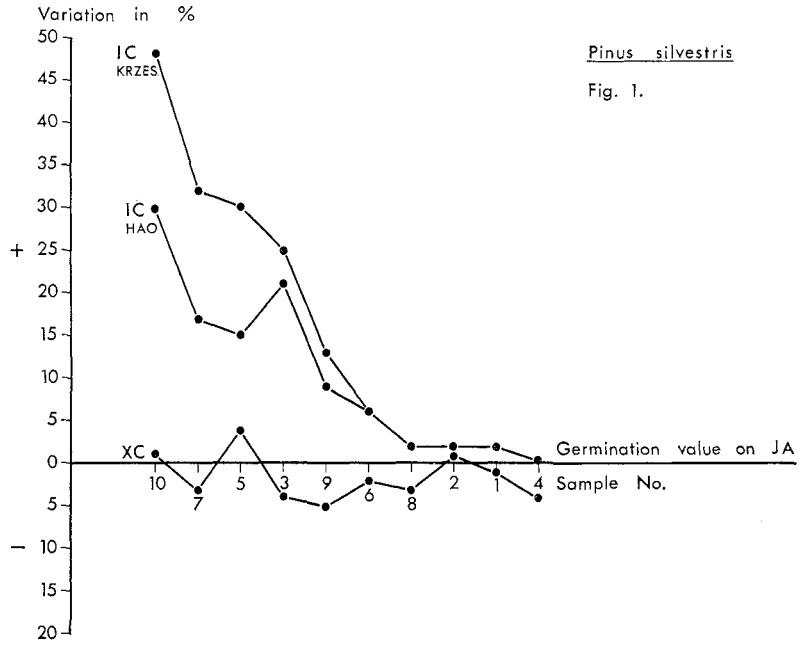
The percentages of the germinability of the samples according to the indigo carmine and the x-ray contrast methods are compared with their germination percentages on Jacobsen apparatus in Table 2.

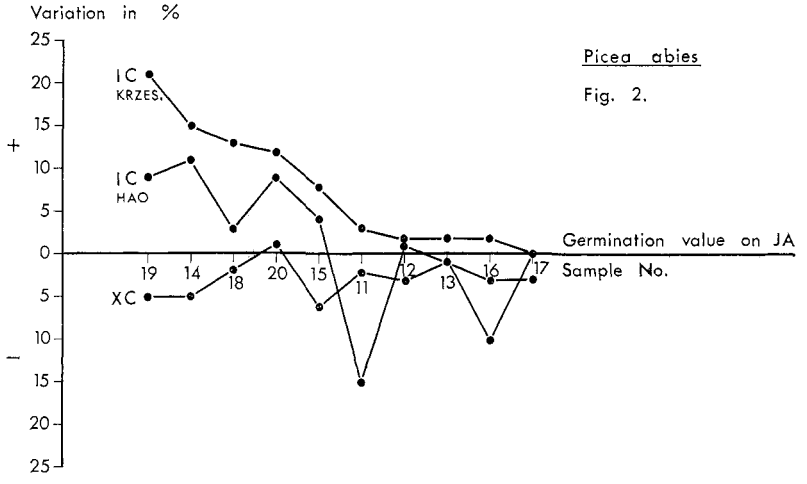
As may be seen from Table 2, the values of germinability of the samples according to the x-ray contrast method agree well with those of germination on Jacobsen apparatus. In the indigo carmine method, the results of germinability agree with those of germination in the case of samples with high viability. For samples with reduced viability, there are considerable differences between the results of the two tests and these differences vary from sample to sample. This question will be gone into in the Discussion.

In the indigo carmine method, the values of germinability according to the criteria of Hao (1939) are equal to or lower than those on the basis of the criteria of Krzeszkiewicz (1939). Mostly, the values of these methods show a better agreement with each other in the case of samples with high viability than in those with reduced viability.

Table 2. Comparison of the germinability percentages of the samples by the indigo carmine (IC) and x-ray contrast (XC) methods with their germination percentages on Jacobsen apparatus (JA).

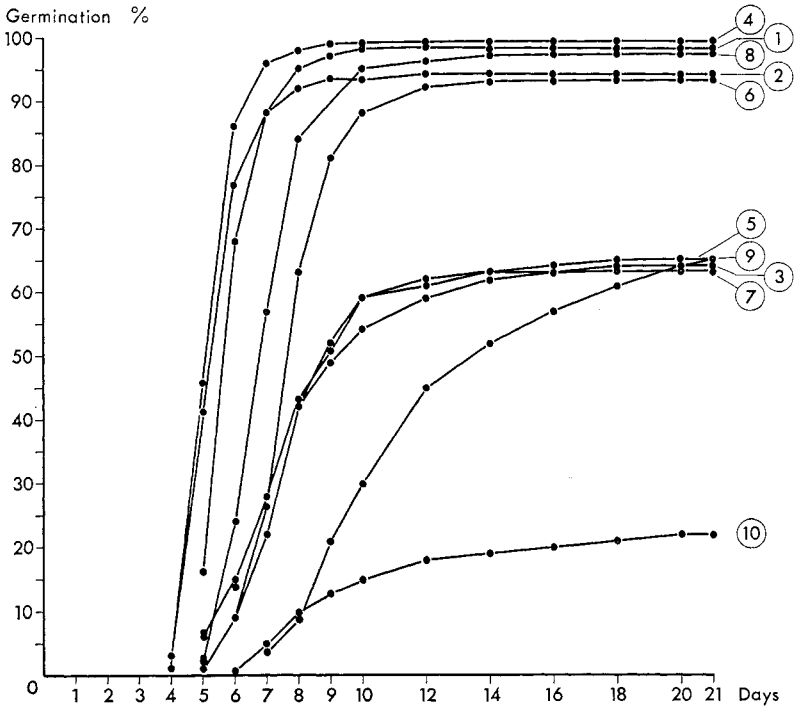
| Sample No. | Germinability by IC method after: | | Germinability by XC method | Germination on JA |
|---|---|---|---|---|
| | Hao | Krzes. | | |
| *Pinus silvestris* | | | | |
| 1 | 100 | 100 | 97 | 98 |
| 2 | 96 | 96 | 95 | 94 |
| 3 | 85 | 89 | 60 | 64 |
| 4 | 99 | 99 | 95 | 99 |
| 5 | 80 | 95 | 69 | 65 |
| 6 | 99 | 99 | 91 | 93 |
| 7 | 80 | 95 | 60 | 63 |
| 8 | 99 | 99 | 94 | 97 |
| 9 | 74 | 78 | 60 | 65 |
| 10 | 52 | 70 | 23 | 22 |
| *Picea abies* | | | | |
| 11 | 44 | 62 | 57 | 59 |
| 12 | 98 | 99 | 94 | 97 |
| 13 | 96 | 98 | 96 | 97 |
| 14 | 68 | 72 | 52 | 57 |
| 15 | 87 | 91 | 77 | 83 |
| 16 | 68 | 80 | 75 | 78 |
| 17 | 100 | 100 | 97 | 100 |
| 18 | 12 | 22 | 7 | 9 |
| 19 | 37 | 49 | 23 | 28 |
| 20 | 50 | 53 | 42 | 41 |



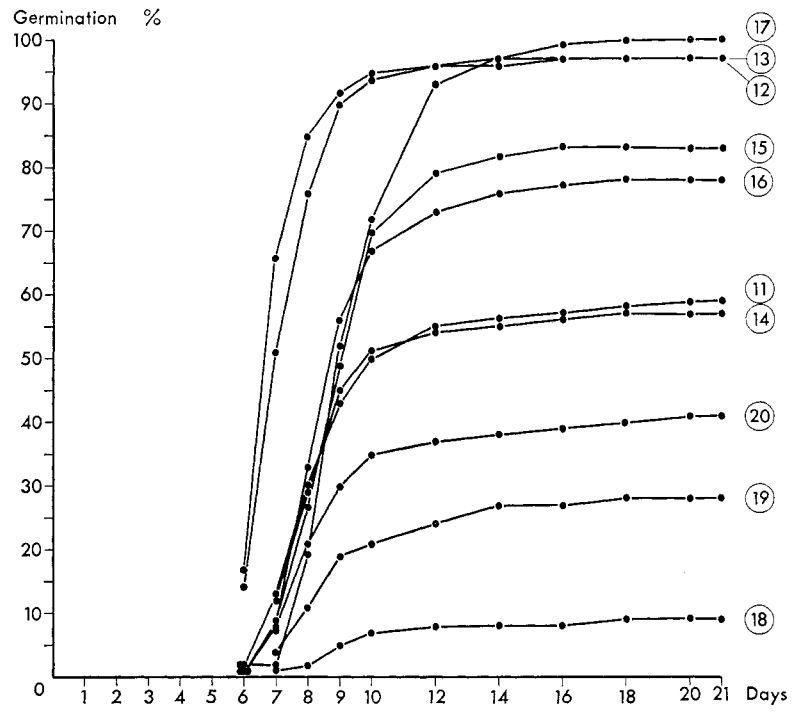

Figures 1 and 2 Variations of the germinability percentages of *Pinus silvestris* and *Picea abies* samples by the indigo carmine method (IC) after Hao and after Krzeszkiewicz, and by the x-ray contrast method (XC), from the germination percentages on Jacobsen apparatus (JA) put equal to zero.

In Figures 1 and 2, the differences between the germinability values of the samples according to the indigo carmine and the x-ray contrast methods on one hand and their germination values on Jacobsen apparatus on the other are presented. The latter values have been put equal to zero, so that only the deviations of the two tests from this standard are visible. As may be observed in the case of Scots pine (Figure 1), the values of germinability according to the indigo carmine method in most of the samples are higher than those of the germination on




Pinus silvestris

Fig. 3.




Picea abies

Fig. 4.

Figures 3 and 4 Rates of germination of the samples of *Pinus silvestris* and *Picea abies* seed on Jacobsen apparatus. The sample numbers are written in the circles.

Jacobsen apparatus. The greatest difference between the values of the two methods is found in sample 10, where the indigo carmine method according to the criteria of Krzeszkiewicz (1939) shows 48 per cent and after the criteria of Hao (1939) 30 per cent higher values than the germination percentage. In Norway spruce (Figure 2), the indigo carmine method does not show such large variations from the standard as in Scots pine. The largest difference between the germination value and that of the germinability by the indigo carmine method is found in sample 19, where the latter method according to the criteria of Krzeszkiewicz shows 21 per cent and after Hao 9 per cent higher values. Samples 11 and 16 show 15 and 10 per cent lower values of germinability after Hao than the germination percentages. In these two samples, the results of germinability after Krzeszkiewicz correspond better with their germination values. The results of germinability by the x-ray contrast method agree well with the germination percentages of the samples both in Scots pine and in Norway spruce, as is shown by Table 2 and Figures 1 and 2.

### 3.2 **Germination rates**

The rates of germination of the samples of Scots pine and Norway spruce are shown in Figures 3 and 4 respectively. As may be seen from Fig. 3, samples 2 and 4 begin their germination already on the fourth day and practically attain their final values on the ninth day. On the fifth day, six samples start germination and of these three (Nos. 1, 6 and 8) germinate rapidly and almost reach their final values on the tenth day. The remaining three samples (3, 5 and 7) germinate relatively slowly and approach their final percentages on the fourteenth day. Sample 10 shows a very slow rate of germination.

In the case of *Picea abies* (Figure 4), the rapidly germinating samples 12, 13 and 17 start on the sixth day and practically attain their final values by the tenth and the twelfth day respectively. Although samples 11, 14, 15 and 16 also begin their germination on the sixth day, they show a slower rate of germination. The remaining samples, 18, 19 and 20, germinate very slowly.

# 4 Discussion

Owing to the close similarity in the structure of Scots pine and Norway spruce seed, the same criteria are usually applied to both these species for judgement of seed germinability. This is done in the tetrazolium as well as in the x-ray contrast methods. Also in the indigo carmine method, Hao (1939) uses the same criteria for *Pinus silvestris* and *Picea abies* seed. Likewise, the criteria of Krzeszkiewicz (1939) should be applicable to seed of both these species. The results given in Table 2 indicate that this is the case. In this respect, the present investigation is an extension of the criteria of Krzeszkiewicz to Norway spruce seed.

When one compares the results of germinability of the samples by the indigo carmine method with their germination values on Jacobsen apparatus (Table 2 and Figures 1 & 2), one finds that they agree with each other in samples with high viability but show considerable differences in some samples with reduced viability. Similar experience was made by Vincent (1957). He found that seed of Scots pine and Norway spruce with reduced viability (below 80 per cent) due to storage, usually showed higher germinability values by the indigo carmine and the tetrazolium methods than the germination percentages. These differences in some cases exceeded the variations allowed in seed testing. In fresh seed with high viability (above 80 per cent), the results of germinability by the indigo carmine and the tetrazolium methods agreed with those of germination. Also the results of Hao (1939), who compared the germinability values of seed samples of some species of forest trees including *Pinus silvestris* and *Picea abies* by the indigo carmine method with the germination tests, indicate a similar trend.

One important reason for the differences between the values of germinability by the indigo carmine method and those of the germination tests, is the fact that the judgement of germinability in this method is based on the staining of the embryo alone, without considering the condition of the endosperm. The results based on such a criterion can only be correct, if the endosperm of the seed is fully sound. If, however, the endosperm is mechanically or physiologically damaged, the above criterion may lead to erroneous results. That the condition of the endosperm is important for judging the germinability of conifer seed, has also been pointed out by Lakon (1950). He states (translated from German): "Comprehensive comparative germination trials have always clearly shown that necrosis, even when small in size, not only on the embryo, but even if present on the endosperm alone, leads to the loss of germination capacity." And at another place in the same paper Lakon states: "The necrosis on the endosperm can be throughout or more or less superficial, in both cases it shows inability of the seed to germinate." Consequently, in the tetrazolium method for conifer seed worked out by Lakon (1950), only those seeds are considered viable, which have fully sound embryo and endosperm. This agrees with the view of Moore (1964) who states: "It appears also worthy to emphasize that in many kinds of seed, the condition of the endosperm can restrict germination of a completely sound embryo."

However, the experience has shown that not all seeds with necrosis on the endosperm are dead (cf. Simak and Kamra 1963 for Scots pine, and Kamra 1971 for Norway spruce seed). Consequently, in the x-ray contrast method, a seed is considered viable, if both the embryo and the endo-

sperm are free of impregnation (= free of necrosis), or in which the embryo is free of impregnation and the endosperm is impregnated in not more than 25 per cent of its total projected area on the x-ray film. That these criteria are dependable is shown by the close correspondence between the values of germinability by the x-ray contrast method and those of the germination tests on Jacobsen apparatus. Moreover, in the x-ray contrast method, in addition to the impregnation behaviour of the embryo and the endosperm, their anatomical development is also taken into consideration. This is done through the use of the embryo and the endosperm classes and their germination values, the so-called "reduction factors". This adds to the accuracy of the x-ray contrast method for determining the germinability of seed.

In order to find out, how the endosperm of the seed with high and reduced viability reacts to treatment with indigo carmine, a pilot experiment was performed. In this, one sample each of Scots pine and Norway spruce with high viability (Nos. 4 and 17, respectively), and the other with reduced viability (Nos. 10 and 18, respectively), were used. Of each sample $2 \times 25$ seeds were soaked in water overnight and after removing the testa, the endosperm was opened and divided into two to four portions, so as to expose the tissues to the dye. The endosperm pieces were then put in a 1 : 1000 solution of indigo carmine for 1 and 20 hours at room temperature, after which they were washed with running tap water and the staining studied. It was observed that after the above two periods of treatment the endosperm in the fresh samples of both the species was only stained along the cut surfaces, as is to be expected. In the case of seed with reduced viability, the endosperm in addition to being coloured along the cut surfaces, was usually also stained deeper in the tissues. Moreover, out of the two samples with reduced viability, sample 18 of Norway spruce showed a larger number of stained endosperm portions than sample 10 of Scots pine, somewhat in relation to their viability. The intensity of the blue colour was stronger after 20 hours than after 1 hour of treatment. This pilot experiment indicates that the endosperm in the case of seed with reduced viability gets stained with indigo carmine. It is therefore possible to take the staining of the endosperm into consideration for testing the germinability of Scots pine and Norway spruce seed by the indigo carmine method.

For treatment with indigo carmine, the embryo has to be dissected out of the seed in the case of *Pinus silvestris* and *Picea abies* seed. That this dissection is a time-consuming operation has already been pointed out (cf. Simak and Kamra 1963). Also some other workers using the indigo carmine method have drawn attention to this difficulty, as mentioned by Gadd (1950). However, Krzeszkiewicz (1939) claims that the dissection of 100 embryos from seeds takes about 20—25 minutes. In the present as well as in the earlier investigation (Simak and Kamra 1963), it took about 2 to 2 1/2 hours for dissecting out 100 embryos. Although through practice this time could be shortened, the dissection of embryos still remains to be a tedious procedure. Moreover, the removal of the embryos from the seeds is connected with the risk of injuring them (e.g. with the preparation needle, etc). Since the injured areas of the embryo get stained with indigo carmine, it is necessary to be able to distinguish them from those stained due to the physiological condition of the embryo, so as to avoid erroneous results. The x-ray contrast method does not require the removal of the embryo from the seed and has therefore the advantage that no damage is caused to the seed while testing its germinability. In addition, the method is easier and quicker to work with.

As described in the Methods, two different criteria, namely those of Hao (1939) and of Krzeszkiewicz (1939) were used for judging the germinability of the samples by the indigo carmine method. These authors consider embryos with various degrees of necrosis as germinable. Their criteria do not agree with the standards laid down in

the tetrazolium and the x-ray contrast methods for determining the germinability of Scots pine and Norway spruce seed (cf. Lakon 1950, Simak 1957 and Kamra 1971). In both these methods, embryos with any kind of necrosis are considered non-germinable. As has been pointed out earlier (Simak and Kamra 1963): "it stands to reason that necrosis, even though small, if it occurs on the meristematic parts of the embryo, can be of serious consequence for the germination of the seed." In view of this, it seems improbable that an embryo with as large a necrosis as 1/4th of its size from the radicle end, could still produce a normal seedling, as is laid down in the criteria of Krzeszkiewicz (1939). On the other hand, a small necrosis on the cotyledons may not always prevent a seed from germinating. In fact, Simak (1957) observed a seed of Scots pine with necrosis on the cotyledons which gave rise to a seedling. Despite this, it is true, that embryos with necrosis, even if they may sometimes germinate, usually fail to produce seedlings with good vitality. Consequently, necrotic embryos are considered non-germinable both in the tetrazolium and in the x-ray contrast methods for Scots pine and Norway spruce.

When one compares the results of the germinability of the samples by the indigo carmine method according to the criteria of Krzeszkiewicz (1939) with those after the criteria of Hao (1939), one finds that they correspond with each other better in samples with high viability than in those with reduced viability. This is due to the fact that seeds with necrotic embryos rarely occur in samples with high viability, but are usually found in those with reduced viability. Since Hao and Krzeszkiewicz consider embryos with different degrees of necrosis as germinable, their results would show greater disagreement in the case of samples where necrotic embryos are of common occurrence than in those in which they are seldom found.

The procedures of Hao (1939) and Krzeszkiewicz (1939) for the indigo carmine method differ in another important aspect also, namely the period of soaking the seeds in water before dissection of the embryos. Krzeszkiewicz recommends a minimum of three hours of soaking in water, whereas Hao uses 24 hours of soaking. In order to find out, if 3 hours of soaking in water is enough for dissecting out the embryos from the seeds, sample No. 17 of Norway spruce was put in water for this period. It was observed that the embryos were not free from the endosperm but adhered to it especially at the radicle end. On treatment with indigo carmine solution, they showed a germinability of 7 per cent after Hao and 10 per cent after Krzeszkiewicz, as against the germination of 100 per cent. However, when the seeds were soaked in water for 24 hours, the embryos separated easily from the endosperm, and on treatment with indigo carmine solution, showed a germinability of 100 per cent both after Hao and after Krzeszkiewicz. This experiment indicated that the soaking of seeds in water for a minimum of 3 hours does not seem to be sufficient and could lead to erroneous results. The soaking of seeds in water for longer than three hours appears therefore to be necessary in the indigo carmine method. However, seeds need not necessarily be put in water for 24 hours. Sample 17 of Norway spruce soaked overnight (16 hours), gave no difficulty in embryo dissection and showed the same value of germinability with indigo carmine as after having been in water for 24 hours. On leaving the sample in water for 48 hours, the seeds had an increased tendency to break easily, but the germinability results were the same as after soaking overnight or for 24 hours. Thus soaking of a seed sample overnight in water was considered satisfactory and was used in this investigation, as described in the Methods.

It may be pointed out that the indigo carmine and the x-ray contrast methods determine the potential germinability of a seed sample. The actual germination percentage can vary depending upon such factors as the germination conditions, dormancy, storage conditions, mechanical

and other damage to seed, etc. The importance of differentiating the potential from the actual germination values has also been stated by Moore (1971) and by Ovcharov *et al.* (1971).

For calculating the germinability of the samples by the x-ray contrast method, the reduction factors for Scots pine and Norway spruce given by Müller-Olsen, Simak and Gustafsson (1954 and 1956) were used. Although these factors have been worked out on Swedish material, they were used both on Swedish and foreign samples in the present investigation. This was 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 99 per cent in the case of Scots pine and 97 per cent in the case of Norway spruce 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 studied here.

Coming now to the rates of germination of the samples, one finds that there are clear differences in them. The samples with high viability show a rapid rate of germination and practically attain their final germination values in 10—12 days. In this category fall sample Nos. 1, 2, 4, 6 and 8 of Scots pine and Nos. 12, 13 and 17 of Norway spruce. Samples with reduced viability show a slower rate of germination. To this group belong samples 3, 5, 7 and 9 of Scots pine and 11 and 14 of Norway spruce. An interesting rate of germination is shown by sample 9 of Scots pine, which begins its germination on the seventh day, as the last of the ten samples of this species, but germinates rapidly and catches up with samples 3, 5 and 7 which had started germinating on the fifth day, that is, two days earlier than this sample. A comparatively slow rate of germination is shown by sample 10 of Scots pine and samples 18, 19 and 20 of Norway spruce. Thus a rapid rate of germination appears to be an indication of high viability of a sample, and a slow rate of reduced viability.

# Summary

1. This paper deals with a comparison of the results of germinability obtained by the indigo carmine and the x-ray contrast methods with the germination values of the samples on Jacobsen apparatus. The latter values were used as the standard for comparison in this investigation.

2. Ten samples each of Scots pine (*Pinus silvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) from different countries and of various years of harvest were used for the investigation. The locality and country of origin, latitude, altitude and year of collection of the samples are given in Table 1.

3. In the indigo carmine method, two different criteria for judgement were used, namely those of Hao (1939) and of Krzeszkiewicz (1939). On an average, the germinability values of the samples after Krzeszkiewicz showed relatively larger variation from the germination values than those after Hao. In general, the germinability values of the samples by the indigo carmine method agreed better with the germination values in the case of samples with high viability than in those with reduced viability. In the latter case, the differences were larger in the samples of Scots pine than in those of Norway spruce.

4. The results of the germinability according to the x-ray contrast method agreed well with the germination values in all the samples both of Scots pine and of Norway spruce.

5. In the indigo carmine method for Scots pine and Norway spruce seed, the condition of the embryo alone is taken into consideration. The importance of considering the condition of the endosperm in addition to that of the embryo, the effect of the embryo and the endosperm necrosis on seed germination, and the influence of the period of soaking seeds in water for dissecting out embryos, etc are pointed out in the Discussion.

6. The investigation has shown that the x-ray contrast method is more reliable than the indigo carmine method for the rapid determination of the germinability of Scots pine and Norway spruce seed.

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# Zusammenfassung

*Vergleichende Studien über die Keimfähigkeit von Kiefern- und Fichtensamen mit den Indigokarmin- und Röntgenkontrastmethoden*

1. Diese Arbeit beschäftigt sich mit einem Vergleich der Keimfähigkeitsergebnisse nach den Indigokarmin- und Röntgenkontrastmethoden mit den Keimwerten derselben Proben auf dem Jakobsenapparat. Die zuletzt genannten Werte wurden als Vergleichsstandard verwendet.

2. Zehn Samenproben jeweils der Kiefer (*Pinus silvestris* L.) und der Fichte (*Picea abies* (L.) Karst.) aus verschiedenen Ländern und aus unterschiedlichen Erntejahren wurden für die Untersuchung verwendet. Die Einzelheiten über die Lokalität und Herkunftsland, Breitengrad, Meereshöhe und Einsammlungsjahr der Proben sind in Tabelle 1 angegeben.

3. Bei der Indigokarminmethode wurden zwei verschiedene Kriterien für die Keimfähigkeitsbestimmung verwendet, nämlich das nach Hao (1939) und das nach Krzeszkiewicz (1939). Im Durchschnitt zeigten die Keimfähigkeitswerte nach Krzeszkiewicz eine größere Abweichung von den Keimwerten als die Werte nach Hao. Im allgemeinen stimmten die Keimfähigkeitswerte nach der Indigokarminmethode mit den Keimwerten besser überein bei Proben mit hoher Keimfähigkeit als bei denen mit relativ niedriger Keimfähigkeit. In dem zuletzt genannten Fall waren die Unterschiede größer bei Kiefern- als bei Fichtenproben.

4. Die Keimfähigkeitsergebnisse nach der Röntgenkontrastmethode stimmten gut überein mit den Keimwerten bei allen Proben sowohl der Kiefer wie der Fichte.

5. Bei der Anwendung der Indigokarminmethode für Kiefern- und Fichtensamen wird ausschließlich der Zustand des Embryos in Betracht gezogen. In der Diskussion wird auf die Bedeutung der Berücksichtigung des Zustands des Endosperms neben dem des Embryos, sowie auf den Einfluß von Embryo- und Endospermnekrosen auf die Samenkeimung, ferner auf die Bedeutung der Einweichungszeit der Samen in Wasser für die Embryopräparation usw. eingegangen.

6. Die Untersuchung hat gezeigt, daß für die schnelle Bestimmung der Keimfähigkeit von Kiefern- und Fichtensamen die Röntgenkontrastmethode zuverlässiger ist als die Indigokarminmethode.

# Sammanfattning

*Jämförande studier över grobarhet hos tall- och granfrö med indigokarmin- och röntgenkontrastmetoderna*

1. I detta arbete jämföres grobarhetsresultaten enligt indigokarmin- och röntgenkontrastmetoderna med samma provers groningsvärden på Jakobsensapparat. De sistnämnda värdena används som jämförelsestandard i denna undersökning.

2. Tio prov vardera av tall (*Pinus silvestris* L.) och granfrö (*Picea abies* (L.) Karst.) från olika länder och av olika skördeår används för undersökningen. Detaljer om lokalitet och härstamningsland, breddgrad, höjd över havet samt insamlingsår av proven finns i tabell 1.

3. Vid indigokarminmetoden används två olika kriteria för grobarhetsbestämning, nämligen efter Hao (1939) och efter Krzeszkiewicz (1939). I genomsnitt visade grobarhetsresultaten efter Krzeszkiewicz större variation från groningsvärdena än de efter Hao. I allmänhet, stämde grobarhetsvärdena enligt indigokarminmetoden bättre överens med groningsvärdena hos prover med hög grobarhet än hos dem med relativt lägre grobarhet. I det sistnämnda fallet, var skillnaderna större hos tallprov än hos granprov.

4. Grobarhetsresultaten enligt röntgenkontrastmetoden stämde väl överens med groningsvärdena hos samtliga prov både av tall och av granfrö.

5. Vid användande av indigokarminmetoden hos tall och granfrö tas endast hänsyn till embryots tillstånd. I diskussionen påpekas betydelsen av att dessutom ta hänsyn till endospermets tillstånd, nekroser hos embryo och endosperm, stöpningstiden osv.

6. Undersökningen har visat att röntgenkontrastmetoden är pålitligare än indigokarminmetoden för snabb bestämning av grobarhet hos tall- och granfrö.

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