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Germination studies on Scots pine (*Pinus silvestris* L.) seed of different provenances under alternating and constant temperatures

Keimungsstudien auf Föhrensamen von verschiedenen Provenienzen unter wechselnden und konstanten Temperaturen

Groningsstudier på tallfrö av olika provenienser under växlande och konstanta temperaturer

by

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Introduction

In a previous investigation (Simak and Kamra 1968), it was observed that Norway spruce seed of different provenances germinates at the constant temperature of 20° C better or as good as at the alternating temperature of 20—30° C, which is prescribed in the ISTA Rules (1966). On account of this result, similar experiments were undertaken to study the germination behaviour of Scots pine seed of different geographical origin at various temperatures, using the ISTA Rules as the standard. The present paper summarizes the results of this investigation.

Material

Ten samples of fresh seed of *Pinus silvestris* L. from autochthonous provenances lying well apart in latitude and situated in different countries were selected for the experiments (Table 1). The distance between the northernmost and the southernmost provenance is about 20 degrees latitude.

Table 1: Details of the material used.

Sample No.	Country of origin	Provenance	Latitude	Altitude in metres	Year of collection
1	Austria	Güssing	47°03′	300	1966/67
2	,,	Obsteig	47°19′	1 000	,,
3	Slovakia	Šaš. Podhradie	48°35′	850	1967/68
4	,,	Bánovce n.B.	48°45′	350	,,
5	Poland	Grybów	49°35′	725	,,
6	,,	Rogów	51°48′	170	,,
7	Sweden	Kristianstad	55°50′	60	,,
8	,,	Kopparberg	60°0′	230	,,
9	,,	Västerbotten	64°19′	210	,,
10	,,	Norrbotten	66°55′	180	,,

Methods

Only filled seeds were used for this study. The empty or otherwise defective seeds were removed from the samples through the application of x-ray radiography.

The germination tests were carried out on 4×100 seeds of each sample on Jacobsen apparatus at the alternating temperature of 20—30° C (ISTA test) and at the constant temperatures of 20° C, 25° C, 30° C and 35° C maintained on the seed beds. The room temperature was 20° C in all the series.

The experiments were conducted in the germination laboratories of the Botanical Institute of the University of Stockholm and of the Institute of Forest Genetics, Royal College of Forestry, Stockholm, with exactly the same equipment and under similar and strictly controlled conditions. The maximum deviation of the temperatures was ± 1° C. The light was given from day-light tubes for 8 hours daily (intensity = 1 000 lux), and the germination period was 21 days.

The counting of the germinated seeds was begun on the third day and carried out every day during the first ten days and every other day thereafter. At each occasion, the counted seeds were removed from the germinator. A seed was considered as germinated, when the length of the root was equal to that of the seed itself.

The distribution of moulds on the seed beds was checked on the seventh day.

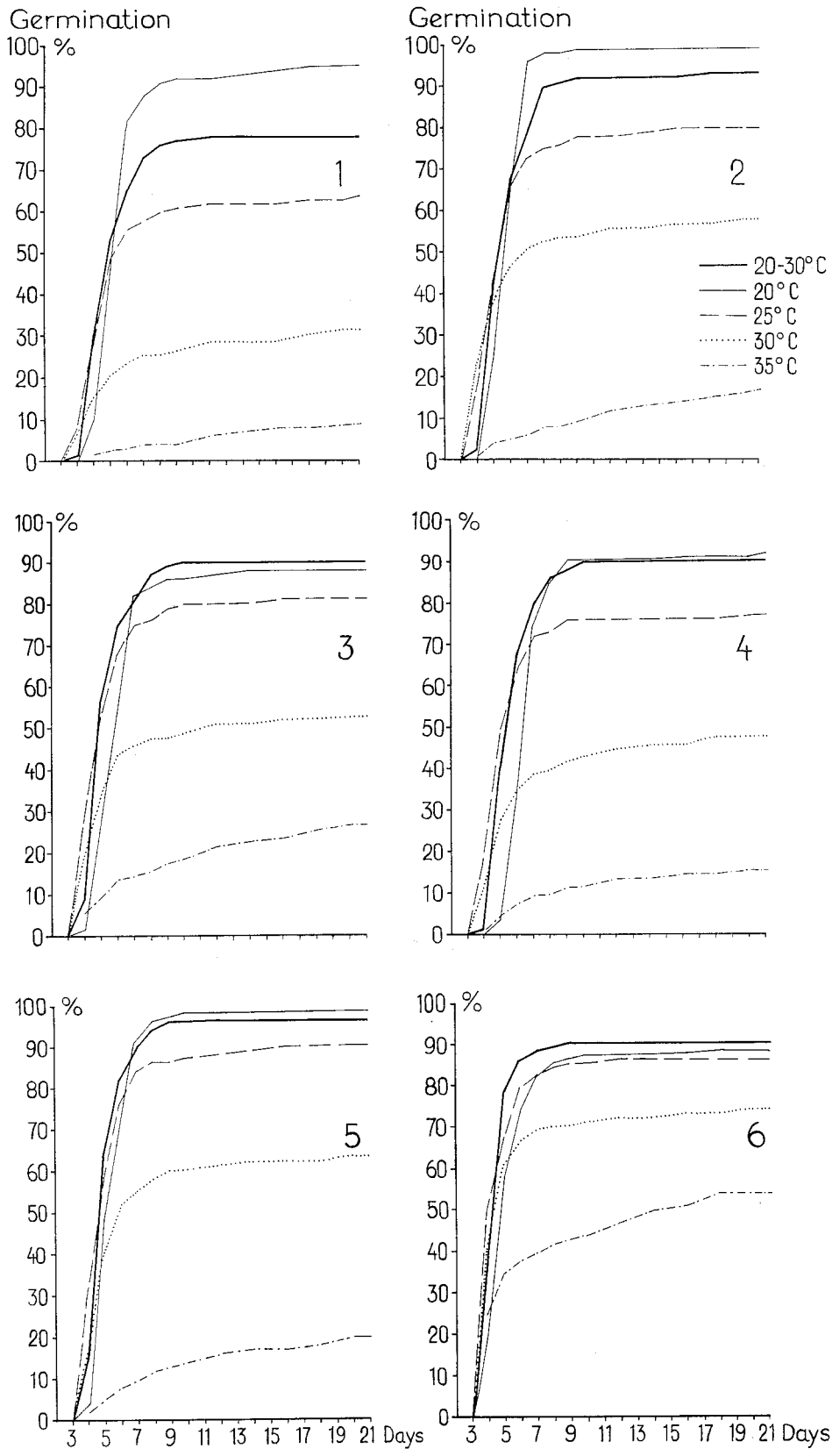
Results

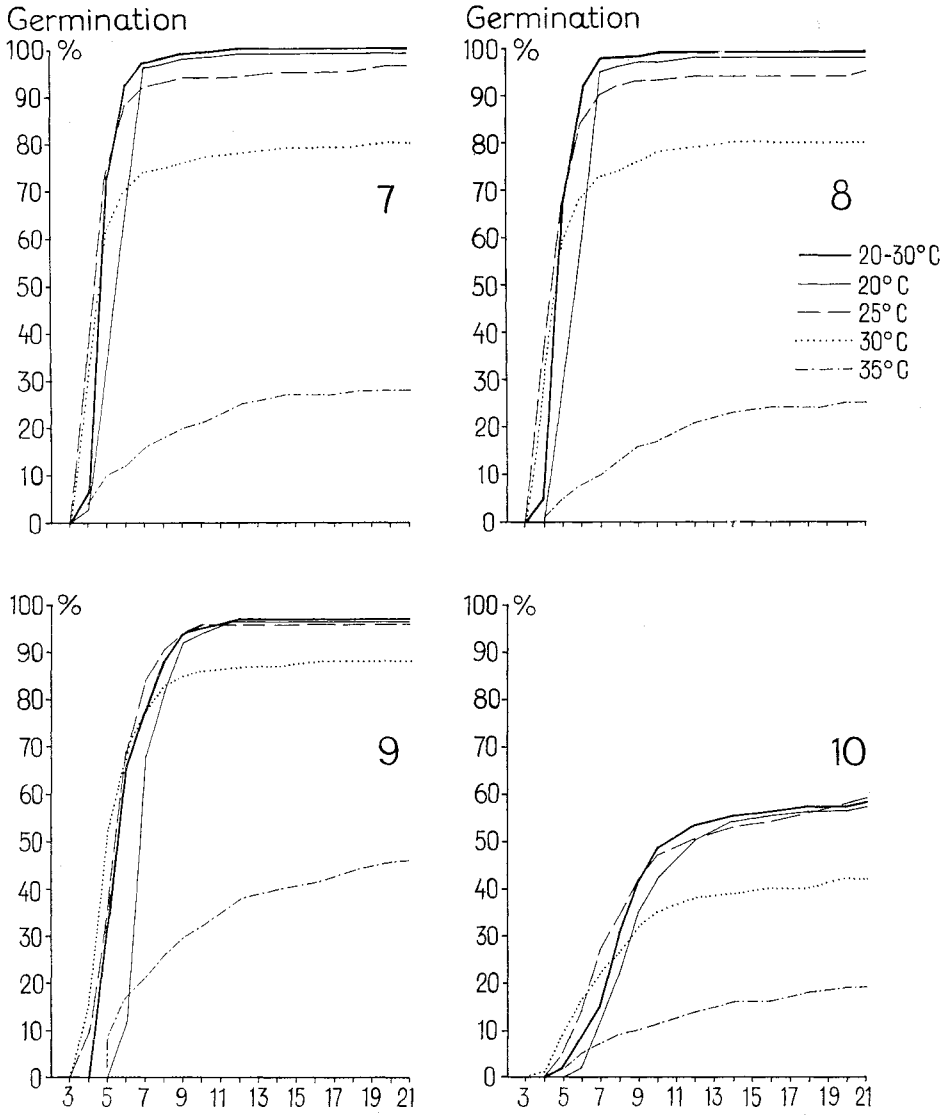
The results of the germination tests are shown in Figures 1—10 individually for each sample. As may be observed, the final germination percentages of the samples are reduced appreciably at 35° C and at 30° C as compared with those of the ISTA test; the reduction being greater at the former than at the latter temperature. At 25° C the germination values of some of the samples are as high as those of the ISTA test, whereas in others they are lower. The temperature of 20° C seems to be suitable for the germination of Scots pine seed, as

the values at this temperature are either higher or equal to those of the ISTA test. However, the rate of germination of all the ten samples is slower in the first few days of the test at 20° C than at 25° C, 30° C and 20—30° C (ISTA test). This as well as the other characteristics of the germination rate are visible from Figures 1—10.

The increase or decrease of the germination at a particular temperature is related with the origin of the samples. As Figure 11 shows, the southern provenances respond to the different constant temperatures used here with greater variation in the final germination values than the northern ones. Thus, the southern provenances show a greater reduction in the germination percentages at 25° C, 30° C and 35° C than the northern provenances. As compared with the ISTA test (20—30° C), the final germination percentages at 20° C are higher for the southern provenances than for the northern ones. Also the elevation of the habitat from which the seed originates, seems to influence the germination result. In our material, there are two pairs of provenances which lie close geographically but have different altitudes; Austria: sample 1: 300 metres above sea level versus sample 2: 1 000 metres; and Slovakia: sample 4: 350 metres versus sample 3: 850 metres. Within the same geographical pair, the decrease of germination at temperatures of 25° C, 30° C and 35° C is lesser for the samples from the higher elevation than for those from the lower one. At 20° C the increase in germination value is greater for the lower elevation than for the higher one within each of the above-mentioned geographical pairs. Thus the germination behaviour of the southern provenances from higher elevations tends to be similar to that of the northern ones.

The results of the distribution of moulds on the seed beds are given in percentages showing the mould-covered area in relation to the total seed bed area for all the provenances together in each of the temperature series. The values found are as follows: At 20—30° C = 23 per cent, at 20° C = 11 per cent, at 25° C = 15 per cent, at 30° C = 27 per cent and at 35° C = 34 per cent.


Germination
100 %
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
20-30° C
20° C
25° C
30° C
35° C
3 5 7 9 11 13 15 17 19 21 Days



Figs. 1—10: Germination of samples 1—10 respectively (cf. Table 1).

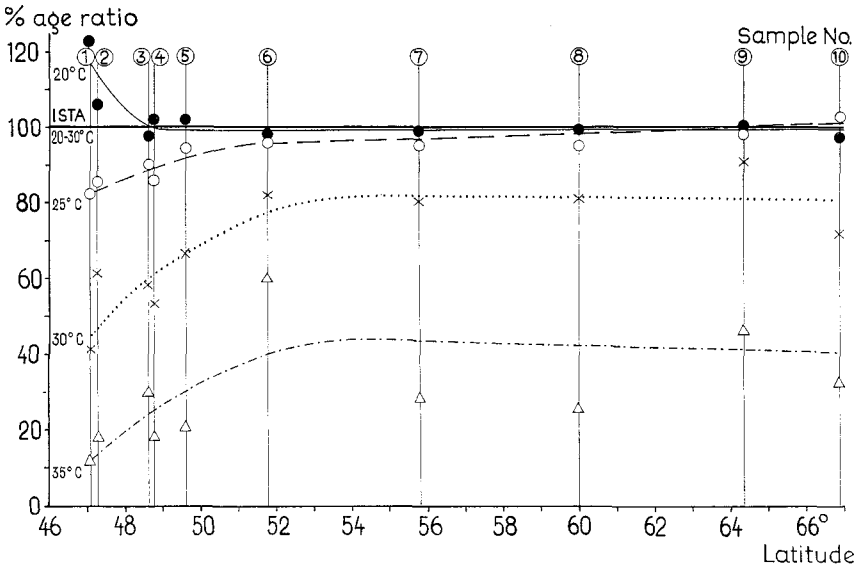


Fig. 11: Comparison of the final germination percentages of the samples at different temperatures with those of the standard (ISTA test) put equal to 100 %.

Discussion

For the present investigation as far as available fresh seed from autochthonous stands was used. This was done to avoid any eventual risk that the age of the seed could influence the experimental results. Rohmeder (1951) states that fresh Scots pine seed of high viability germinates equally good at the alternating temperature of 20—25° C (20° C for 18 hours and 25° C for 6 hours) as at the constant temperature of 25° C. However, he observed slight variations in the germination values of the old seed under the above-mentioned conditions. Kamra (1967) working with fresh and old Scots pine seed of Swedish origin did not find any differences in the germination percentages of the samples at the constant temperatures of 20° C and 23° C and at the alternating temperature of 20—30° C (ISTA Rules, 1966), the light intensity (= 1 000 lux), the quality and the duration (8 hours daily) being the same in all the three cases. It seems, therefore, that special investigations on larger material could be valuable for the clarification of this question.

A point which needs special mention is that the results published by different workers have been obtained under different germination

conditions (e.g. intensity, quality and duration of light, germination medium, etc.), even if the temperatures may be comparable. The fact that the exact germination conditions are either not strictly controlled or not properly described by some workers, makes any comparison of the results uncertain. This point may be kept in mind in connection with the present discussion.

As can be well understood, a homogeneous material is a prerequisite for getting dependable results in comparative experiments of the type as the present investigation. If the samples contain an indefinite number of empty or otherwise defective seeds, the results cannot be considered as dependable or comparable. In view of this, only filled seeds were used for the present experiment, the empty and defective ones being removed from the samples by x-ray radiography. As can be seen from the results, all the samples except No. 10 have a nearly 100 per cent potential germination capacity. The northernmost sample No. 10 has a lower potential germination because of the presence of a high amount of incompletely developed seeds (mostly embryo classes II and III, cf. Simak and Gustafsson 1954). Due to the fact that such incompletely developed seed is of common occurrence in high latitudes, sample No. 10 was taken as the representative for these regions.

In addition to the use of x-ray radiography for the selection of the experimental material, the germination conditions were kept strictly under control, as described in the Methods, so that dependable results could be obtained, even if the number of samples had to be limited to ten due to technical reasons.

On an average for all the ten samples, the highest germination percentages are attained at 20° C, 25° C and 20—30° C (ISTA test). However, in the southern provenances the germination at 20° C is higher and at 25° C lower than that at 20—30° C. The germination values of the samples of the northern provenances were the same at all the three temperatures. The temperature around 20° C has been found to be the optimum for the germination of Scots pine seed also by other workers (cf. Nobbe 1890, 20° C; Heit 1958, 20° C, cf. Table 2, darkness series; Eliason and Heit 1940, approx. 18° C; Heit and Eliason 1940, about 20° C with continuous light). Perner (1966) who studied the germination of Scots pine seed at 16° C, 24° C and at other temperatures, considers 16° C to be the optimum. However, due to the large difference between these two temperature steps (16° C and 24° C), the possibility cannot be excluded that the optimum could lie somewhere in between. Similar comments apply to the paper of

Jamblinne and Elens (1953) who tested the germination of *Pinus silvestris* at 8° C, 15° C, 25° C, 33° C and 44° C, and consider the temperature of about 25° C as the optimum. However, in agreement with our results and those of Jamblinne and Elens (1953), Perner (1966), Eliason and Heit (1940), Heit (1958), Baldwin (1942), etc., the temperatures above 25° C reduce the germination percentage of Scots pine seed. Our preliminary studies show that the germination rate and percentage at 15° C are lower than those at 20° C. This is supported by the results of some other authors.

Baldwin (1942) states that the daily alternation of temperatures is more favourable for the germination of most seeds than constant temperatures. Also Heit (1958, Table 4) recommends an alternating temperature of 20—30° C with light for testing the germination of *Pinus silvestris* seed. However, in his investigation, there is no test series at 20° C with light which could be compared with that at 20—30° C with light. Even if it cannot be denied that the alternating temperatures are beneficial or necessary for the germination of certain species of seeds (e.g. some grasses, cf. Morinaga 1926), there does not seem to be any binding evidence that this is the case with the seed of *Pinus silvestris*. Moreover, Nobbe (1890) showed that the constant temperature of 20° C is slightly better for the germination of Scots pine seed than the alternating temperature of 20—30° C (30° C for 6 hours daily). The present investigation in agreement with that of Kamra (1967) shows that 20° C (constant) is at least as good and in some provenances even better than the alternating temperature of 20—30° C for testing the germination of Scots pine seed. It is, therefore, suggested that the constant temperature of 20° C is also included in the ISTA Rules along with the alternating temperature of 20—30° C for testing the germination of *Pinus silvestris* seed, as has been done for several other species of tree seed (cf. ISTA Rules, 1966). The use of constant temperatures has the advantage that it does away with the effect of the rate of change of temperature and is also often easier to maintain without the help of special equipment. Moreover, the mould development is lesser at 20° C than at 20—30° C, which is another advantage.

The earlier start of germination at higher temperatures, as has been observed by many workers (e.g. Perner 1966, Jamblinne and Elens 1953, etc.) does not necessarily mean that also the final germination percentage will be higher at these temperatures. In this connection, Baldwin (1942, p. 120) writes: "High temperatures usually induce the earliest germination in seeds which are fully prepared for

sprouting, but when long maintained they apparently injure or inhibit less thoroughly after-ripened seeds, so that the final germination is less than for lower temperatures." Our results agree with this statement (cf. Figs. 1—10).

The question of provenance in relation to germination at different temperatures is of special interest and has been described in detail in the Results. Haasis and Thrupp (1931) observed in *Pinus contorta murrayana* that the seed from the higher elevations showed much better germination at 19° C than at 41° C as compared with the seed from the lower elevations and vice versa. Karschon (1949) tested *Pinus silvestris* seed from different elevations at 41° C in water. After 24 hours' test, he found that the provenances from the lower elevations had higher percentage of seeds with bursted seed coats and with visible or just emerged root tips (called by him as "germinated") than the seed from the higher elevations. Also Perner (1966) states that the seed of different *Pinus* species originating from northern regions has a lower optimum germination temperature than that for seed of pines which inhabit southern regions. According to the above authors, it seems that there is a correlation between the temperature requirements for optimum germination and the natural climatic conditions of the region from which a species or a provenance originates. However, due to the fact that the material and/or the methods used by the above authors are different from ours, a direct comparison with our results, which do not quite agree with the above viewpoint, cannot be made. Since this problem is important for seed provenance studies, further investigations on larger material are necessary.

Summary

In this investigation, the germination behaviour of fresh Scots pine seed from ten autochthonous provenances lying between 47° and about 67° latitude was studied (cf. Table 1). The tests were carried out on Jacobsen apparatus at the constant temperatures of 20° C, 25° C, 30° C and 35° C and compared with those at the alternating temperature of 20—30° C (ISTA Rules, 1966), which was used as the standard. All germination values were calculated on the basis of the filled seeds only. It was found that:

1. The germination percentages at 20° C, 25° C and 20—30° C were the same for the northern provenances. Seed from the southern

provenances germinated higher at 20° C and lower at 25° C than at 20—30° C (Figs. 1—11). The constant temperature of 20° C was found to be very suitable for the germination of Scots pine seed.

2. At 30° C and 35° C the germination values of the samples were lower than those at 20—30° C.
3. The origin of a seed sample seems to influence its germination percentage at a particular temperature. Thus the samples from the southern latitudes or the lower elevations were more sensitive to the temperatures used than those from the northern latitudes or the higher elevations.
4. The rate of germination at 20° C is in the first about seven days slower and later on equal or faster than that at 25° C, 30° C and 20—30° C.
5. The mould development on seed beds increased with increase in the temperature.
6. On the basis of the germination results reported in this paper, it is suggested that the constant temperature of 20° C is also included along with the alternating temperature of 20—30° C in the ISTA Rules for testing the germination of Scots pine seed.

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Zusammenfassung

Keimungsstudien auf Föhrensamen von verschiedenen Provenienzen unter wechselnden und konstanten Temperaturen

In dieser Arbeit wurde das Keimungsverhalten von frischem Föhrensamen untersucht, der aus zehn autochthonen Provenienzen zwischen 47° und 67° nördlicher Breite stammte (vgl. Tafel 1). Die Keimprüfungen wurden auf dem Jacobsen-Apparat bei konstanten Temperaturen von 20° C, 25° C und 35° C ausgeführt und mit solchen bei wechselnder Temperatur von 20—30° C (ISTA-Vorschriften 1966), die als Standard dienten, verglichen. Alle Keimwerte wurden ausschliesslich auf die gefüllten Samen bezogen. Es ergab sich folgendes:

1. Die Keimungsprozente bei 20° C, 25° C und 20—30° C waren bei den nördlichen Provenienzen gleich. Samen südlicher Provenienzen hatte bei 20° C einen höheren und bei 25° C einen niedrigeren Keimwert als bei 20—30° C (Fig. 1—11). Die konstante Temperatur von 20° erwies sich als sehr günstig für die Keimung von Föhrensamen.

2. Bei 30° C und 35° C waren die Keimwerte der Proben niedriger als bei 20—30° C.

3. Die Herkunft einer Samenprobe scheint ihren Keimwert bei einer bestimmten Temperatur zu beeinflussen. So waren die Proben aus südlichen Breiten oder geringer Höhe empfindlicher gegen die benutzten Temperaturen als die aus nördlichen Breiten oder größeren Höhen.

4. Die Keimungsgeschwindigkeit bei 20° C ist in den ersten ca. sieben Tagen langsamer, später aber gleich schnell oder schneller als die bei 25° C, 30° C und 20—30° C.

5. Die Schimmelbildung auf den Keimbetten nahm mit steigender Temperatur zu.

6. Auf Grund der hier berichteten Keimungsergebnisse wird vorgeschlagen, die konstante Temperatur von 20° C neben der wechselnden Temperatur von 20—30° C in die ISTA-Vorschriften für die Keimprüfung von Föhrensamen aufzunehmen.

Sammanfattning

Groningsstudier på tallfrö av olika provenienser under växlande och konstanta temperaturer

Groningen studerades hos nyskördat tallfrö av 10 autoktona provenienser från platser belägna mellan latituderna 47° och ca 67° (jfr tab. 1). Groningsförsöken utfördes på Jacobsens apparat vid konstanta temperaturer av 20° C, 25° C, 30° C och 35° C och jämfördes med försök vid alternerande temperatur på 20—30° C (ISTA-regler, 1966), vilken användes som standard. Alla groningsvärden beräknades på basis av matat frö.

Det konstaterades att:

1. Groningsprocenten vid 20° C, 25° C och 20—30° C var desamma för de nordliga provenienserna. Grobarheten hos frö från de södra provenienserna var högre vid 20° C och lägre vid 25° C än vid 20—30° C (fig. 1—11). Den konstanta temperaturen på 20° C tycks därför vara mycket gynnsam för groning av tallfrö.

2. Vid 30° C och 35° C var groningsvärdena lägre än vid 20—30° C.

3. Fröproveniensen förefaller att påverka grobarhetsprocenten vid en viss temperatur. Sålunda var provenienser från södra latituder eller lägre höjder känsligare för de använda temperaturerna än de från nordliga latituder eller högre höjder.

4. Groningshastigheten vid 20° C är under de ca sju första dagarna långsammare, och senare samma som eller snabbare än den vid 25° C, 30° C och 20—30° C.

5. Mögelutvecklingen på groningsbäddarna ökade med höjning av temperaturen.

6. På grundval av de groningsresultat som framlagts i denna uppsats föreslås att en konstant temperatur på 20° C inkluderas i ISTA-reglerna för groning av tallfrö tillsammans med den alternerande temperaturen på 20—30° C.