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Ripening process in relation to temperature and sugar content in seeds of Scots pine (Pinus silvestris L.)

Mognadsprocessen hos tallfrö (Pinus silvestris L.) i relation till temperaturklimatet samt fröets innehåll av sockerarter

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Abstract

ODC 232.318: 174 Pinus silvestris +161.4

The investigation was performed on seeds of Scots pine (Pinus silvestris L.) from one and the same stand of trees in three consecutive years with samplings during 1 September—1 April. Germination capacities, relative germination rates and concomitant changes of carbohydrates during the actual ripening period were studied. The end of the ripening process during September—October has been discussed in relation to frost temperatures during the same period. It has been stressed that unfavourable temperatures during sensitive phases of the ripening process can damage the seeds' further development, and jeopardize the well-known correlation between the mean temperatures for the months June—September and the ripeness. Quantitative changes of glucose, fructose, sucrose, raffinose and stachyose were studied with gas-chromatography. For starch a colorimetric technique was used. The content of glucose and fructose evidently decreased with increasing ripeness during September—October. A concomitant new formation of sucrose, raffinose and stachyose was observed. Starch was found in all samples, irrespective of the sampling dates, with a minimum content in September and with a more irregular but similar trend of new formation. To correlate the degree of ripeness as expressed in the germinability with some coincidental content of one of the carbohydrates has not been possible.

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Contents

1 Introduction

The relationship between the temperature climate and the ripening of seeds in Scots pine is a problem that has interested Scandinavian forestry researchers for a long time. Holmerz and Örtenblad reported as early as in 1886 that seeds from cones harvested in the winter of 1885—86 were of low quality, and attributed this to the wet, cold weather of the previous summer. Detailed studies of the way in which the temperature affects the ripening of pine seeds have since been made by *Hagem* (1917) and *Eide* (1923, 1932) in Norway, *Kujala* (1927) in Finland and *Wibeck* (1920, 1928, 1929) in Sweden. Wibeck (op.cit.) showed that the mean temperature for the period 1 June—1 October in the second year of the cones' development must be in the +10.5 — +11.5°C range if the seeds are to develop favourably. In general this means that for Swedish localities south of 60° lat. at any altitude, and south of 65° lat. at altitudes below 200 m, the seeds are of good quality.

It was further shown by *Wibeck* (op.cit.), *Oldertz* (1921), *Heikinheimo* (1921) and *Kujala* (op.cit.) that the low germinability of seeds from colder climates was correlated with inhibited embryo development, e.g. the occurrence of a high percentage of seeds with small embryos or polyembryoni. This correlation was confirmed by *Simak* and *Gustafsson* (1954) with the help of an X-ray technique which permitted studies to be made of the morphological development and the germinability of the same individual seeds. The same subject has been studied further by *Simak* (1966).

In an attempt to overcome the problems connected with the low quality of the seed material yielded by stands in notherly localities and other climatically unfavourable parts of the country, studies have been made concerning the best time for harvesting and the way in which pine cones should be treated to bring about the most favourable development of the seed.

Thus *Nordström* (1955), *Edlund* (1959) and *Kardell* (1967) were able, by harvesting the cones earlier and storing them in suitable conditions, to obtain seeds of higher germinability than was possible in the case of cones collected during the winter season. Even a stratification procedure for the extracted seeds led to an improvement in the germinability of badly developed material from higher altitudes (*Bergman* 1960a, *Simak* and *Gustafsson* 1957).

The present investigation is an attempt to describe further some of the complicated connections between the temperature climate and the germinability of pine seeds and simultaneously their changing content of various types of sugar. To limit the ecological variation as far as possible, with the exception of the temperature factor, the studies have been based on material collected from one and the same pine stand. Collections of pine cones were made during three consecutive years, and the local temperatures were taken and recorded at the same time. The study is part of a more extensive investigation into the ripening conditions of Scots pine seeds at higher altitudes in northern Sweden (*Kardell* 1973 a, b).

2 Material and methods

2.1 The experimental material

The experimental material consisted of seeds of Scots pine (*Pinus silvestris* L.) from cones, which were collected at various times from the same locality at Siksjö, Åsele parish, Västerbotten, latitude 64° 20′ north, 405 m altitude. A survey of the material is given in Tab. 1. Some introductory experiments on the analytical method were also made on seeds from Malå krp., Malå parish, Västerbotten, latitude 65° 08′ north, 360 m altitude, collected in October, 1969 cf. Tab. 3).

Table 1. Collecting data for the experimental materials

Cones of Scots pine (*Pinus silvestris* L.) collected at Siksjö, Åsele parish, Västerbotten, latitude 64° 20′ north, 405 m altitude. Collections from the years 1967—1970.

Collection No.	Collecting date		
	Year	Month	Date
I-01	1967	August	28
I-02		September	16
I-03		September	28
I-04		October	14
I-05		October	25
I-06		December	13
I-07	1968	January	31
I-08		March	15
I-09		April	19
II-10	1968	August	28
II-11		September	13
II-12		October	01
II-13		October	14
II-14		November	05
II-15	1969	February	03
II-16		April	01
III-17	1969	September	02
III-18		September	15
III-19		October	08
III-20		October	20
III-21		December	01
III-22	1970	February	01
III-23		April	04

2.2 Cone sampling

The cones were collected each year from the same stand of trees (about 800 stems in an area of 2 ha). At the beginning of each new collection period all trees with an estimated cone crop of more than 100 cones were numbered and 20 trees were selected at random. From each donor tree 20 cones—randomly distributed in the crown—were collected on each of the dates during the period stated. Because of meagre cone production, and a strong correlation between years and cone production of individual trees, the same trees have in the main donated the seed material gathered on the three occasions. From the total of 400 cones collected on each occasion 15 cones randomly sampled were taken, sealed in plastic bags and stored at —20°C until it was time to make the sugar analyses. A further 100 cones were selected at random, and these supplied the material for the immediate germination analyses.

2.3 Seed extraction

Seed extraction from the cones collected during September to December was done by manual breaking-up of each individual cone. Cones from the other collecting dates were opened by heat treatment at +42°C in a ventilated kiln for 3×24 hours. Cones which had not opened after the first two treatment periods were treated separately in tapwater (+11°C) for 20 minutes and then returned to the kiln. Cones that remained closed after the final treatment period were opened manually as above. The seed recovery was about 1 350—1 750 seeds per 100 cones.

2.4 Seed dewinging

Seed dewinging of each individual seed was done with preparatory needles.

2.5 Seed sampling

Seed sampling was done by transferring the seeds individually to each of nine consecutive groups to a total of 100 in each group. Four of these (4×100 seeds) were used for each determination of the germination capacity—the others were used in experiments reported elsewhere.

Seeds from cones that had been kept in cold storage were extracted in the same way. From each sample of 15 cones the recovery was 170—350 seeds, which after dewinging as above were distributed at random between two samples with the same number of seeds and then treated separately as described below for sugar analyses.

2.6 Determination of the percentage of empty seeds

Determination of the percentage of empty seeds in the samples used for the germination analyses was done by X-ray photography according to *Simak* (1966). The seeds used for the sugar analyses were individually cut immediately before the analyses and the empty ones discarded.

2.7 Germination tests

Germination tests were done in conventional Jacobsen apparatuses at room temperature (+18°C) and supplemented with heating equipment for raising the water temperature to +35°C once each 24 hours. The distance between the water level and the germination beds was 8 cm. The tests were performed in ordinary room light. The germination results were recorded every fifth day and finally after 30 days. On each occasion seeds with radicles of 3 mm were regarded as germinated.

2.8 Sugar analyses

After cutting the individual seeds (cf. above) and adding melibiose (generally 100 seeds and 10.0 mg melibiose per sample) the material was homogenized in a mortar with the addition of analytic sand. The homogenate in petroleum ether (Shellysolve B, b.p. +60—80°C) was then fat extracted with the same solvent for 10 hours in Soxhlet apparatuses according to *Nyman* (1966). After air drying overnight the residues were further extracted with 80% (v/v) ethanol for 4 hours according to *Nyman* (1969). The extraction tubes had been pre-extracted for 2 hours using the same solvent and then tested for the absence of soluble sugars. The extracts were evaporated to dryness in vacuum at +40°C and the residues dissolved in 5.00 ml dried pyridine (with KOH). To 1.00 ml of this solution, 0.90 ml hexamethyldisilazane (Applied Science, Inc.) and 0.10 ml trifluoracetic acid (Merck, Für Synthese) were added. The mixture was shaken for 30 minutes and stored at +4°C until not later than the following day, when the GLC analyses were made (the mixture is stable for one week). Standard solutions of different sugars (cf. *Nyman* 1969) were prepared in pyridine and treated as above.

The analyses were made in a gas chromatograph Perkin-Elmer Model 880 with a flame ionizing detector and a Hitatchi Perkin-Elmer recorder (Model 159, paper speed 10 mm · $min.^{-1}$) equipped with a Disc Chart Integrator (Model 246/D). Three percent SE-52 (w/w) on Chromosorb G-AW (80—100 mesh; 0.6 m, 1/8″ steel tubes) were used as columns. The carrier gas was nitrogen, 30 ml · $min.^{-1}$. The detector was supplied with hydrogen, 30 ml · $min.^{-1}$ and air, 600 ml · $min.^{-1}$. Injector and detector temperature was +280°C and the column temperature was programmed for 10°C · $min.^{-1}$ between +120—350°C. The attenuator was ×100 and the injected volumes were mostly 2 μl. The analytical method used was adopted and modified from *Otter & Taylor* (1967), partly according to *Dahlgren* and *Äyräpää* (unpublished). The results in Fig. 1 are given as an example.

For the qualitative evaluation, the retention times for the sample compounds and for pure substances were compared in sep-

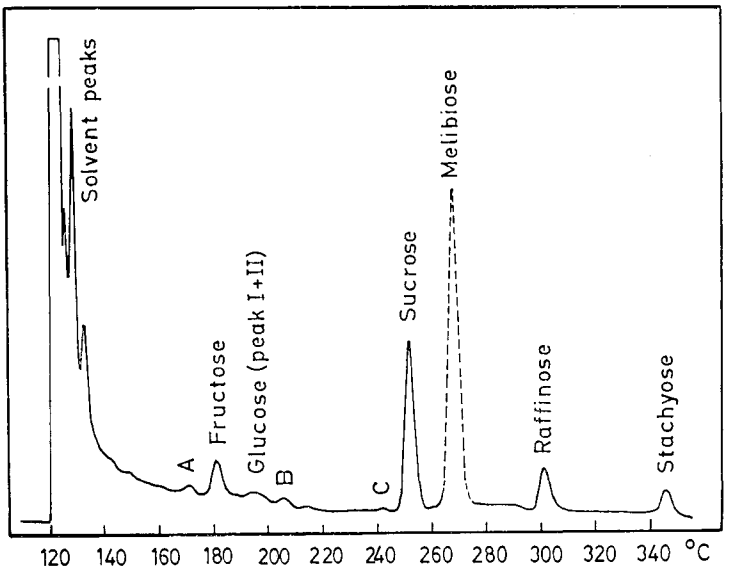

Figure 1. A GLC separation pattern for hexamethyldisilazane derivatives of sugars in seeds of Scots pine *(Pinus silvestris* L.). Column: 0.6 m×1/8″, steel with 3% SE-52 on Chromosorb G-AW, 80—100 mesh. Carrier gas: nitrogen, 30 ml · min.$^{-1}$. Temperature: +120 — +350°C with a program rate of 10° · min.$^{-1}$. Injector and detector temperature: +280°C. Flame ionizing detector with hydrogen, 30 ml · min.$^{-1}$ and air, 600 ml · min.$^{-1}$. Attenuator: ×100. Instruments: Perkin-Elmer Model 880 with a Hitatchi Perkin-Elmer Recorder (Model 159, paper speed 10 mm · min.$^{-1}$). Sample from Siksjö, collected 3rd Feb., 1969. Injected volume: 0.6 µl. Peaks A, B and C unidentified. Melibiose added as an internal standard.

Table 2. Relative peak areas of glucose, fructose, sucrose, melibiose, raffinose and stachyose at GLC.

Derivatives with hexamethyldisilazane in standard solutions, 1 µg · µl^{-1}. For details, see the methods.

Substances	Peak area · µg^{-1}	Relative peak area
Glucose (I+II)	1 414	1.52
Peak I	956	1.03
Peak II	458	0.49
Fructose	1 313	1.41
Sucrose	1 167	1.25
Melibiose	930	1.00[1]
Raffinose	853	0.92
Stachyose	761	0.82

[1]) Melibiose used as an internal standard.

Table 3. Relative errors at the GLC-determinations of sugars in standard solutions and in seed samples of Scots pine (*Pinus silvestris* L.).

The same conditions as in Table 2. Relative errors calculated after *Stahl* (1962, p. 56).

Substances	Relative errors %		
	In standard solutions[1])	In seed samples[2])	
Glucose (I+II)	± 1.5	± 13.8	(6.5)[3])
Peak I	± 1.8	—	—
Peak II	± 0.9	—	—
Fructose	± 2.8	± 5.2	(7.8)
Sucrose	± 3.7	± 3.2	(78.5)
Melibiose	± 5.0	—	—
Raffinose	± 6.7	± 3.5	(20.0)
Stachyose	± 7.5	± 6.4	(20.3)

[1]) at a level of 1 µg of the substances, respectively in the analytical samples n = 4.

[2]) at a corresponding level of 0.01—0.60 µg n = 4.

[3]) the sugar content, respectively as µg · seed^{-1}.

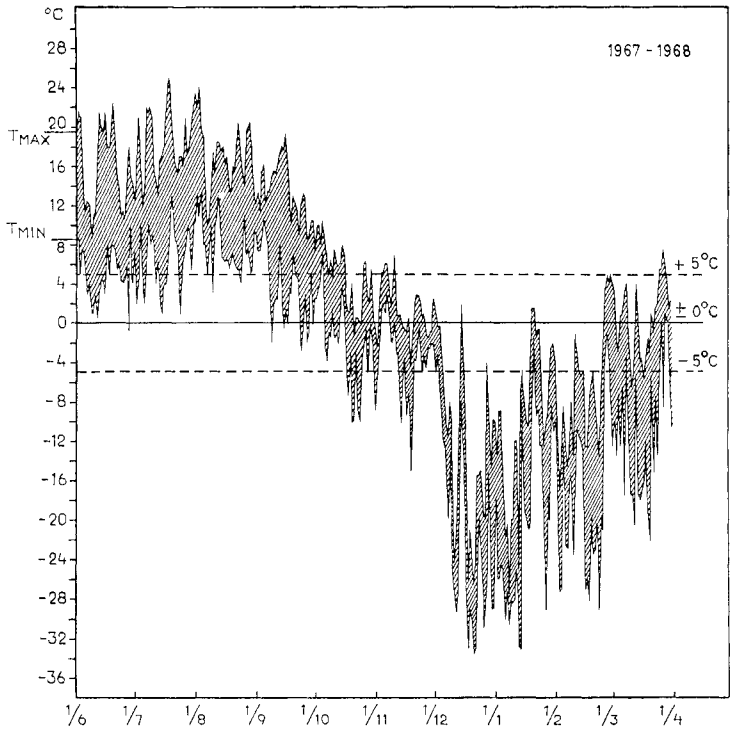

Figure 2. The daily, maximum and minimum temperature at Yxsjö meteorological station, Åsele parish, Västerbotten, 64° 16′ north, 350 m altitude during the period 1st June 1967 —1st April 1968.

arate experiments and at co-chromatography. For the quantitative determinations, the relations between injected amounts of standard compounds and corresponding peak areas were studied and related to the peak area of the added melibiose used as an internal standard (Tab. 2). In preliminary experiments the absence of melibiose in the original samples was established. The contents of the individual compounds were calculated as $\mu g \cdot seed^{-1}$. The relative errors were estimated in separate experiments (Tab. 3). All analyses were made at least twice from parallel samples. The results are given as mean values.

2.9 **Starch analyses**

These were made on the sugar free extracts, which were further extracted with boiling distilled water for 2 hours according to *Nyman* (1969). The extracts were evaporated as above. The residues were then dissolved by warming in 3×5 ml distilled water and quantitatively transferred to another vessel, evaporated to dryness and finally dissolved in 6.00 ml distilled water. The starch was colorimetrically determined with KJ_3 at 578 nm after *Nyman* (1971).

2.10 **Temperature measurements**

These were made with a recording thermohygrograph (W. Lambrecht, type 252) from 1 June to 1 October during the vegetation periods under investigation. The instrument was placed 1.4 m above the ground in a white painted standard cabinet (SMHI). It was placed in the central part of the stand and more than 3 m from the surrounding trees. It was operating for one week at a time and was checked twice a week. During the winter season, corresponding data had for practical reasons to be taken from the nearest meteorological station in Yxsjö, 350 m altitude and 12 km from the experimental

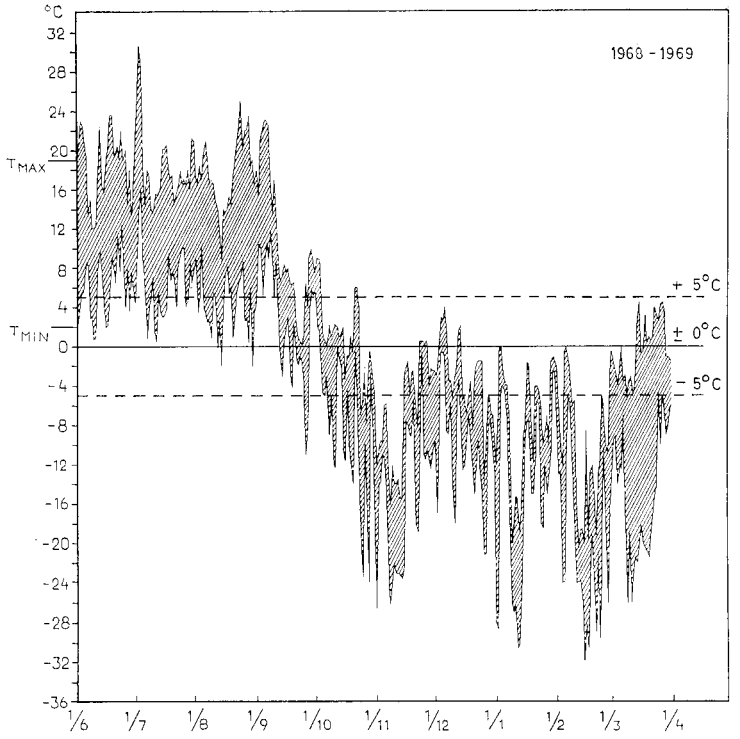

Figure 3. Corresponding data as in Figure 2 but for the period 1st June 1968—1st April 1969.

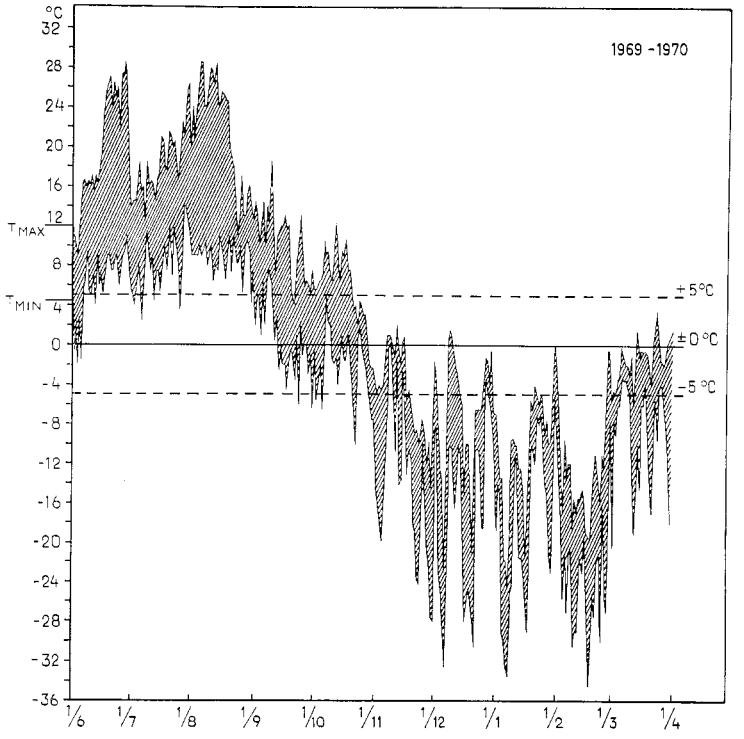

Figure 4. Corresponding data as in Figure 2 but for the period 1st June 1969—1st April 1970.

Table 4. The mean temperature per month during the period 1st June—1st October of the years 1967—1969 at Yxsjö meteorological station, Åsele parish, Västerbotten and the growth unit sums for the Yxsjö locality and the experimental plot at Siksjö, Åsele parish, Västerbotten during corresponding periods.

Growth unit sums calculated according to *Mork* (1941).

Year	Month	Locality Yxsjö 64° 16′ north, 350 m altitude			Locality Siksjö 64° 20′ north, 405 m altitude		
		Mean temp. °C	Growth unit sum per month	Cumulative growth unit sums	Mean temp. °C	Growth unit sum per month	Cumulative growth unit sums
1967	June	10.7	74.4	—	—	—	—
	July	12.8	95.9	170.3	—	—	—
	August	12.4	91.7	262.0	—	—	—
	September	8.3	58.7	320.7	9.0	57.9	—
1968	June	12.6	92.4	—	12.4	92.1	—
	July	12.7	90.1	182.5	12.0	86.0	178.1
	August	10.9	89.5	272.0	10.9	87.0	265.1
	September	6.3	49.2	321.2	5.3	41.3	306.4
1969	June	13.6	120.5	—	13.1	121.7	—
	July	13.3	97.3	217.8	12.3	90.5	212.2
	August	15.6	155.0	372.8	16.0	153.1	365.3
	September	5.6	39.2	412.0	5.3	34.7	400.0

plot. A survey of the daily maximum and minimum temperatures at the Yxsjö meteorological station during the three investigated periods is given in Figs. 2, 3 and 4, respectively. For a comparison between the climates at this station and in the experimental plot, mean temperatures per month and growing units, calculated after *Mork* (1941), for the months June—September are presented in Tab. 4.

3 Results

As an arbitrary expression of the Scots pine seeds' degree of ripeness, which has been studied as a function of the date of collection, the germination percentage up to 30 days under standard conditions was used. Simultaneously a study was also made of the relative, cumulative germinability for each 5-day period expressed as a percentage of the terminal germinability after 30 days. This is referred to as the relative germination rate, and the results are shown in Figs. 5 and 6, respectively. The analyses do not take empty seeds into account.

Material from the first collection, made 28 August 1967, had a degree of germination of only 21%, indicating a low degree of ripeness. During the period up to October 14 this increased to 79%, a level which with the one exception later discussed was maintained during the remaining winter months.

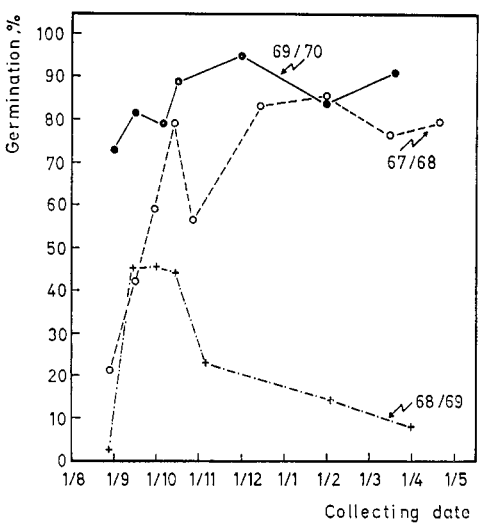

Figure 5. The relation between the germination percentage after 30 days and the collecting date for seeds of Scots pine (*Pinus silvestris* L.).

Material from three consecutive years (1968—1970) of the same locality at Siksjö, Åsele parish, Västerbotten, latitude 64° 20′ north, altitude 405 m. For details of the germination tests, see the methods.

At the next collection exactly a year later the degree of germinability was lower, i.e. 3%. This increased relatively quickly during September to 42% and this level was maintained during October. This was followed by a successive decrease during the remaining winter months.

The first collection made in 1969—70 (2 September) showed that the degree of ripeness was much higher, and 73% of the seeds germinated. This value increased slightly during September and reached a level between 80 and 90%, where it remained during the late autumn and winter.

Concerning the relative germination rate (Fig. 6), none of the samples from the material collected during 1968—69, irrespective of the date of collection, showed a relative germination of 50% or more after 5 days' germination time. It should also be mentioned that where the samples collected 28 August 1968 are concerned, none of the few seeds that germinated did so until 25 days had elapsed.

It should also be noted that in the material from 1967—68, which as regards ripeness occupied an intermediate position, only the samples from the three earliest collection dates showed an inhibited germination rate with less than 50% relative germination after 5 days.

In the best developed material from 1969—70, none of the samples, irrespective of the date of collection, showed a lower relative germination than 50% after 5 days. If a comparison is made between the relative germination rates of the various samples, it will be found that there is a tendency for

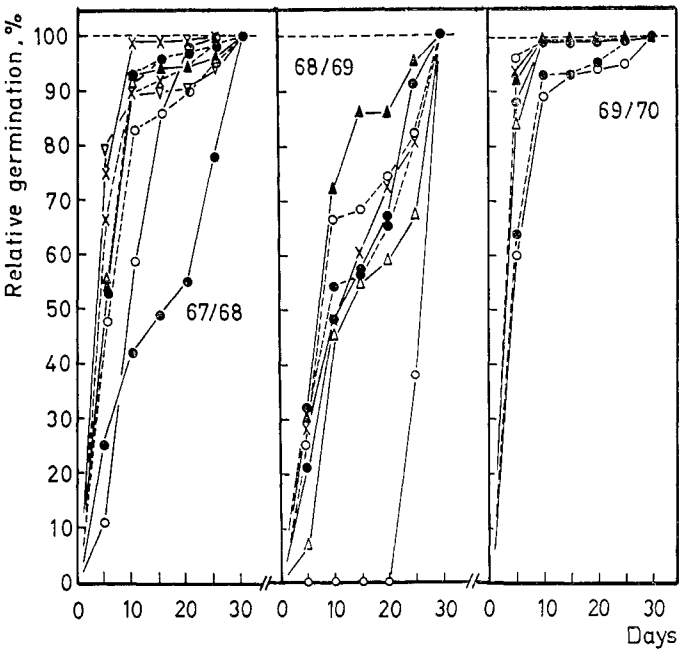

Figure 6. The relative germination rate of Scots pine seeds (*Pinus silvestris* L.) in relation to the collecting date.

All material from the same stand (cf. Figure 5). The relative values given as percent of the final germination capacities after 30 days for each individual collection.

Symbols	Collection period and date		
	1967—1968	1968—1969	1969—1970
○——○	28/8	28/8	2/9
●——●	16/9	13/9	15/9
○----○	28/9	1/10	8/10
●----●	14/10	14/10	20/10
△——△	25/10	5/11	1/12
▲——▲	13/12	3/2	1/2
X——X	31/1	1/4	4/4
X----X	15/3	—	—
▽----▽	19/4	—	—

the seeds with an earlier collection date to have a lower relative germination rate.

As the seed material in the three cases was collected from the same stand under statistically unbiased conditions, and the germinability of the material was tested under comparable conditions and with as brief a storage period as possible between the collection dates and the tests, it may be said that the variations in the results reflect differences in the conditions prevailing during the ripening phase. It may also be stated that under certain conditions an important part of the ripening process takes place during September, and finally that the weather during the following winter can affect the germinability of local seed material.

Coincidental with the ripening process, which until now has been referred to as the germinability of the seed material, a large number of biochemical changes take place. Such changes have been studied in a large number of species, see e.g. *Crocker* and *Barton* (1953) and *Barton* (1967), but where forest seeds are concerned, relatively few studies have been made (*Lyr* et al. 1967). For this reason a parallel investigation was made to study the changes in the content of the more common sugars and starch in the seeds of Scots pine. Such changes during the acual germination process have previously been studied by one of the authors (*Nyman* 1969). For the current studies a somewhat modified GLC technique after *Otter* and *Taylor* (1967) was used. The results of these analyses are shown in Fig. 7, and represent the average values obtained from double samples of seed material before germination commenced. In these analyses glucose, fructose, sucrose, raffinose and stachyose were ten-

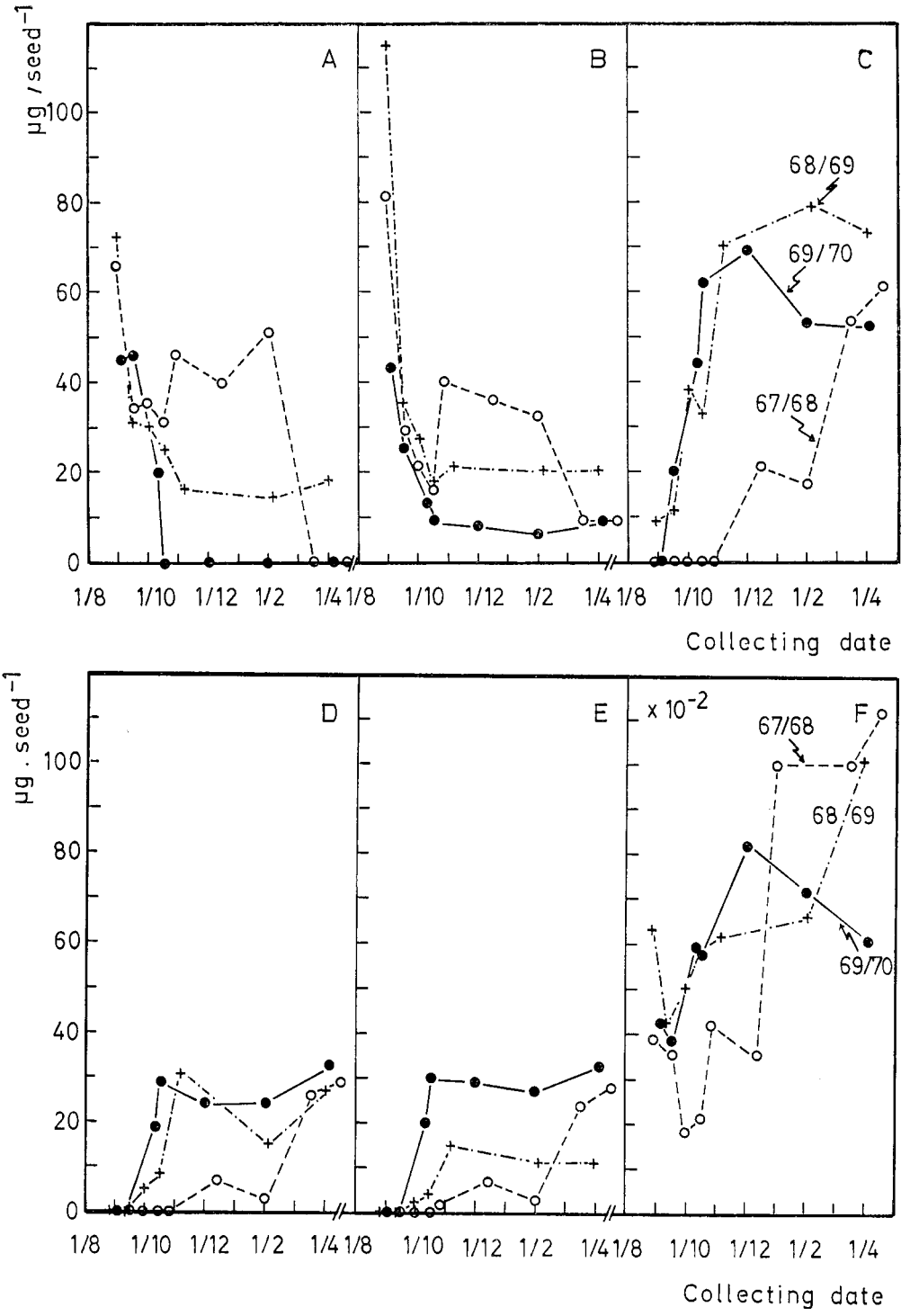

Figure 7. The content of different sugars and starch in seeds of Scots pine *(Pinus silvestris* L.) in relation to the collecting date.
A: glucose, B: fructose, C: sucrose, D: raffinose, E: stachyose, F: starch. Determinations done with GLC and melibiose as an internal standard (cf. Figure 1 and the methods). Materials and sampling periods the same as in Figure 5.

tatively identified together with small quantities of hitherto unidentified substances (cf. Fig. 1).

In the material from the 1967—68 collection period, the only identifiable sugars present up to 25 October were glucose and fructose (Figs. 7A and B, respectively). In time, both substances decreased in quantity, and germinability increased during the same period (cf. Fig. 5). After 25 October there was a certain increase in the quantity of both glucose and fructose, and at the same time the presence of stachyose could be proved (Fig. 7E). In the same series sucrose (Fig. 7C) and raffinose (Fig. 7D) were present first in the samples collected 13 December. Later in the winter period there was a fall in both glucose and fructose, at the same time as increasing quantities of sucrose, raffinose and stachyose were noted.

With the exception of the accumulation of glucose and fructose mentioned above, and differences in the time scale, the material for the three consecutive years showed roughly the same changes in the content of common sugars. Thus the initial formation of sucrose, raffinose and stachyose occurred simultaneously, and in the case of the material collected in 1968—69 and 1969—70 this was between the end of September and the beginning of October, whereas for the material collected in 1967—68 this was delayed until the beginning of February.

As regards the starch content (studied with a colorimetric technique—Fig. 7F) there was an initial decrease and a minimum content was reached in September; this was followed by a somewhat erratic new formation during the rest of the period covered by the study. A tendency towards a lower starch content after 1 December was particularly noticeable in the case of the 1969—70 material, and this should be compared with the corresponding decrease in sucrose during the same period.

4 Discussion

As mentioned in the introduction, the mean temperature during the period June to September must be between +10.5° and +11.5°C if the seed of Scandinavian Scots pine is to achieve acceptable germinability. Similar conclusions were also reached by *Kohh* (1968), who found that Scots pine seeds require a mean temperature of at least +11.6°C during the above period in the second summer of their development if they are to reach 80% germination after harvest the following winter. A comparison between the outcome of Kohh's investigation and of the present one can be made with the help of the following figures: 1967—68 mean temperature +11.0°C, germination 85% (cone harvest 1 February); 1968—69 mean temperature +10.8°C, germination 14% (harvest as above); 1969—70 mean temperature +12.0°C, germination 84% (harvest as above). As temperatures for the Siksjö test plot for the summer of 1967 are not available (cf. Tab. 4), the mean temperatures mentioned above are from Yxsjö, the nearest meteorological station. A comparison between the data from the two places does not suggest however that there are any striking differences between them, this irrespective of the climate being expressed as mean temperatures or growth unit sums according to *Mork* (op.cit.). It might therefore be worth noting the great difference in the germinability of the seed material collected in the winters of 1968 and 1969, this despite the fact that there was only a slight difference in summer temperature (+11.0° and +10.8°C, respectively). On the other hand, the greater difference in mean temperature between 1967 and 1969 (+11.0° and +12.0°C, respectively) did not affect the germinability of the seed to any great extent (85% and 84%, respectively). These results may demonstrate the importance of deviations in local temperatures, which can occur irrespective of general calculations based on a more extensive statistical material. Neither has the use of *Mork's* (op.cit.) growth unit sums in this investigation produced such results that they allow a close correlation with physiological observations, despite the fact that *Opsahl* (1951) in the case of spruce and *Mork* (1957), *Bergman* (1960b) and *Kardell* (1967) in the case of pine have found a good relationship between the growth unit sum and the germination percent of the seed. Thus the growth unit sums for the June—September period in 1967, 1968 and 1969 in this material were 320, 321 and 412 respectively, values that do not agree with the germination capacity values.

However, the importance of this relationship between the mean temperature of the June—September period and germinability can be affected by unfavourable temperatures of short duration at critical stages of the seed development. It has already been pointed out that the temperature conditions during the month of September, at least during moderately warm summers, seem to coincide with the phase in seed development when the greatest increases in germination capacity occur (cf. Fig. 5). For this reason it may be of interest to study the frequency of night frost in September 1967 and 1968. In 1967 there was night frost on 3 occasions (9, 24 and 27 September with temperatures of —1.0°, —1.9° and —1.5°C respectively, according to data supplied by the Yxsjö station). The frequency was considerably greater in August—September 1968, there being night frost on no fewer than 13 occasions (14 August, —1.9°C; 12, —0.9°C; 13, —1.1°C; 14, —3.5°C; 18, —3.1°C; 19, —3.4°C; 21,

—1.0°C; 22, —0.8°C; 23, —1.8°C—frost during 24 hours; 24, —2.2°C; 25, —6.7°C; 26, —9.0°C and 27 September, —6.2°C, cf. also Figs. 2 and 3). There were also several cases of night frost in September 1969 (Fig. 4), but as the germination data given in Figs. 5 and 6 show, these occurred only after the seeds had reached a higher degree of ripeness. Where the 1968—1969 material is concerned it should also be noted that the increased germinability between the two dates of collection, namely 3% on 28 August and 42% on 13 September, was preceded by only one 4 hour period of night frost on 14 August. The further inhibited development of the seed coincided with the frost periods mentioned above. The subsequent reduction in the germinability of this year's seed material, which occurred during the autumn and winter period, and the correspondence of which has previously been proved by *Nordström* (1955) and *Kardell* (1967), was perhaps due to the undeveloped embryo being killed by unfavourable temperatures (*Kardell*, unpublished) or by a state of dormancy being induced in the seed, a dormancy which was not possible to interrupt with the temperatures that prevailed during the following germination tests (cf. *Vegis* 1965). Also the results produced by *Simak* (1972) indicate a deleterious effect; his experiments with the controlled freezing of Scots pine cones on trees during August and September reduced the germination capacity of the seeds.

It is not possible to determine with the help of the data available whether a state of induced dormancy can be the cause of the temporary reduction in germinability of the material collected 25 October, 1967.

It can be proved, however, that this reduction may to some extent be the result of several extremely sharp frosts during October that year (cf. Fig. 2), which induced only a lower degree of dormancy in that year's ripe seed material (cf. Figs. 5 and 6, 67/68). In such a case it may be possible that the state of dormancy was relieved by the fluctuating temperature conditions which prevailed during the period up to the middle of December, when the original level of germinability returned.

The above discussion of the complex relationship between temperature conditions and the pine seed's germinability indicates a need for more analyses of the ripening process. An experiment in this direction is the one made here, where a study of the sugar and starch content of the seed was made parallel with the development of the germinability.

Apart from the hitherto unidentified substances (cf. Fig. 1), which without exception occurred in the samples in small quantities, the GLC analyses made in conjunction with the present study have tentatively identified all the sugar types previously found in ripe, ungerminated Scots pine seeds (*Nyman* 1969). As regards the amounts of the various sugar types, it can be mentioned that these are compatible with those resulting from earlier experiments using a different analysis technique (*Nyman*, op.cit.).

Attempts to correlate the ripeness of individual samples with their carbohydrate content were unsuccessful, although it should be noted that, where glucose and fructose are concerned, these reduced in quantity during the three years under investigation at the same time as germinability increased between September—October. A corresponding reduction in glucose and fructose in developing seeds of *Pinus Roxburgii* Sarg. has already been described by *Konar* (1958), but he also found sucrose during the entire period under investigation and the formation of an unidentified trisaccharide. As regards sucrose, the results of Konar's experiments are not compatible with those of the present investigation using Scots pine seeds, where sucrose was evidently synthesized during the continuing ripening process (cf. Fig. 7C). Konar's material is also interesting as it shows the presence of a trisaccharide, which can be compared with the formation of raffinose in Scots pine. Also *Radecke's* (1967) study of ripening seeds of *Tilia cordata* L. demonstrated a high initial content of glucose and fructose simultaneously with a high sucrose content. Following an initial decrease in all of these there was

a secondary formation of sucrose concomitant with the production of raffinose. *Jensen* et al. (1967a, b) have studied the chemical changes in ripening seeds of *Picea abies* (L.) Karst. and were able to demonstrate the formation of a trisaccharide when ripening had reached an advanced stage (in October, ibid. 1967b), although the presence of glucose and fructose in this material seemed to vary according to the origin of the seeds. Parallel investigations using seeds of *Picea sitchensis* (Bong.) Carr. showed however that glucose and fructose disappeared at the same time as sucrose and a trisaccharide increased in quantity. Without identifying the individual sugars, *Rediske* (1961) and *Rediske* and *Nicholson* (1965) studied the ripening process of *Pseudotsuga menziesii* (Mirb.) Franco and *Abies procera* Rehd. respectively and its relationship with the sugar content of the seeds. They found a decreasing content of reducing sugars with increasing ripeness. In the case of non-reducing sugars the *Pseudotsuga* seeds showed a decreasing trend with increasing ripeness while the *Abies* seeds showed a transient reduction.

Judging from the above comparisons there appear to be certain similarities between the results obtained from experiments with Scots pine seeds and those using other tree species. These similarities may be expressed by a decreasing content of monosaccharides (glucose and fructose) simultaneously with or followed by a synthesis of oligosaccharides (sucrose and sugars belonging to the raffinose family) during the progressive ripening process. It would appear however that there is only a limited possibility of using the seed's content of different sugar types as an indication of its degree of ripeness expressed as germinability (cf. also *Jensen* et al. 1967b). Concerning the relationship between temperature and the anabolic and catabolic changes in sugars and starch shown in this study, the results suggest that they are dependent on a limited incidence of positive temperatures. A more detailed evaluation of these results would call for more specific knowledge of the temperature conditions inside the cones themselves and/or the seeds.

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Sammanfattning

Föreliggande undersökning har utförts på frön av vanlig tall (*Pinus silvestris* L.), som insamlats från ett och samma bestånd av 20 träd i Siksjö, Åsele socken, Västerbotten (64° 20′ n.b., 405 m.ö.h.) under tre på varandra följande år (1967—68, 1968—69, 1969—70). Insamlingarna har under varje period genomförts under tiden 1 september—1 april. Avsikten med undersökningen har varit att studera tallfröets utveckling med avseende på groningsegenskaper i relation till insamlingstidpunkten samt parellellt löpande förändringar i frönas innehåll av sockerarter och stärkelse. Groningsförmågan ökade snabbt under september månad i de prov, som insamlades åren 1967 och 1969 och synes ha uppnått full mognad under oktober månad. Materialet insamlat under 1968 uppvisade emellertid redan under september en hämmad utveckling, som efter avstannande övergick i minskad groningsförmåga under de följande vintermånaderna. Resultaten har diskuterats i relation till uppmätta temperaturförhållanden under aktuella tidsperioder. Det har framhållits att olämpliga temperaturer (frostförekomst) under känsliga faser av fröets mognadsprocess kan skada fröets vidare utveckling varigenom allmänt konstaterade samband mellan temperaturerna under de föregående sommarmånaderna juni, juli och augusti och fröets mognadsutveckling kan omintetgöras.

Med användande av gaskromatografisk analysteknik har glukos, fruktos, sackaros, raffinos och stachyos kunnat identifieras försöksvis och studeras kvantitativt. Även stärkelse har påvisats och analyserats kvantitativt med kolorimetrisk teknik. Frönas halt av glukos och fruktos har uppenbarligen minskat med ökande mognad under september månad. Med undantag för ett av materialen har halterna för dessa huvudsakligen förblivit oförändrade från mitten av oktober till slutet av vintern. Ingen sackaros kunde påvisas i de tidigaste proven från två av de undersökta åren och i proven från åren 1968 och 1969 kunde en tydlig nybildning av sackaros påvisas under månaderna september—oktober. I materialet insamlat 1967 var dock denna nysyntes fördröjd tre månader. Även raffinos och stachyos saknades i de tidigare proven men uppträdde i ökande mängder med fortskridande mognad på sinsemellan likartat sätt och parallellt med nybildningen av sackaros. Stärkelse har kunnat påvisas i samtliga prov oberoende av insamlingstidpunkten men med minimala halter under september. Den därefter insättande nybildningen har varit mer oregelbunden än för de ovan nämnda substanserna men dock med parallella drag. Det har ej varit möjligt att korrelera graden av mognad hos de undersökta frömaterialen uttryckt genom deras groningsegenskaper med någon av deras samtidiga halter av de aktuella sockerarterna eller stärkelse.

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