

Studies on storage of mechanically
damaged seed of Scots Pine
(*Pinus silvestris* L.)

*Studien über die Lagerung von mechanisch
beschädigten Kiefern Samen*

Studier över lagring av mekaniskt skadat tallfrö

by

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Introduction

Irregular seed production is a rather common phenomenon in forest trees, including several species of conifers. Good and poor crops follow each other at intervals. This makes it necessary to store seed of desired species, so that regular supplies are available each year for planting purposes. In order that seed may not lose its germination capacity during storage, suitable conditions must be provided, e.g. low temperature, low relative humidity, etc. Reviews of literature pertaining to storage of forest tree seed, including that of conifers, are given by BALDWIN (1942) and BARTON (1961).

An important factor affecting the germination capacity of stored seed is the amount of mechanical damage it possesses (e.g. from dewinging, etc.). In an earlier study (KAMRA, 1963), it was observed that mechanically damaged seed of Scots pine can lose its germination capacity rather rapidly under storage. This problem was investigated further by storing seed of *Pinus silvestris* both undamaged and with different degrees of mechanical damage under similar conditions in laboratory and in cold room for three years. Germination tests were carried out on Jacobsen apparatus. Parallel studies were made by the x-ray contrast method, using as contrast agents urografin (which impregnates mechanically damaged seeds of Scots pine, cf. KAMRA, 1963), and barium chloride (which impregnates both mechanically and physiologically damaged seeds of this species, cf. SIMAK, 1957). The results of these investigations are reported in this paper.

Material and Methods

Cones of Scots pine (*Pinus silvestris* L.) were collected in 1963 from Bogesund experimental area near Stockholm. Seed was extracted by subjecting them to 49°C overnight in an extractor. The control sample was dewinged by being rubbed carefully between the hands, in order not to injure it mechanically. Dewinging of other samples was carried out with two dewingers, a new and an old machine (for description of the dewingers, see Huss 1957 and 1950 respectively). Seed was dewinged with the new machine until it became free of wings (which took about one minute), and with the old machine for five minutes.

From each of the treatments (i.e. control, dewinged with new and old machine) representative samples (3×100 seeds) were put for germination on Jacobsen apparatus at 20—22°C under continuous light (about 1000 Lux) for 30 days. Germinated seeds were counted every day during the first ten days and every other day thereafter. A seed was considered as germinated when the length of the root was equal to that of the seed itself. Germination tests were made at the beginning of the experiment and after 6 months, 1, 2 and 3 years of storage. The germination percentages were calculated on the basis of filled seeds only.

Studies were undertaken on representative samples of 200 seeds each by the x-ray contrast method, using as contrast agents urografin (60 %) and barium chloride (20 %, cf. SIMAK 1957). These tests were carried out at the start of the investigation and after 6 months, 1, 2 and 3 years of storage. The procedure was the same as that described earlier (KAMRA, 1963).

The water content of the samples was determined by heating 2×50 seeds at 105°C for 16 hours. This estimation was performed at the beginning of the experiment and at the end of each year.

When the material for the above tests had been taken out at the start of the investigation, the remainder of each sample was divided into two parts: one for storage in the laboratory at about 22°C (relative humidity = 50 % approximately), and the other in the cold room at about 4°C (rel. humidity = 60 % approx.). The samples were put in small plastic bottles and the lids screwed on. The bottles were not sealed.

Results

1. Germination

(a) *Germination percentage*

The germination percentages of all samples at the start of the investigation and after 6 months, 1, 2 and 3 years of storage are given in Figure 1. It may be observed from this figure that the germination values of the samples remained practically unchanged throughout the experiment under cold storage. However, there was a decrease in the germination percentages of the samples kept at room temperature for three years. Thus in this case, the control lost 21 %, seed dewinged with the new machine 42 % and that dewinged with the old machine 56 % germination.

(b) *Germination rate*

When one compares the rates of germination of the different samples at the beginning of the experiment (Fig. 2), it will be found that seed dewinged with the new machine germinates faster than the control up to the fifth day. On the sixth day, both attain almost the same value, and thereafter, the control germinates more quickly than the seed dewinged with the new machine. The latter, however, has a more rapid rate of germination, on the whole, than that of the sample dewinged with the old machine.

Germination rate of samples stored in the laboratory:

After storage for six months in the laboratory, seed dewinged with the new machine germinated faster than the control up to the ninth day, and thereafter, more slowly than the latter (cf. Fig. 3).

At the end of one year, seed dewinged with the new machine kept its faster rate of germination up to the sixth day, as compared with the corresponding control, after which the latter was more rapid than the former (Fig. 4).

Testing after two years showed that seed dewinged with the new machine was faster in germination up to the tenth day. Later, the control was the quicker (Fig. 5).

However, at the end of three years, seed dewinged with the new machine was no longer faster in germination than the control, but instead lagged behind the latter from the start of the test (cf. Fig. 6).

As compared with seed dewinged with the old machine, that dewinged with the new machine germinated faster in all tests conducted from the start of the experiment and up to three years of storage in the laboratory (Figs. 2—6).

Germination rate of samples stored in the cold room:

For samples stored in the cold room, the rate of germination of seed dewinged with the new machine was either lower than that of the control (cf. Fig. 3), or was higher up to the fifth or the seventh day of the germination test, and was thereafter lower (cf. Fig. 4, and Figs. 5 and 6 respectively). As compared with seed dewinged with the old machine, that dewinged with the new machine was, on the whole, faster in germination at the beginning of the experiment and after six months of storage. However, at the end of 1, 2 and 3 years, the sample dewinged with the new machine showed a quicker rate of germination than that of seed dewinged with the old machine only in the first few days of germination. Thereafter, either the latter was faster than the former (cf. Fig. 4: from the tenth day onwards, and Fig. 6: Eighth to fourteenth day) or both had an equally fast rate of germination (cf. Fig. 5: from the fourteenth day onwards, and Fig. 6: from the sixteenth day onwards).

Mould development during germination:

Seed samples were not treated with any fungicide, in order to avoid introducing a factor the effect of which on germination could not be ascertained correctly when comparing the results of the various treatments. However, mould development on samples was carefully noted.

It was observed that fungal growth began on about the sixth or the seventh day of germination, usually in samples dewinged with the old or the new machine. Seed stored at room temperature showed earlier and greater mould development than that kept in the cold room. The control samples were usually the last to be affected, if at all, and even they showed the same tendency as stated above. Judging by the whole period of the germination tests, seed dewinged with the old machine often had the greatest mould development, followed by that dewinged with the new machine. The control had either no, or

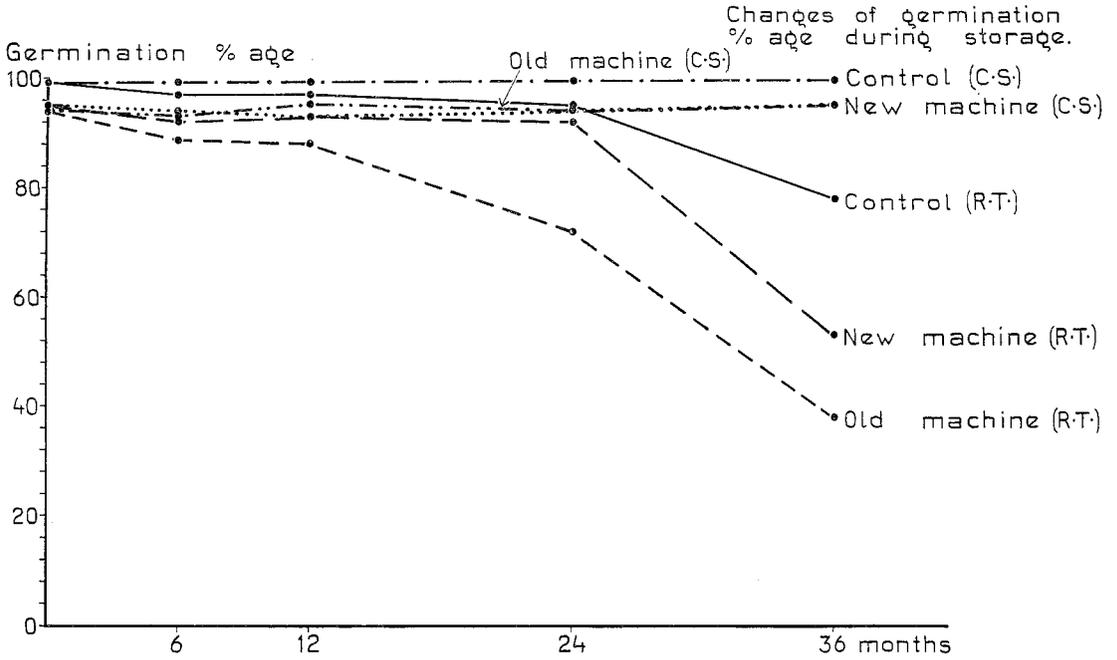


Figure 1: Rate of change of germination percentage during 3 years of storage. C. S. = Cold Storage. R. T. = Storage at Room Temperature.

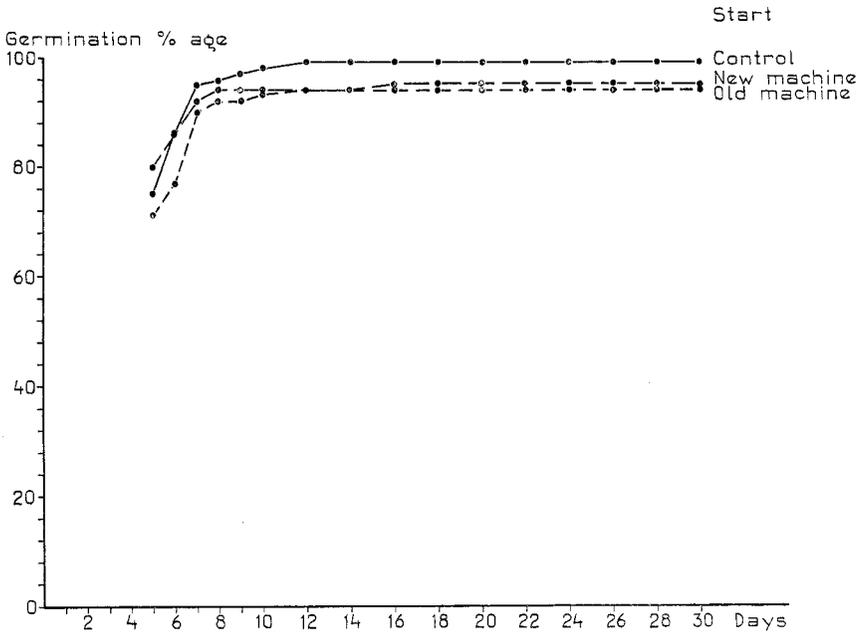


Figure 2: Germination rate of samples at the start of the investigation.

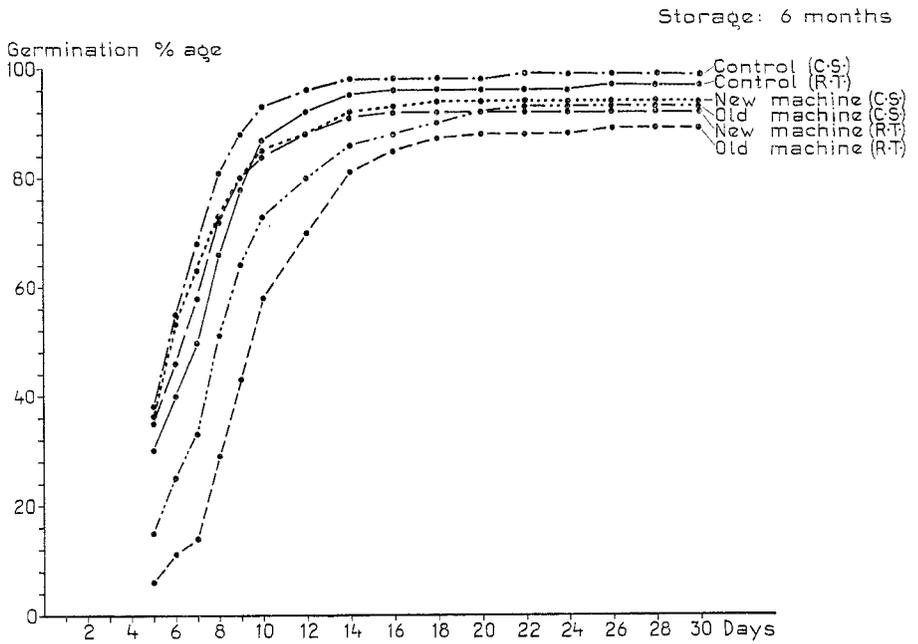


Figure 3: Rate of germination of samples after 6 months of storage.

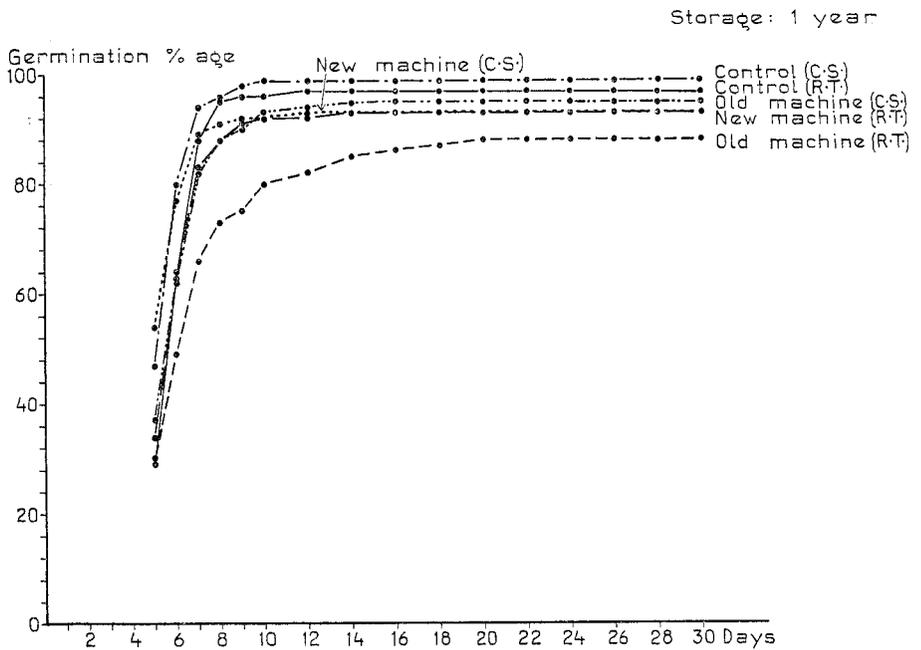


Figure 4: Germination rate of samples after 1 year of storage.

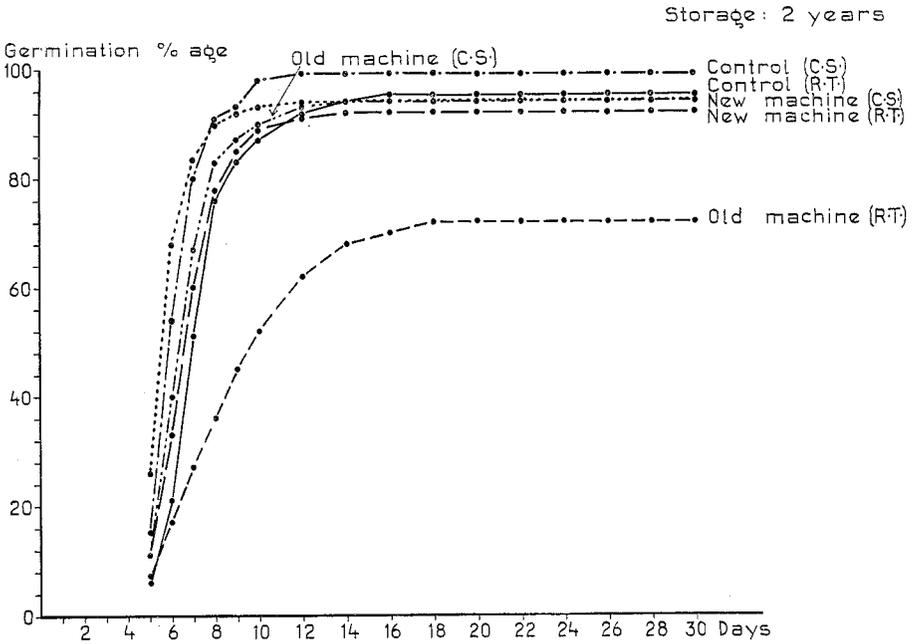


Figure 5: Rate of germination of samples at the end of 2 years of storage.

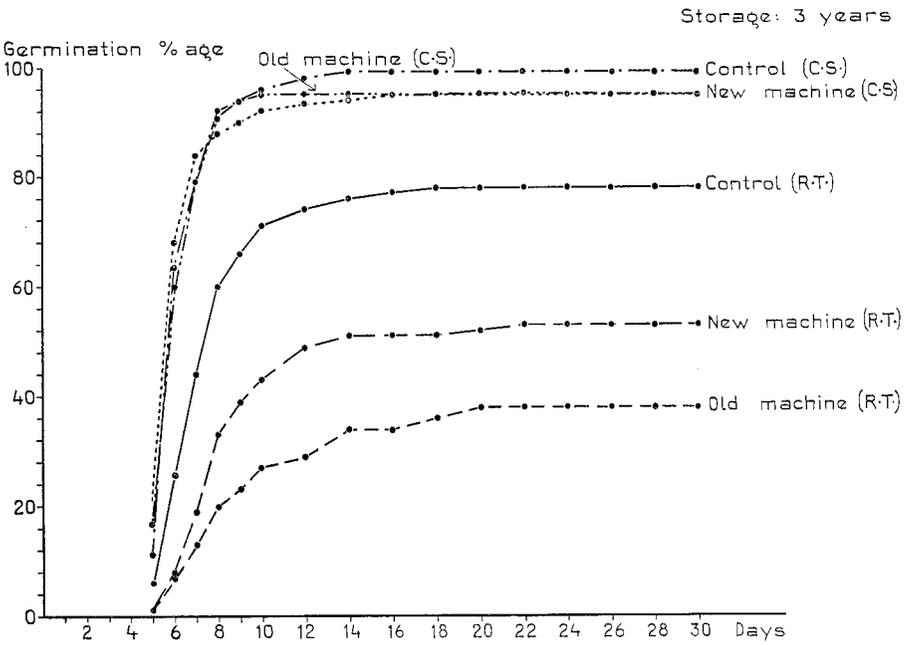


Figure 6: Germination rate of samples after 3 years of storage.

only slight, fungal growth. These observations agree, in principle, with those made earlier (KAMRA, 1963).

2. X-ray contrast method

Tests by the x-ray contrast method, using urografin and barium chloride, were undertaken to study the impregnation behaviour of samples during storage. Detailed investigations of this would need a larger material than was available for the present experiment. Consequently, only the tendencies of the impregnation behaviour of samples are described below.

(a) Impregnation with urografin

The amount of mechanical damage to each sample was determined by the x-ray contrast method, using urografin as contrast agent (cf. KAMRA, 1963). This test was made at the start of the experiment and after 6 months, 1, 2 and 3 years of storage. The results showed that at the beginning of the investigation, the control had 0.8 %, and the samples dewinged with the new and the old machine 13.6 % and 38.5 % impregnation, respectively. These values increased progressively in all samples with the length of storage. Relatively seen, the increase was very small in samples of the control, greater for those dewinged with the new machine, and greatest for those dewinged with the old machine. The percentages and rates of impregnation were higher for the samples stored in the laboratory than for the corresponding ones kept in the cold room.

(b) Impregnation with barium chloride

On each occasion, all samples were treated simultaneously with barium chloride and urografin. The tendencies of impregnation were the same as those described above for urografin. However, the values of impregnation with barium chloride were throughout higher than those with urografin for the corresponding samples.

3. Water content

The water content of all samples was determined at the start of the investigation and at the end of each year. The values obtained are given in the following table.

Table 1: Values of water content of samples

Treatment	Initial value	Value after storage for		
		1 year	2 years	3 years
<i>Storage in the laboratory:</i>				
Control	6.0 %	5.7 %	5.2 %	5.8 %
Dewinged with the new machine	5.4 %	5.5 %	5.2 %	5.6 %
Dewinged with the old machine	5.3 %	5.6 %	5.1 %	5.4 %
<i>Storage in the cold room:</i>				
Control	6.0 %	6.2 %	6.4 %	7.1 %
Dewinged with the new machine	5.4 %	5.9 %	6.1 %	6.6 %
Dewinged with the old machine	5.3 %	5.9 %	5.7 %	6.4 %

As may be seen from the above table, the water content of the samples did not show any great variations during the three years of storage. The values lie within the limits of the "lower and upper critical damp quotients: 4.5 % and 8 % respectively" (cf. Huss, 1954), which were found by the cited author to be favourable for the retention of germinability of conifer seed during storage.

Discussion

From the germination values given above (cf. Fig. 1), it may be observed that both mechanically damaged and undamaged (control) samples of Scots pine seed retained their germination capacity almost unaltered for three years when stored at 4°C, but suffered a loss at laboratory temperature. This observation, that seed of Scots pine during cold storage maintains its viability longer than when it is kept at room temperature, agrees with the experience of many workers. Thus HAACK (1909) found that there was a severe decrease in the germination percentage of Scots pine seed after storage for three years at room temperature, less when it was kept in an unheated room and least when it was stored cool or on ice. That cold storage is favourable for the maintenance of viability of seeds of conifers and hardwoods was also reported by CIĚSLAR (1897), ZEDERBAUER (1910), SHIRASAWA and KOYAMA (1915) and others. MILLER (1930) states that generally temperatures of 0—5°C and low relative humidity are the best conditions for storage of dry seed. BARTON (1935) concluded from her experiments with conifer seed, that sealed storage at low temperatures (5°C, or—4 to —15°C) was effective in maintaining vitality. HUSS (1954) also reported the favourable effect of cold storage on the preservation of germinability of conifer seed.

The reason why seed can keep its viability longer at low than at room temperature seems to be that biological and biochemical processes (e.g. respiration, etc.) proceed progressively slower as the temperature is lowered. Thus BARTON (1953) found that of the three temperatures tried by her for storing seed of *Pinus echinata* and *Pinus taeda*, the loss of viability was slower at 5°C than at room temperature, and still slower at —4°C. HUSS (1954) obtained similar results with seeds of conifers.

In addition to low temperature, a favourable effect of air-tight or sealed storage on the maintenance of viability of seed of conifers (including that of *Pinus silvestris*) has been reported by several workers (CIĚSLAR, 1897; HAACK, 1909; TILLOTSON, 1921; BARTON, 1935; etc.). The author made the following observation in this connection:

In an earlier investigation (KAMRA, 1963) a sample (No. 13) of Scots pine seed with 22 % mechanical damage was stored in a thin paper bag for five years at about 4°C (rel. humidity = 60 % approx.). Although initially its potential germinability (on the basis of "Ap" value) was

98 %, it germinated only 44 % after five years of storage (a loss of 54 %). This rather rapid decrease in germination occurred even when the sample was kept at a low temperature. As against this, Scots pine seed with even greater mechanical damage (38.5 %, due to dewinging with the old machine), kept in a closed plastic bottle under the same conditions in the present experiment, did not show any apparent loss in germination percentage up to three years of storage.

The favourable effect of air-tight or sealed storage is believed to be due to the preservation of a proper moisture content, and the prevention of fungal contamination of seed, etc. (cf. BALDWIN, 1942). However, it is necessary at the time of storage that seed has a suitable moisture content. The question of moisture content and storage of conifer seed has been investigated in detail by HUSS (1954).

In the case of samples with different degrees of mechanical damage stored at room temperature for three years (Fig. 1), it may be observed that the decrease in germination percentage becomes greater as the mechanical damage to seed increases. Thus in three years, the control lost 21 % germination, whereas the samples dewinged with the new and the old machine lost 42 % and 56 %, respectively. These observations agree with those of HUSS (1950), who finds in the case of pine and spruce that "the plant percents of the experimental material are lower for the mechanically dewinged seed samples than for the corresponding hand de-winged ones, and that the plant percents decrease strongly with the strength of the treatment."

When one compares the rates of germination of the different samples, one finds that the seed dewinged with the new machine germinates faster than the control, at least in the first few days of germination, up to two years of storage at room temperature (cf. Figs. 3—5). After three years (Fig. 6), the control germinates more rapidly than the sample dewinged with the new machine. The explanation for such differences in the percentage and the rate of germination of samples dewinged mechanically has been suggested by BALDWIN (1942, p. 69) to be as follows: "Dewinging by mechanical abrasion tends to scratch or mar the seed coat in tearing off the wing, and this results in greater permeability, quicker germination, more active respiration, and hence poorer capacity to stand storage."

However, seed dewinged with the old machine germinated more slowly than that dewinged with the new machine, and than the control, at the start of the experiment and after being stored up to three years in the laboratory. Probably this sample had suffered too much mechanical damage (38.5 %) during dewinging with the old machine.

The corresponding values for sample dewinged with the new machine and for the control were lower, 13.6 % and 0.8 % respectively.

That the effect of the degree of mechanical damage on the rate and percentage of germination was less marked in samples stored at 4°C than those kept at room temperature, is not surprising. This is because the biological and biochemical processes leading to the reduction in the percentage and the rate of germination proceed more slowly at lower than at room temperature. Consequently, a longer time would be necessary for observing their effect clearly at 4°C than at 22°C. For the same reason, the amount of impregnation of seed with urografin and barium chloride was higher in samples kept at laboratory temperature than in those stored in the cold room (cf. results).

The percentages of impregnation with barium chloride were throughout higher than those of urografin for the corresponding treatments (cf. results). This is because barium chloride enters both mechanically and physiologically damaged seeds of Scots pine (cf. SIMAK, 1957), whereas urografin impregnates only those with mechanical damage (cf. KAMRA, 1963).

In an earlier paper (KAMRA, 1963), it was mentioned that a correlation between the impregnation of seed with urografin, umbradil and other contrast agents, and its germination capacity, was being worked out. Experience in this connection showed that although impregnation with urografin or umbradil is an indication of mechanical damage to a seed, it cannot predict how this damage will affect the germination capacity of the seed in question. This is because the effect of mechanical damage on germination of seed is variable from case to case. Thus, for example, while a seed the root or shoot-forming regions of which are seriously damaged, may fail to germinate, one with slight damage to the testa could grow. Moreover, for stored seed, the effect of mechanical damage on the germination capacity would vary with the length and the conditions of storage. In view of the variable nature of the effect of mechanical damage on the germination capacity of seed, it would be misleading to try to predict it on the basis of impregnation with urografin or umbradil. The value of this method lies in its estimating the amount of mechanical damage to a sample, which information, for example, can indicate whether it is profitable to store a particular sample or not. As in the earlier paper (KAMRA, 1963) no attempt is made here to calculate the germination percentage of a sample on the basis of impregnation with urografin or umbradil.

In reference to the water content of the samples (Table 1), it will be found that it increased slightly more in those stored in the cold room

than in the corresponding ones kept in the laboratory for three years (except for the control in the latter case). This difference in rise of water content could be because the relative humidity in the cold room was higher than that in the laboratory (60 % and 50 % respectively).

One might ask whether any seasonal rhythm in germination was observed, as has been reported by SCHMIDT (1930) and REHACKOVA (1954) for Scots pine seed. It may be pointed out in this connection, that all samples in the present investigation were germinated at the same time each year (in October), except for those tested after six months of storage. Consequently, seasonal differences could hardly be expected in the above cases. Moreover, the samples (except for the control) were mechanically damaged to different degrees, the effect of which on germination may vary from one case to another. In view of these facts, it would not be correct to draw conclusions about the seasonal rhythm in Scots pine from the results of the present investigation.

In order not to cause mechanical damage to seed, dewinging is sometimes done with water. In this connection, BALDWIN (1942, p. 69) states that: "When seeds are moistened to facilitate wing removal they become partly imbibed and stimulated to begin germination. Interrupted in this process they become weakened. Wing removal then usually results in increasing the readiness of seeds to germinate, and in decreasing the duration of their viability in storage;" But dewinging machines usually also cause some mechanical damage to seed, which affects its germination capacity during storage. Thus both methods have advantages and disadvantages which may be kept in mind while selecting a method of seed dewinging to suit one's needs.

In conclusion, it may be said that the present investigation has shown that mechanically damaged seed of Scots pine loses its germination capacity earlier when stored at room temperature, than in cold storage. Moreover, the higher the amount of mechanical damage to a sample, the greater is the loss of germination during the corresponding period of storage. This decrease is especially clear if samples are kept at room temperature. Thus it is profitable to store seed at low temperature and with as little mechanical damage as possible. In the case of *Pinus silvestris*, seed could be stored in the winged condition, in order to avoid injuring it during dewinging, as is done in *Pinus palustris* (cf. BALDWIN 1942, p. 69). Dewinging could then be carried out just before seed is to be sown. Moreover, the storage of seed in air-tight or sealed containers might help to preserve its viability longer than storage in containers where air and moisture have easy access to it.

Summary

This paper deals with the storage of seed of Scots pine (*Pinus silvestris* L.) in laboratory at about 22°C (relative humidity = 50 % approximately) and in cold room at about 4°C (rel. hum. = 60 % approx.) for three years. The control was dewinged by hand, in order not to injure it mechanically. Two dewingers (a new and an old machine) were used for the other samples, and they caused different amounts of mechanical damage to seed.

During cold storage, germination values of all samples remained practically unchanged up to three years. As against this, there was a decrease in the germination percentages of the samples kept at room temperature. Thus during three years in the latter case, the control lost 21 %, and the samples dewinged with the new and the old machine 42 % and 56 % germination, respectively. The rates of germination of all the samples are shown in Figs. 2—6.

The amounts of mechanical damage, and mechanical plus physiological damage together, to all samples, were determined by the x-ray contrast method using urografin and barium chloride respectively as contrast agents. These tests were carried out at the start of the experiment, and after 6 months, 1, 2 and 3 years of storage. In all cases, there was a rise in impregnation percentage with the period of storage, although this increase was less for samples stored in the cold room than for those kept in the laboratory.

The water content of samples was determined at the beginning of the investigation and at the end of each year. The results are given in Table 1.

The investigation has shown that mechanically damaged seed of Scots pine loses its germination capacity earlier when kept at room temperature than when kept under cold storage. Moreover, the higher the amount of mechanical damage to seed, the greater is the decrease in germination percentage during the same period of storage. Thus it is profitable to store seed at low temperature and with as little mechanical damage as possible. In the case of Scots pine, seed could be kept in the winged condition, and be dewinged just before sowing. Storage in air-tight or sealed containers might help to preserve the viability of seed longer than storage in containers where air and moisture have easy access to it.

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Zusammenfassung

Studien über die Lagerung von mechanisch beschädigten Kiefernsemen

Die vorliegende Arbeit beschäftigt sich mit der Lagerung von Kiefernsemen (*Pinus silvestris* L.) im Laboratorium bei ca. 22° C (rel. Feuchtigkeit ca. 50 %) und im Kühlraum bei ca. 4° C (rel. Feuchtigkeit ca. 60 %) für 3 Jahre. Die Kontrolle wurde mit der Hand entflügelt, um die Samen nicht mechanisch zu beschädigen. Zwei Entflügelungsmaschinen (eine alte und eine neue) wurden für die anderen Proben benutzt; sie verursachten verschiedene Grade von mechanischen Schäden.

Unter Kühlung erhielten sich die Keimwerte aller Proben beinahe unverändert bis 3 Jahre. Dagegen trat eine Verminderung der Keimfähigkeit bei den im Laboratorium aufbewahrten Proben auf. So hatten nach dreijähriger Lagerung die Kontrolle 21 % und die mit der neuen und der alten Maschine entflügelten Proben 42 % bzw. 56 % an Keimfähigkeit verloren. Die Keimungsgeschwindigkeit der Proben ist in Figuren 2—6 angegeben.

Die Mengen der mechanischen Schäden, und mechanischen plus physiologischen Schäden zusammen bei allen Proben, wurden mit der Röntgenkontrastmethode durch Anwendung von Urografin bzw. Bariumchlorid als Kontrastmittel festgestellt. Diese Bestimmung wurde zum Anfang des Versuches sowie nach 6 Monaten, 1, 2 und 3 Jahren der Lagerung durchgeführt. In allen Fällen stiegen die Imprägnierungsprozente mit der Dauer der Lagerung. Diese Steigerung war weniger für die im Kühlraum aufbewahrten Proben, als für die, die bei Zimmertemperatur gelagert wurden.

Der Wassergehalt der Proben wurde zum Anfang der Untersuchung sowie nach jedem Jahr bestimmt. Die Ergebnisse sind in Tabelle 1 angegeben.

Die Untersuchung hat gezeigt, dass mechanisch beschädigte Kiefernsemen bei Lagerung in Zimmertemperatur ihre Keimfähigkeit früher verlieren, als im Kühlraum. Ausserdem, je stärker die mechanischen Schäden bei Samen sind, desto grössere Verluste der Keimfähigkeit treten unter der gleichen Lagerungsdauer auf. Es ist also vorteilhaft, Samen mit so wenig mechanischen Schäden wie möglich bei niedriger Temperatur zu lagern. Bei Kiefer könnte das Saatgut mit Flügeln aufbewahrt und unmittelbar vor dem Aussäen entflügelt werden. Ausserdem, die Lagerung von Samen in luftdichten Gefässen könnte dazu beitragen, dass die Keimfähigkeit für längere Zeit erhalten bleibt, als in solchen Gefässen, in denen Luft und Feuchtigkeit leichten Zutritt zu Samen haben.

Sammanfattning

Studier över lagring av mekaniskt skadat tallfrö

Föreliggande uppsats behandlar lagring av tallfrö (*Pinus silvestris* L.) i laboratorium vid ca. 22° C och ca. 50 % rel. fuktighet samt i kylrum vid ca 4° C och ca. 60 % rel. fuktighet under en tid av tre år. Kontrollen avvingades för hand för att inte skada den mekaniskt. Två avvingare (en ny och en gammal maskin) användes för de andra proven och de åstadkom olika grader av mekaniska skador på fröet.

Vid förvaring i kylrum förblev grobarheten på alla prov praktiskt taget oförändrad under tre år. Däremot sjönk grobarhetsprocenten på de prover som förvarats vid rumstemperatur. Efter tre års lagring hade kontrollen förlorat 21 % och proverna avvingade med ny och gammal maskin respektive 42 % och 56 % av grobarheten. Groningshastigheten på de olika proverna visas i figurerna 2—6.

Storleken av mekaniska skador och mekaniska plus fysiologiska skador tillsammans bestämdes på de olika proverna med röntgenkontrastmetoden, varvid urografin respektive bariumklorid användes som kontrastmedel. Dessa tests utfördes vid experimentets början samt efter 6 månader, 1, 2 och 3 års lagring. I samtliga fall ökade impregneringsprocenten med förvaringstiden fastän denna ökning var mindre för de prover som lagrats i kylrum och större för dem som lagrats i laboratorium.

Provernas vattenhalt bestämdes vid undersökningens början och vid slutet av varje år. Resultaten är sammanställda i tabell 1.

Undersökningen har visat att mekaniskt skadat tallfrö förlorar sin grobarhetsförmåga tidigare när det förvaras vid rumstemperatur än i kylrum. Dessutom visar resultaten att ju högre grad av mekaniska skador fröet har desto större är grobarhetsförlusten under samma lagringsperiod. Det är alltså bättre att förvara frö vid låg temperatur och med så få mekaniska skador som möjligt. För tall kunde detta göras genom att lagra fröet med vingar och avvinga det omedelbart före sådd. Lagring i lufttät behållare kunde hjälpa till att bevara fröets groningsförmåga längre än om det förvarades så att luft och fuktighet lätt har tillträde.