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Determination of mechanical damage on Scots pine seed with x-ray contrast method

Bestämning av mekaniska skador på tallfrö med röntgenkontrastmetoden

by

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For profitable practice of forestry and agriculture, it is necessary to obtain as high percentage of seedlings from the sown seed as possible. One important factor determining this is the amount of mechanical damage present on the seed from the threshing or dewinging operations. It has been observed, that forest tree seed with slight or moderate mechanical damage, which may as a result show only 5—10 % loss in germination capacity on Jacobsen apparatus, usually produces 40—50 % fewer seedlings in the soil, as compared to undamaged seed (cf. Huss 1956). In terms of economics it means that the cost of producing an equal number of seedlings from damaged seed is about twice as high as that for undamaged seed. This cost increases still further with greater mechanical damage on seed, as the percentage of seedlings produced then is less. Not only is the seedling percentage reduced because of mechanical damage on seed, but also the height and development of the plants produced from mechanically damaged seed in the earlier stages are inferior to those of plants raised from undamaged seed.

The effects of mechanical damage on seed increase if the damaged seed is stored, as has often to be done in practice. During storage, the mechanically damaged seed loses its germination capacity more rapidly than the undamaged seed, and as a result the seedling percentage decreases. Forest sowings may sometimes fail more or less completely if stored seed with strong mechanical damage is used.

In spite of such great consequences of mechanical damage on seed, there is so far no method available for determining its extent on seed except by direct microscopical examination, which is fairly time-consuming. There is, therefore, an urgent need for developing quick and reliable methods for the estimation of mechanical damage on seed.

In an earlier paper (Kamra 1963), the author pointed out that when a sample of Scots pine seed was treated with organic contrast agents (e.g. Urografin, Umbradil, etc.), only mechanically damaged seed got impregnated whereas the undamaged seed remained free of impregnation, as shown by the x-ray analysis. This observation was subjected to a critical study, the results of which are reported in this paper.

Material

Three samples of the seed of Scots pine (*Pinus silvestris* L.) were used for the experiment. Sample 1 was fresh seed from the harvest year 1962 and sample 11 was dead seed after long storage. Sample 13 had mechanical damage from the dewinging operation and had been stored for 5 years at 4° C. Further details about the samples are given in Table 1.

Table 1

Sample No.	Locality	Latitude	Altitude in metres	Year of harvest
1	Västergötland	58° 45′	180	1962
11	Västernorrland	62° 4′	500	1946
13	Västernorrland	62° 5′	—	1957

It has been established that there is a direct relationship between the development of seed (embryo and endosperm) and its germinability (Simak and Gustafsson 1954, Müller-Olsen and Simak 1954). The different embryo and endosperm classes (0—IV, A & B) along with their germination values on Jacobsen apparatus are given in an earlier paper (Simak and Kamra 1962). With the help of these values it is possible to calculate the so-called "Anatomical potential" ("Ap") of any seed sample for which the embryo and endosperm spectrum is known. The "Ap" value indicates the theoretical probability with which a seed sample would germinate under given conditions. This value is calculated on the basis of the development of embryo and endosperm in the seed and hence corresponds with the actual germination percentage if the seed is physiologically sound. In physiologically damaged seed, the actual germination percentage is lower than this value. The embryo and endosperm spectrum and the "Ap" values for the samples investigated are given in Table 2.

Table 2

Sample No.	Embryo and endosperm class									"*Ap*" value	Germination
	0	I	IIp	IIA	IIIA	IVA	IIB	IIIB	IVB		
1	5	—	—	5	8	82	—	—	—	95 %	96 %
11	4	—	1	2	4	88	—	1	—	96 %	0 %
13	8	—	—	2	3	87	—	—	—	98 %	44 %

Note: Calculations of "*Ap*" value and germination percentage are based on the number of full seeds only (Embryo classes I—IV).

Methods

Before describing the methods, it is considered necessary to explain the technical terms used in this paper.

The term "mechanical damage" means the damage which the seed suffers during the mechanical treatments (e.g. threshing, dewinging, etc.). This may be divided into two categories: (a) Visible mechanical damage—which can be observed on the seed with or without the help of a microscope (e.g. breaks in testa, portions of seed missing, etc.), and (b) Invisible mechanical damage—which cannot be observed directly on the seed.

"Physiological damage" is a collective term which refers to the damage which seed may suffer not from mechanical treatments but from such factors as unfavourable storage conditions, ageing, high temperature during drying, etc., and which may weaken or impair its germination capacity. This kind of damage is usually not directly visible on the seed.

Experiment 1. Impregnation of seed possessing different degrees of mechanical damage

Sample 1. Dewinging was done in various ways, thus causing different degrees of mechanical damage to the seed. In the "control", the wings of the seed were removed carefully with the help of a pair of forceps while holding the seed gently between fingers. Another sample was dewinged by rubbing the seed between hands ("hand-dewinged"). Water-dewinging was applied to the third sample. It was soaked in water for 30 minutes followed by spreading the seed overnight for drying in the laboratory. The wings fell off from the seed through these treatments and were blown away. This sample was thus "dewinged by water." Machine dewinging was carried out with the help of two kinds of dewingers: an old machine (for details, see Huss 1950, Fig. 21) and a new machine (for details, see Huss 1957, p. 363). Seed was dewinged in the old machine for 1, 3 and 5 minutes to produce different degrees of mechanical damage and in the new machine until it was free of wings (about 1 minute). All samples were divided into two groups, one for germination test on Jacobsen apparatus and the other for studying the impregnation behaviour with different x-ray contrast agents.

(a) *Germination test:* 4×100 seeds were put for germination on Jacobsen apparatus at 20—22° C under continuous light (1 000 Lux) for 30 days. The germinated seeds were counted every day during the

first 10 days and every second day later on. A seed was considered as germinated when the length of the root was equal to the length of the seed itself.

(b) *Impregnation studies:* Chemicals used: Urografin, Umbradil and Barium chloride. (The first two of these are used as x-ray contrast agents in human radiography).

Seed was soaked in water for 16 hours at room temperature. After drying its surface with filter paper, it was divided into three groups. One was treated with 60 % Urografin, the other with 50 % Umbradil for 3 hours and 15 minutes, and the third with a concentrated solution of Barium chloride for 1 hour at room temperature. For each treatment a sufficient quantity of the chemical was taken (20 ml. for about 200 seeds). After treatment, the seed was washed with slowly-running tap water for about half a minute to remove traces of the chemical from the surface. This was followed by wiping off the extra water from the seed surface with a filter paper and drying the seed in a thermostat at 70° C for 1 ½ hours. Finally, the seed was radiographed using soft x-rays (kV = 14, mA = 10, focus = 25 cm, time of exposure = 1 ½ seconds) (Simak and Gustafsson 1953).

Experiment 2. Demonstration of impregnation on seed with controlled mechanical damage.

Samples 1 and 11. Fresh and dead seed, undamaged and damaged by pressing under fingers till the seed coat bursted, was treated with Urografin, Umbradil and Barium chloride and radiographed in the same way as described above for Expt. 1. The pictures are given in Plates 1 and 2.

Experiment 3. Storage of mechanically damaged seed.

Sample 13. This sample possessed mechanical damage and had been stored for 5 years at 4° C. For the present study it was treated with Urografin and Umbradil to determine the extent of mechanical damage in the same way as described above for Expt. 1. The germination capacity of the seed was determined on Jacobsen apparatus in 1962 and 1963 under the conditions stated above. The "Ap" value, which is a reliable index of the theoretical germination capacity of a sample, has been used for comparison.

Results

Expt. 1. Impregnation of seed possessing different degrees of mechanical damage.

In this experiment, a portion of the seed dewinged by various methods and possessing different degrees of mechanical damage was treated with Urografin, Umbradil and Barium chloride, and the other was germinated on Jacobsen apparatus. The results are summarized in Figures 1 and 5 and Table 3.

Table 3

Treatments.	Impregnated seed in %			Unimpregnated seed in %			Germination in %
	Urografin	Umbradil	Barium chloride	Urografin	Umbradil	Barium chloride	
Control	1.6	1.6	2.4	98.4	98.4	97.6	96
Hand-dewinged	3.2	2.6	5.7	96.8	97.4	94.3	96
Water-dewinged	2.6	3.1	5.2	97.4	96.9	94.8	96
Dewinged by New machine	9.4	9.3	15.4	90.6	90.7	84.6	90
Dewinged by old machine for — 1 min	17.9	18.5	23.0	82.1	81.5	77.0	85
" — 3 min	30.1	29.5	33.5	69.9	70.5	66.5	75
" — 5 min	36.1	33.7	39.5	63.9	66.3	60.5	63

Regarding the pattern of impregnation, it was observed that Barium chloride showed a diffuse type of impregnation with a difficult distinction between weakly-impregnated and unimpregnated seed or portions of a seed, whereas the organic contrast agents gave a clear difference between impregnated and unimpregnated areas. Moreover, the impregnation was often in patches in the latter case (Figure 2).

An observation of special value for the seed testing work needs mentioning here. The endosperm rests, often present in the empty seed, usually swell up during soaking in water and can get impregnated with organic contrast agents in case of damaged seed (Fig. 2). Care is, therefore, necessary to keep such empty seed apart from the impregnated full seed.

Figure 4 shows the radiograph of a sample of pine seed treated with Urografin. It is easily possible to distinguish between mechanically damaged—impregnated seed (dark in colour) and undamaged—unimpregnated seed (light in colour).

The fungal attack during germination of the seed samples is photographed in Figure 3. It will be observed that there is no fungus on the "control" and very little on the samples "dewinged by hand and water". In contrast, the machine-dewinged seed shows a steady in-

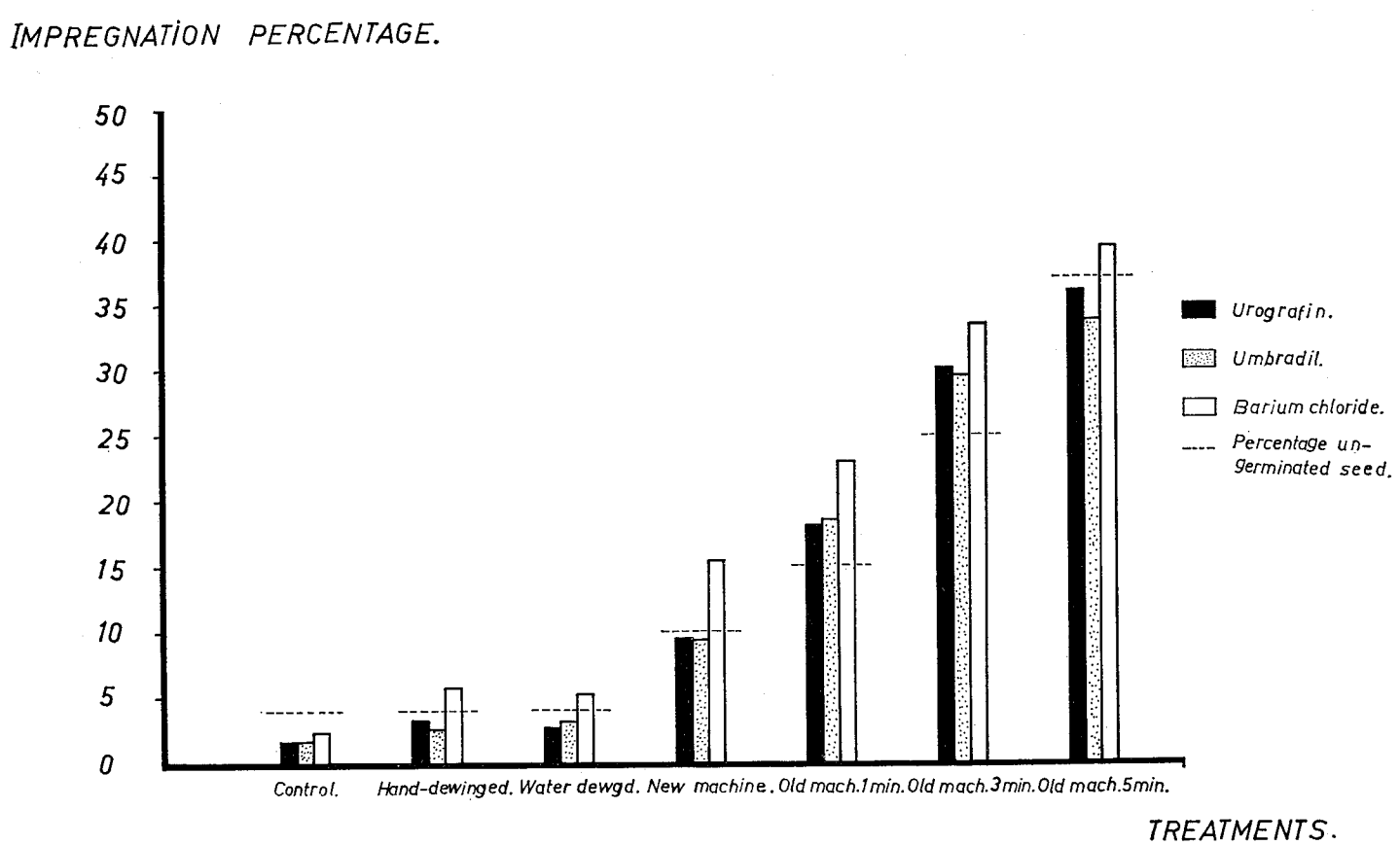


Figure 1. Comparison of the values of impregnation with Urografin, Umbradil and Barium chloride for seed possessing different degrees of mechanical damage due to various methods of dewinging. (Expt. 1).

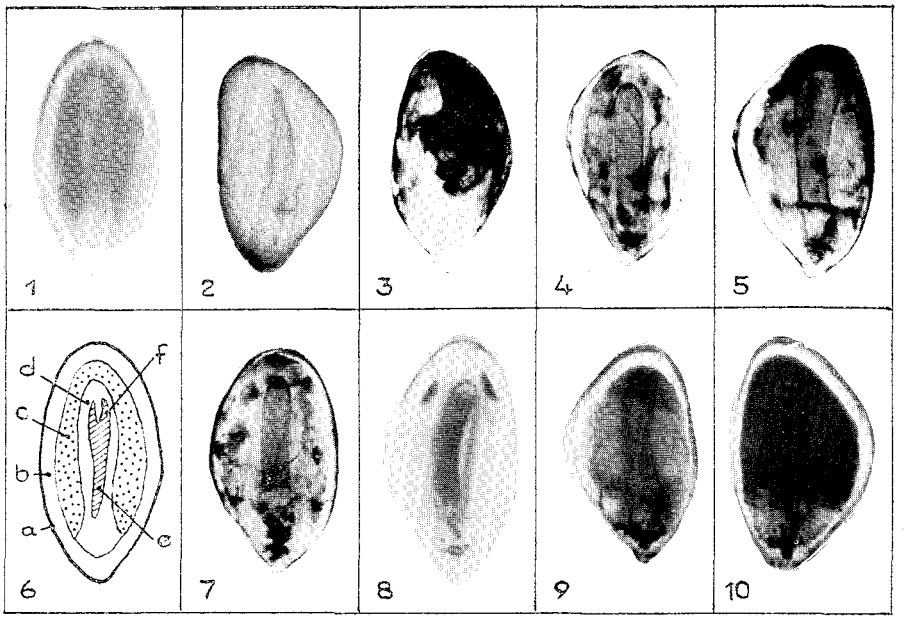


Figure 2. Various patterns of impregnation with Urografin and Barium chloride:
1. An unimpregnated full seed.
2. An unimpregnated empty seed. Note endosperm rests.
3. An empty seed. Note endosperm rests impregnated with Urografin.
4, 5 and 7. Seeds treated with Urografin. Note cracks in the testa and patched impregnation in Nos. 4 & 7.
6. Diagrammatic representation of a full seed:
a. Seed coat. b. Cavity between seed coat and endosperm. c. Endosperm. d. Embryo cavity. e. Radicle. f. Cotyledons.
8—10: Seeds treated with Barium chloride.
8. Embryo impregnated, endosperm practically free of impregnation.
9. Embryo and endosperm impregnated. Note the specially streng impregnation towards both ends of the seed.
10. Practically complete impregnation of the seed.

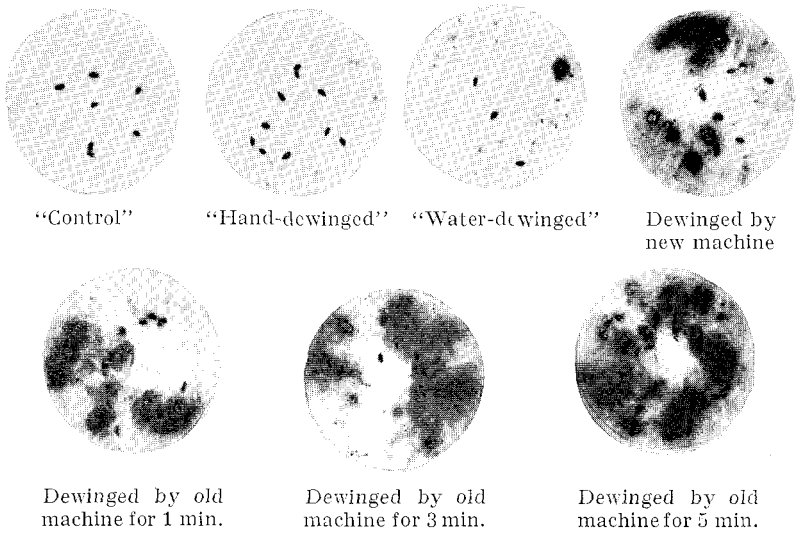
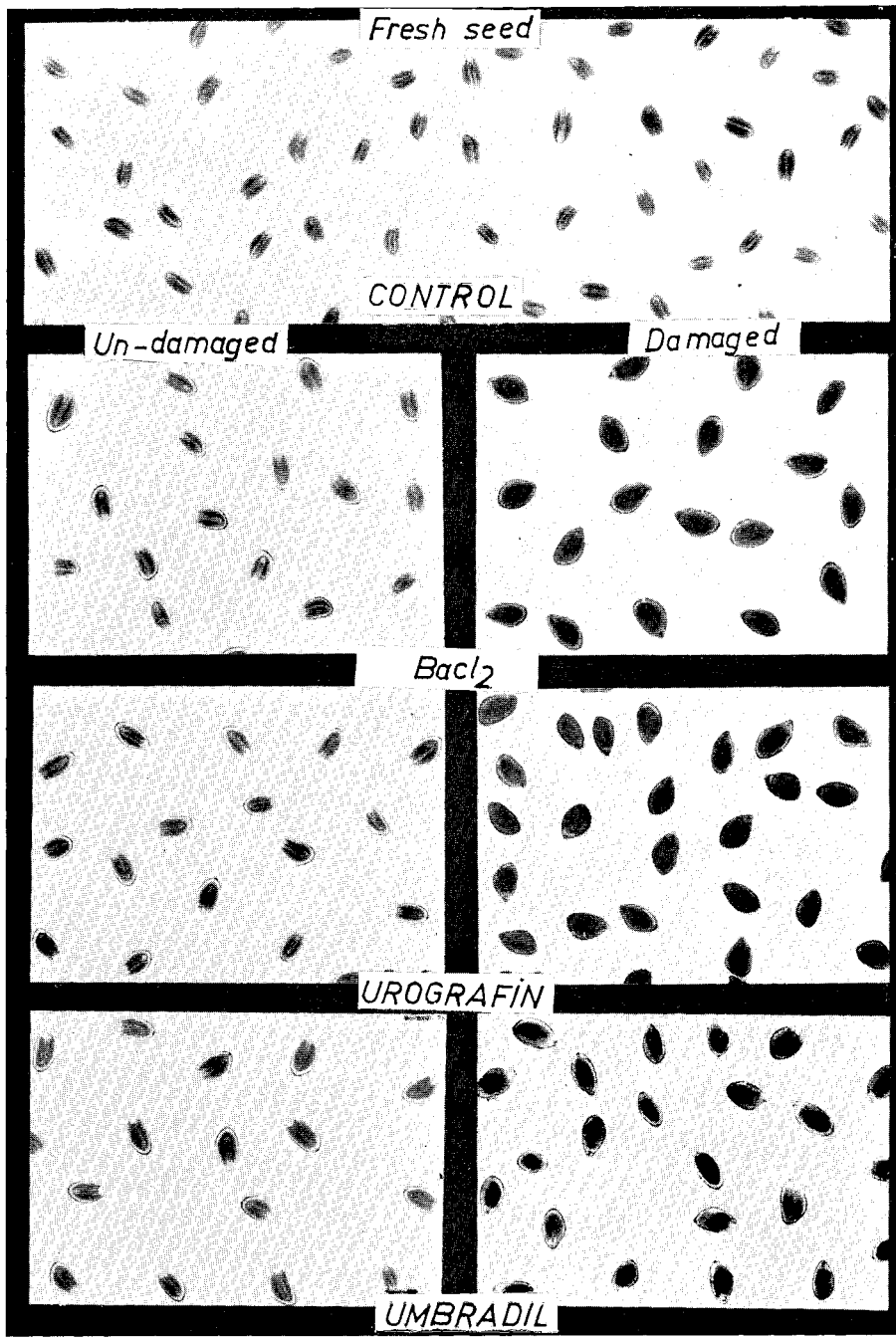


Figure 3. Fungal attack on seed with different degrees of mechanical damage due to various methods of dewinging. (Expt. 1). Photo taken after 25 days of germination on Jacobsen apparatus.



Plates 1 and 2. Fresh and dead, undamaged and damaged seed treated with Barium chloride, Urografin and Umbradil. Note that the damaged seed is impregnated in all cases and the undamaged is free of impregnation in all except the sample of dead seed treated with Barium chloride (Plate 2, Top left). (Expt. 2).

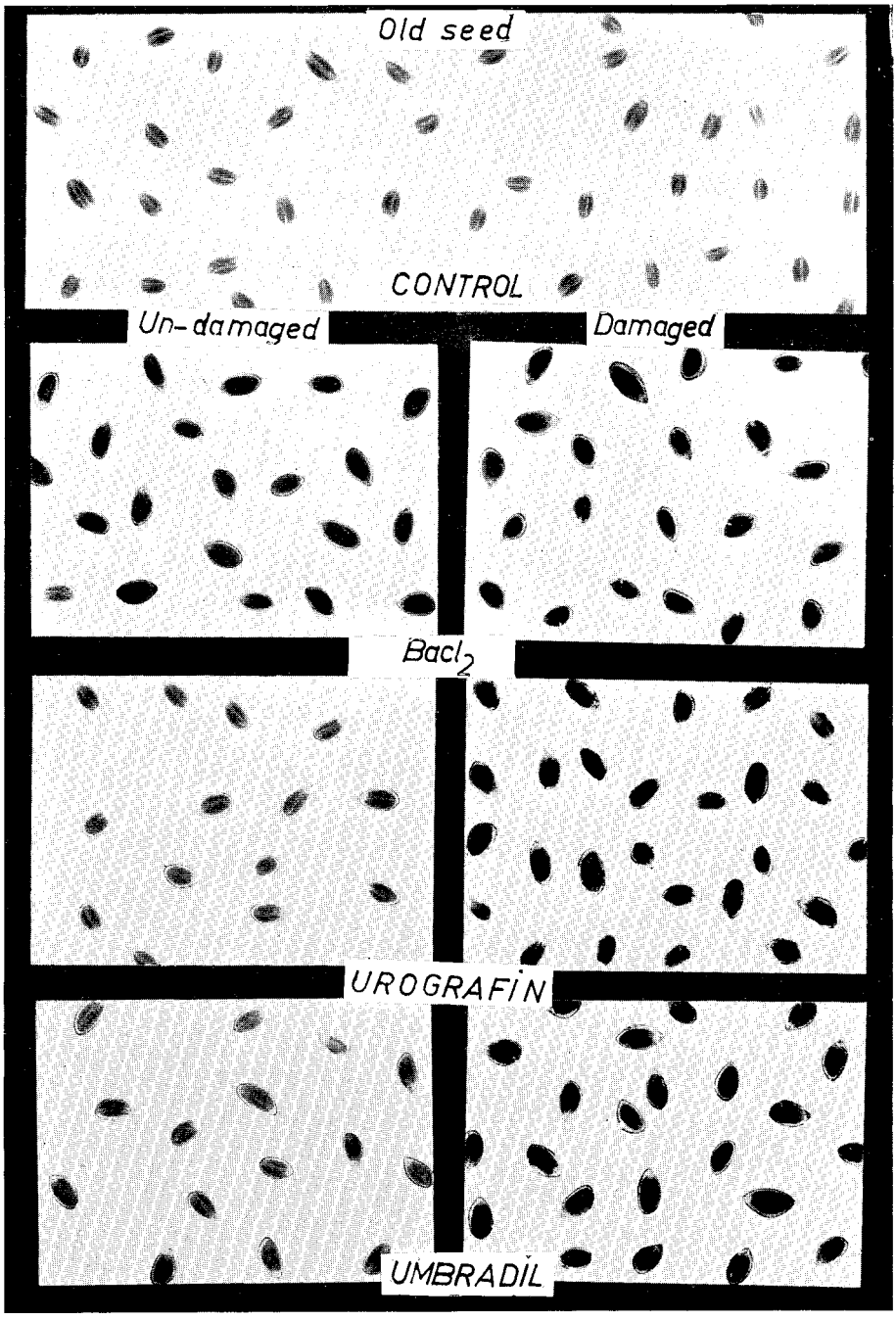

Old seed
CONTROL
Un-damaged
Damaged
$BaCl_2$
UROGRAFIN
UMBRADIL

Plate 2

Figure 4. A sample of pine seed treated with Urografin. The impregnated seeds (dark in colour) are mechanically damaged, the unimpregnated ones (light in colour) are undamaged.

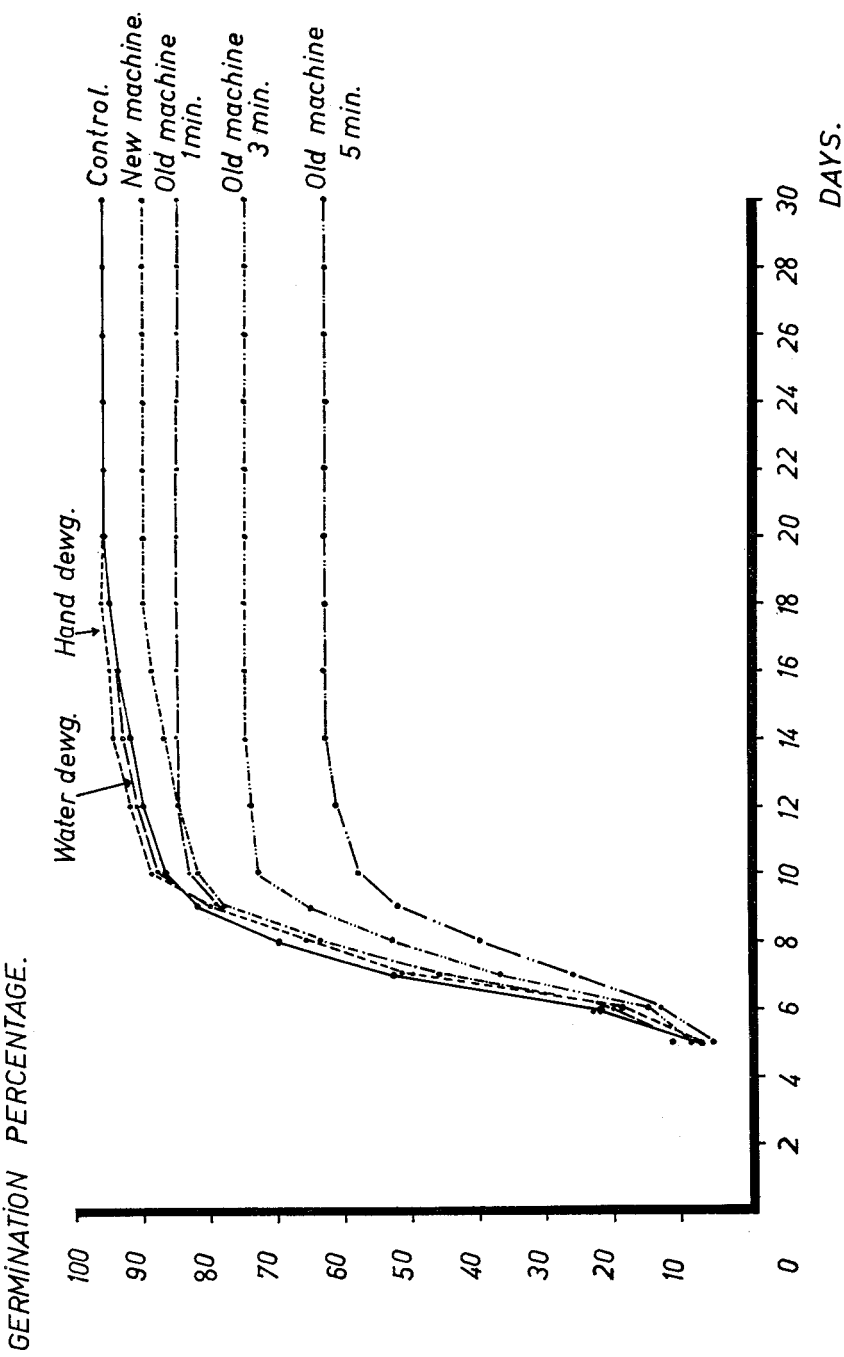


Figure 5. Germination rates of seed dewinged by different methods. (Expt. 1).

crease in the fungal attack with the prolongation of the time of dewinging (cf. 1, 3 and 5 minutes treatment with the old machine).

In Figure 5, the rates of germination of the different seed samples are given. As will be seen, the germination rates of the samples of "control", "hand-dewinged" and "water-dewinged" seed are closely similar and they all attain the same ultimate value. On the other hand, the seed dewinged with machines germinates more slowly, the longer it has been treated with the machine, and reaches a lower final value, as compared with the "control."

Expt. 2. Demonstration of impregnation on seed with controlled mechanical damage.

In order to make sure that Urografin and Umbradil impregnate only mechanically damaged seed and that the impregnation obtained with these contrast agents in the case of pine seed is a reliable indication of mechanical damage, another experiment was carried out. In this, fresh and dead seed undamaged and damaged by pressing under fingers until the seed coat bursted was treated with Urografin, Umbradil and Barium chloride. The photographs of this experiment are given in Plates 1 and 2. It will be observed that the undamaged seed samples show practically no impregnation with Urografin and Umbradil, whereas all samples of the damaged seed are impregnated. Regarding the treatment with Barium chloride, the undamaged fresh seed does not show impregnation, whereas the undamaged dead seed is impregnated. This is to be expected, as Barium chloride enters the seed through the process of diffusion. In living cells, its entry is prevented by the action of semipermeability, which is not the case with dead cells. From the damaged samples, both fresh and dead seed is impregnated with Barium chloride.

Expt. 3. Storage of mechanically damaged seed.

Sample 13 possessing mechanical damage has been stored for 5 years at 4° C. The details about germination capacity, "Ap" value etc. are given in Table 4.

Table 4

Year	"*Ap*" value	Germination on Jacobsen apparatus	Calculated germinability after Barium chloride method	Impregnation with Urografin. (Damaged seed)
1962	98 %	63 %	50 %	—
1963		44 %	39 %	22 %

The potential germinability of the sample on the basis of its "Ap" value was 98 %, which would correspond with the germination percentage on Jacobsen apparatus for the freshly-collected mechanically and physiologically undamaged seed. Apparently the sample has lost about half of its initial germination capacity during five years of storage, as is shown by the value on Jacobsen apparatus in 1963 and the calculated germinability according to the Barium chloride impregnation method. This rather rapid fall in germination capacity of the damaged seed has occurred in spite of storage under favourable conditions, where an undamaged seed sample would be expected to keep its germination capacity much longer. Under unfavourable storage conditions, therefore, damaged seed would lose its germination capacity much more quickly and become unsuitable as sowing material in a rather short time. Detailed comparative studies of the loss of germination capacity during storage of undamaged and mechanically damaged seed according to the present method will be pursued by the author.

Discussion

From what has been said above it will be observed that there appears to be a direct relationship between the extent of mechanical damage to seed, the amount of impregnation obtained with Urografin and Umbradil, and the decrease in the germination capacity. As Experiment 1 showed, with increasing mechanical damage not only the values of impregnation rise, but there is also a regular decline in the germination percentage. This observation is of practical value, as it shows that great care is necessary during mechanical treatments of the seed (such as seed extraction, dewinging, threshing, etc.), if the germination capacity of the seed is to be kept unaffected. Further, as Experiment 3 showed, if damaged seed is stored, there is a rapid loss of germination capacity. In practice, as seed has often to be stored for short or long periods, the determination of the extent of mechanical damage on a sample with the help of Urografin or Umbradil prior to storing would give useful clues regarding the suitability of the sample for storage. Consequently, seed should be stored with as little mechanical damage as possible. If, however, mechanically damaged seed has to be kept, this should be done for the shortest possible time only. For pine seed, it might be useful to store it in winged condition, so as to avoid the loss of germination capacity because of the mechanical damage from the dewinging operation. Another alternative is to dewing the seed with water treatment. This suggestion is supported by the

results of Expt. 1 (Table 3), viz. that the values of impregnation for seed dewinged by hand and water are considerably lower than those for the seed dewinged by machines. Apparently, these treatments do much less damage to the seed than the mechanical dewingers. However, for practical use the water-dewinging method would need to be technically developed. Perhaps the mechanical damage to seed from dewinging machines could also be reduced by regulating the moisture content of the seed for the dewinging operation (cf. Nilsson 1963).

Regarding the impregnation of mechanically damaged seed, it will be seen from Fig. 1, Tables 3 and 4, that the values of impregnation with Urografin and Umbradil are lower than those of Barium chloride for the corresponding treatments. This is due to the fact that whereas the organic contrast agents used impregnate seed with mechanical damage only, Barium chloride, in addition, enters seed with physiological damage.

It may be pointed out that germination test of damaged seed on Jacobsen apparatus often gives different results than those expected on the basis of impregnation with organic contrast agents or Barium chloride (cf. Tables 3 & 4). This is due to the fact that not all damaged seeds are dead. It has been shown previously (Simak 1957, Simak and Kamra 1962) that also seeds with endosperm partially impregnated with Barium chloride (up to 25 % of the projected area on an x-ray picture) may germinate. Similar correlations for impregnation with Urografin, Umbradil and other organic contrast agents are being worked out by the author. In this paper no attempt is made to calculate the germination percentage of a sample on the basis of the impregnation obtained with Urografin or Umbradil.

Regarding sample 13, it was observed in an earlier investigation (Simak and Kamra 1962) that the percentage of impregnation with Barium chloride increased with prolongation of the time of treatment. Thus the calculated germinability of the sample for 1 hour's treatment was 50 % and for 2 hours' treatment 31 %. It was stated (p. 9) that "This sensitivity of the seeds to the time of treatment, which did not show any effect in case of other samples, can eventually be explained in that way, that the seeds with weak vitality or mechanical damage are killed with the prolongation of the time of treatment in barium chloride." The present study has shown that the amount of mechanical damage to the sample is 22 % (cf. Table 4). This observation, therefore, supports the statement made above regarding the presence of mechanical damage on the seed belonging to sample 13.

Conclusion

To conclude, it may be stated that the present investigation has shown, that it is possible to determine reliably the extent of mechanical damage on pine seed with the help of organic contrast agents like Urografin and Umbradil. Moreover, it appears from the present study that the damaged seed loses its germination capacity rather rapidly under storage. Consequently, it would be profitable to store seed with as little mechanical damage as possible, so as to reduce the loss of germination capacity during storage. For pine seed this might be achieved by storing seed in winged condition or by dewinging it with water.

Summary

The present paper deals with the determination of mechanical damage on Scots pine seed (*Pinus silvestris* L.) with the x-ray contrast method. The contrast agents used were: Urografin, Umbradil and Barium chloride.

In Experiment 1, seed dewinged in various ways (by hand, water, new and old machine) and possessing different degrees of mechanical damage, was treated with Urografin, Umbradil and Barium chloride. It was observed that whereas the "control", "hand-dewinged" and "water-dewinged" seed showed very little impregnation, the values of impregnation in case of machine-dewinged samples increased from new to old machine and with the time of dewinging in the latter (cf. 1, 3 and 5 minutes' treatments). The results of germination for these samples on Jacobsen apparatus agreed in principle with the values of impregnation obtained with organic contrast agents. The method is being developed further in order to study the problem of partial impregnation of seed. However, the present experiment has shown that it is possible to estimate reliably the mechanical damage on Scots pine seed with the help of Urografin and Umbradil.

In order to make sure that Urografin and Umbradil impregnate only mechanically damaged seed, another experiment (No. 2) with controlled mechanical damage on fresh and dead pine seed was carried out using also Barium chloride for comparison. It was observed that whereas undamaged seed remained free of impregnation, the damaged seed got impregnated with Urografin and Umbradil. The experiment thus showed that the impregnation with Urografin and Umbradil is a reliable indication of the mechanical damage on Scots pine seed.

Regarding the storage of mechanically damaged seed, Experiment 3 showed that the damaged seed can lose its germination capacity rather rapidly even under favourable conditions of storage. It would be profitable, therefore, to store seed with as little mechanical damage as possible, in order to reduce the loss of germination capacity during storage. For pine seed, this could be done by storing it in winged condition or by dewinging it with water.

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Zusammenfassung

Bestimmung der mechanischen Schäden auf Kiefersamen mit der Röntgenkontrastmethode.

Die vorliegende Arbeit beschäftigt sich mit der Bestimmung der mechanischen Schäden auf Kiefersamen (Pinus silvestris L.) mit Hilfe der Röntgenkontrastmethode. Als Kontrastmitteln wurden Urografin, Umbradil und Bariumchlorid angewandt.

In Experiment 1 wurde das auf verschiedene Weise (mit Hand, Wasser, Neue- und Altemaschine) entflügelte Saatgut, das unterschiedliche mechanische Schäden hatte, mit Urografin, Umbradil und Bariumchlorid behandelt. Es ergab sich daraus, dass während die Kontrolle und die mit Hand und Wasser entflügelte Proben sehr wenig Impregnation zeigten, waren die Impregnationswerte beim Maschinentflügelten Saatgut höher, und sie stiegen von der neuen zu der alten Maschine und bei dieser mit der Zeit der Behandlung (vergl. 1, 3 und 5 minutige Behandlung). Die Keimprozenten für diese Proben auf Jacobsens-apparat stimmen mit den Werten der Impregnation durch organische Kontrastmitteln im Grossen und Ganzen überein. Diese Methode wird weiter entwickelt, um das Problem der Partialimpregnation der Samen zu studieren. Der Jetztige Versuch hat gezeigt, dass eine zuverlässige Bestimmung der mechanischen Schäden auf Kiefersamen mit Hilfe von Urografin und Umbradil möglich ist.

Um sicherzustellen, dass Urografin und Umbradil nur mechanisch-beschädigte Samen impregnieren, wurde ein andrer Versuch (Expt. 2) durchgeführt. Hier wurden frische und alte Kiefersamen, die kontrollierte mechanische Schäden besassen, mit Urografin, Umbradil und Bariumchlorid behandelt. Es ergab sich daraus, dass während die unbeschädigten Samen keine Impregnation zeigten, waren die beschädigten Samen mit Urografin und Umbradil impregniert. Aus diesem Versuch lässt sich schliessen, dass die Impregnation der Kiefersamen durch Urografin und Umbradil eine zuverlässige Indikation der mechanischen Schäden ist.

Was die Lagerung der mechanisch-beschädigten Samen betrifft, so wurde beobachtet (Expt. 3), dass das beschädigte Kiefersaatgut trotz der guten Lagerungsbedingungen seine Keimfähigkeit ziemlich schnell verlieren kann. Es wäre daher vorteilhaft, das Saatgut mit möglichst wenig mechanische Schäden zu lagern (z. B. ohne Entflügelung oder die Entflügelung mit Wasser), um den Verlust der Keimfähigkeit zu erniedrigen.

Sammanfattning

Bestämning av mekaniska skador på tallfrö med röntgenkontrastmetoden.

Föreliggande uppsats behandlar påvisande av mekaniska skador hos frö av tall (Pinus silvestris L.) med hjälp av röntgenkontrastmetoden. Kontrastmedel som använts äro: urografin, umbradil och bariumklorid.

I experiment 1 behandlades frön, som avvingats på olika sätt (för hand, i vatten, i ny och i gammal maskin) och skadats i varierande grad, med urografin, umbradil och bariumklorid. Det observerades att medan »kontroll», »handavvingade» och »vattenavvingade» frön impregnerades i mycket liten utsträckning, graden av impregnering i de maskinavvingade proven steg från den nya till den gamla maskinen liksom även med avvingningstiden i den senare maskinen (jfr 1, 3 och 5 minuters behandling). Resultaten av groningsförsök med dessa fröprov i Jacobsens apparat överensstämde i huvudsak med de impregneringsvärden som erhållits med organiska kontrastmedel. Metoden kommer att utvecklas vidare varvid problemet med partiell impregnering av fröet kommer att studeras. Det utförda experimentet har emellertid visat att det är möjligt att relativt säkert uppskatta mekaniska skador på tallfrö med hjälp av urografin och umbradil.

För att fastställa att urografin och umbradil impregnerar endast skadat frö utfördes ytterligare ett försök (nr 2) med kontrollerade mekaniska skador på färskt och dött tallfrö varvid även bariumklorid användes som jämförelse. Det observerades att oskadat frö ej impregnerades alls medan skadade frön impregnerades med urografin och umbradil. Försöket visade sålunda att impregnering med urografin och umbradil är en säker indikation på mekaniska skador hos tallfrö.

Beträffande lagring av mekaniskt skadat frö visade experiment 3 att sådant frö relativt snabbt förlorar sin groningsförmåga även under gynnsamma lagringsförhållanden. För att reducera förlusten i groningskapacitet under lagringen borde det sålunda vara fördelaktigast att lagra frö med så få skador som möjligt. Exempelvis kunde tallfrö lagras med vingar eller avvingningen ske i vatten.