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A study on the temperature response of pollen mother cells in Norway spruce

En studie av temperaturens verkan på meiosförloppet i pollenmoderceller hos gran

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Abstract

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The effect of temperature upon the pattern of meiotic development was studied in PMC in Picea abies. *The twigs were collected from grafts and exposed to different temperature conditions in a climatic cabinet. (The thermoperiods used were +20°C/+15°C and +15°C/+10°C respectively.) The rate as well as the temperature sum requirement of the development within given limits were compared. The data obtained are compiled in Figure 31. Significant differences obtained in some instances indicate that at least some phases of development are accelerated by a rise in the temperature. However, the results were somewhat contradictory with respect to different phases of the meiotic development. For example a shorter time and a smaller temperature sum were needed for the development from diplotene to interkinesis (compared at the 50 per cent level) in the experiment carried out at the higher temperature. On the other hand, the reaching of tetrads proceeded faster and had a lower temperature sum requirement in the lower temperature conditions. The exposure of the material to −5°C caused a delay in the meiotic development.*

Furthermore, the induction of irregularities in PMC following the treatment at −5°C was studied. Data on the frequency of different types of irregularity, the frequency of irregularities at different meiotic stages as well as the relationship between the occurrence of sensitive stages during the treatment at −5°C and the frequency of irregularities were presented. The relationships between the origin of the material on the one hand and the pattern of meiotic development or the frequency of irregularities induced on the other hand were discussed.

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Contents

1 Introduction

A number of investigators have concluded that the temperature conditions prevailing during the pollen formation play a decisive role in the induction of irregularities in the pollen mother cells (PMC) of Norway spruce (Andersson 1966 and 1974, Andersson *et al.* 1969, Ekberg *et al.* 1970, Eriksson *et al.* 1970 b, Eriksson *et al.* 1972). The result of these irregularities is a poor pollen quality and few chances of a good seed set. It is, therefore, important to obtain the information on the temperature responsible for the normal development of PMC in introduced spruce provenances. This information makes it possible to select provenances suited to the climatic conditions in a new locality and in this way contribute to a good seed set.

On the basis of cytological studies performed in conifers (mainly in PMC) it can be stated that the temperature affects the pattern of meiotic development and that extreme temperatures, both low and high, are the reason for the occurrence of irregularities (cf. literature cited above as well as: Ekberg *et al.* 1972, Eriksson 1970, Eriksson *et al.* 1969, Eriksson *et al.* 1970a). The most common types of irregularity are also known as well as the most temperature sensitive meiotic stages.

Up to now the information on meiosis in conifers has mostly been obtained by studying material cultivated outdoors. However, the basic principles concerning the temperature influence on PMC can not be elucidated until we have an opportunity of carrying out the experiments under controlled temperature conditions. The main interest will then be focused on the following problems:

1. the action mechanism of the temperature on the pattern of meiosis—the limits within which the increasing temperature causes an increase in the rate of development and the way in which the rate increases (exponentially, linearly)
2. the critical temperature and the exposure time for induction of various types of irregularity as well as the action mechanism of a temperature with respect to induction of irregularities (linear, exponential or threshold effect)—cf. Eriksson 1970.

In the present investigation an attempt was made to elucidate some of the above mentioned problems. The effect of different temperatures on the pattern of meiotic development as well as the effect of a below zero temperature on the induction of irregularities were studied. Experiments were carried out under controlled temperature conditions in a climatic cabinet on Norway spruce of both Swedish and introduced provenances. The different origin of the material also allowed one to investigate whether or not the origin affected the temperature response of PMC.

2 Material and methods

Norway spruce grafts, of both Swedish and foreign origin (cf. Table 1) growing at Röskär (latitude 59°25′, longitude 18°11′ and altitude 30 m) were selected for this investigation. Each graft represented one clone.

The twigs (approximately 20 cm long) were collected from grafts on three different occasions during April 1971 *viz.* on April 14th (the first experiment) on April 20th (the second experiment) and on April 26th (the third experiment). The twigs were then exposed to different temperature conditions in a climatic cabinet. In Figure 1 the temperature conditions prevailing during the three experiments are shown.

The temperature figures refer to the day and night temperatures respectively. As seen from Figure 1 the night temperature was lower than the day one.

The above zero temperatures (+20°C/+15°C and +15°C/+10°C) have been chosen in the light of the results obtained for PMC of larch during an investigation performed at +5°C, +10°C, +15°C and +22°C respectively (Eriksson *et al.* 1972). After the breakage of dormancy the temperature determined the rate of the meiotic development in such a way that meiosis was completed earlier at a higher temperature. The choice of a below zero temperature (−5°C) was based on the reports by Andersson (1954, 1966 and 1974) in which the critical temperature for the induction of irregularities in PMC of *Picea abies* was estimated to be −4°C.

The photoperiod in the climatic cabinet was changed once a week according to the conditions prevailing outdoors. The air humidity was approximately 75 per cent. The velocity of the air current did not exceed 0.6 metres per second.

The temperature within the male strobili was recorded every tenth minute with the aid of thermoelements put into the strobili. The air temperature was also recorded. The voltage of the thermoelements was punched on a tape and translated to a temperature in a computer. For more information concerning the registration of the temperature the reader is referred to the paper by Eriksson *et al.* (1970 b). The temperature sum

Table 1. The data on the origin of the material investigated.

Graft No.	Provenance	Latitude	Longitude	Altitude (m)	Origin index
53-766	Plánice	49°20′	13°30′[a]	700—800	56.8
53-2256	Griva	55°58′	26°15′	160	57.6
53-2891 54-2854	Hjuleberg	56°56′	12°44′	50—100	57.6
54-4684	Crucea	47°21′	25°40′	720	54.5
54-4798	Svinošice	49°20′	16°30′	300—400	52.8
S 3348[c]	Mangslidberget	60°31′	5°16′[b]	350	64.0
S 49-437[c]	Mangslidberget	60°31′	5°16′	334	63.8
S 49-594	Mangslidberget	60°30′	5°16′	420	64.7

[a] East of Greenwich
[b] West of Stockholm
[c] Grafts excluded from the third experiment

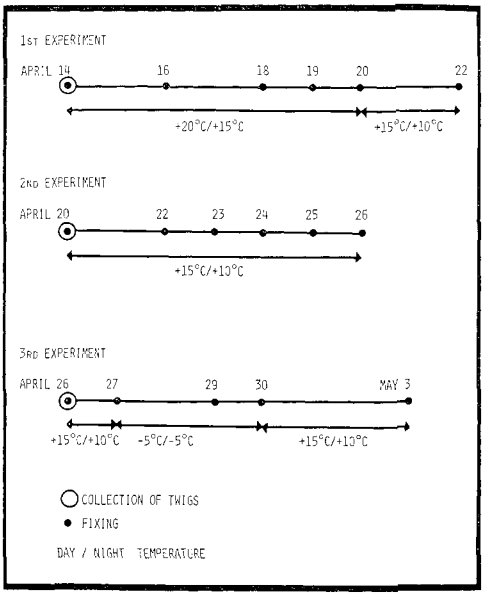

Figure 1. The data on the collections of twigs, the fixings of male strobili as well as day and night temperatures during the three experiments.

was calculated as a sum of products of the hours with an average temperature exceeding +5°C and degrees above +5°C. The temperature recorded within the male strobili was used for the calculation.

The first fixing of the male strobili in 3 : 1 alcohol : acetic acid took place before the twigs were put in vessels containing water and placed in the climatic cabinet. The fixings were then carried out at intervals of 1—3 days (cf. Figure 1). In most cases ten strobili were fixed from every graft on each occasion. The fixed material was stored in a refrigerator at +4°C.

The method of preparing the slides and counting the different meiotic stages as well as the irregular PMC has been described by Eriksson *et al.* (1970 b).

The meiotic stages and irregularities were classified according to the method used by Eriksson (1968).

3 Results and discussion

3.1 The pattern of meiotic development in different temperature conditions

3.1.1 *The effect of an above zero temperature on the pattern of meiotic development*

According to Sarvas (1967) each stage of the annual development in forest trees is passed through in the main part of its range at a relative temperature sum specific to the species in question.

Therefore, it was of interest to study a relationship between the pattern of meiotic development and the temperature sum (an equivalent term used in literature is heat sum, cf. Boyer 1972). The temperature sums based on the critical temperature of +5°C seemed to give the best agreement with a normal distribution in the paper by Eriksson *et al.* (1970 b). The same critical temperature was, therefore, selected in this investigation.

To illustrate the pattern of meiotic development the cumulative percentages were calculated for three stages—diplotene, interkinesis and tetrads. (The cumulative percentages refer to all PMC that have once reached the stage in question.)

Two approaches were used to study the effect of the above zero temperature on the pattern of meiotic development. The first one was to compare graphs in which the cumulative percentages of diplotene, interkinesis and tetrads were plotted against the time in days. The second one was to compare analogous graphs with the temperature sum in degree-hours on the abscissa. No difference revealed in the pattern of meiotic development between different temperature conditions would in this case mean, that the magnitude of the temperature sum alone played a decisive role in the development, while the temperature level (within the limits studied) was of little or no importance.

According to Ekberg *et al.* (1972) a suitable method of comparison of the meiotic development in different grafts was to measure the temperature sum needed for the passing of a certain stage by 10 per cent of PMC or the reaching of a certain stage by 90 per cent of PMC. In the present investigation the twigs were collected at three points in time (cf. Figure 1). This means that the material included in the three different experiments had reached a different temperature sum outdoors before the start of the forcing in the climatic cabinet. The above mentioned method of comparison of the meiotic development was therefore unsuitable. In the present report, both time and temperature sum required for the development within given limits during the experiments were compared. It would be desirable to study the material taken in simultaneously and forced in climatic cabinets at different temperatures. However, only one climatic cabinet was available when the experiments were carried out.

For each graft included in the first and the second experiments both graphs were drawn with the time and the temperature sum on the abscissa. (The former were used for further calculation but are not shown. The latter are shown in Figures 2—10. For the comparison of graphs with the time and the temperature sum see Figures 11 and 12 showing the average cumulative percentages of diplotene, interkinesis and tetrads.) The time or the temperature sum required for the development from diplotene to tetrads, from diplotene to interkinesis and from interkinesis to tetrads, were measured at the 50 per cent level (cf. Figure 13) and compared for both experiments.

Furthermore, the slopes of the curves for

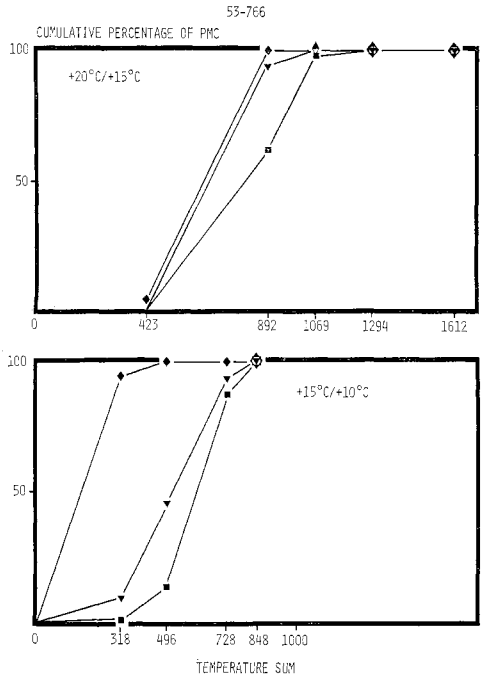

Figure 2. Graft 53-766. The cumulative percentages of PMC at diplotene, interkinesis and tetrads plotted against the temperature sum in degree—hours. Above: The first experiment (+20°C/+15°C). Below: The second experiment (+15°C/+10°C). Symbols: ◆ diplotene, ▼ interkinesis, ■ tetrad.

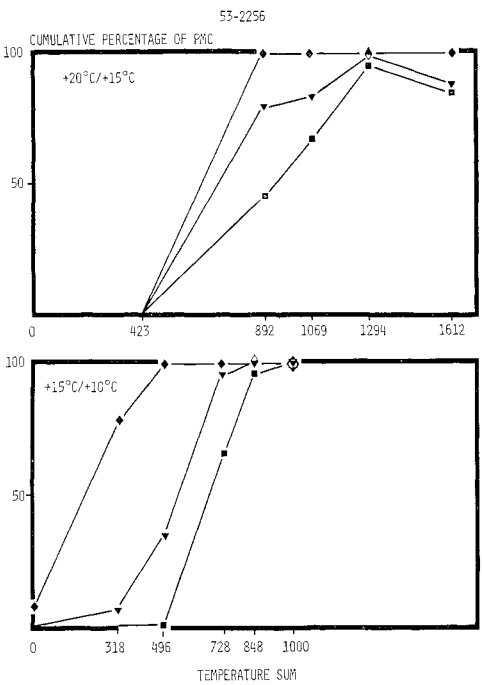

Figure 3. Graft 53-2256. Legend as in Figure 2.

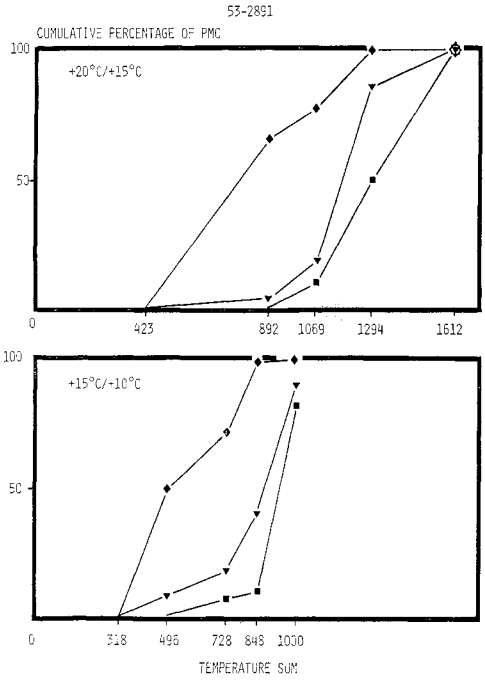

Figure 4. Graft 53-2891. Legend as in Figure 2.

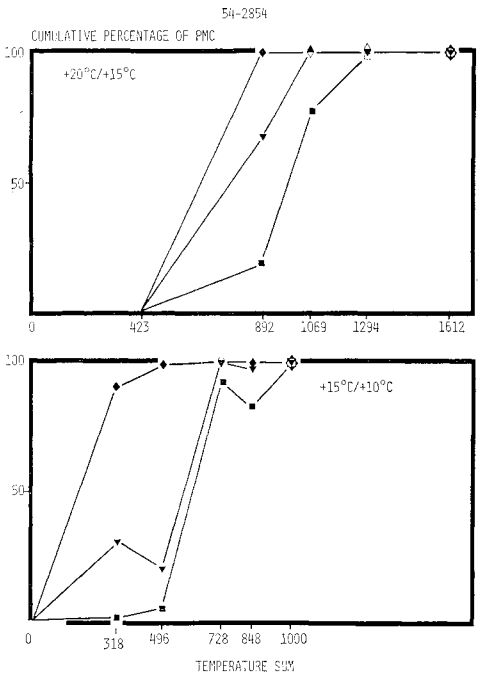

Figure 5. Graft 54-2854. Legend as in Figure 2.

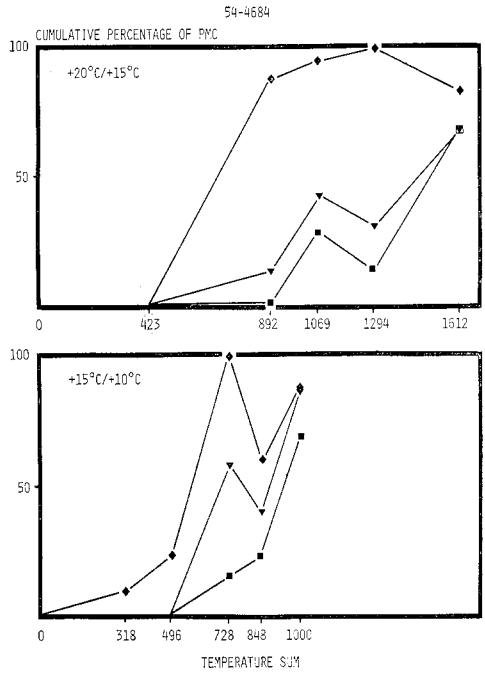

Figure 6. Graft 54-4684. Legend as in Figure 2.

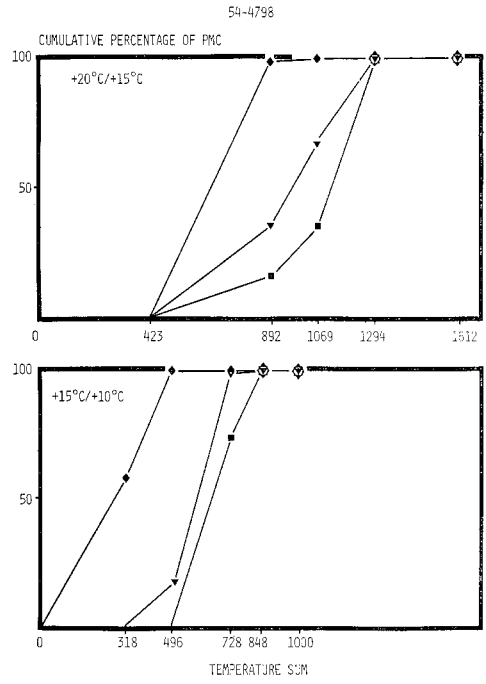

Figure 7. Graft 54-4798. Legend as in Figure 2.

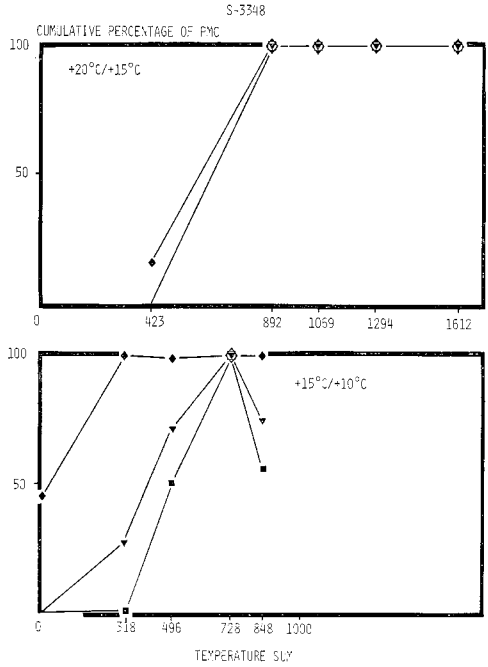

Figure 8. Graft S 3348. Legend as in Figure 2.

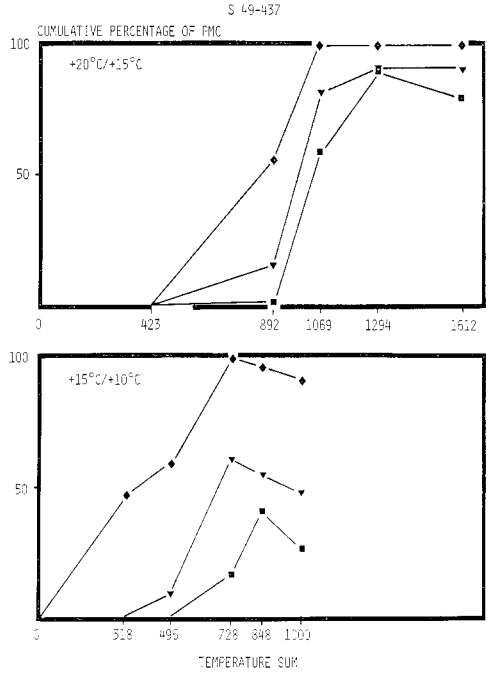

Figure 9. Graft S 49-437. Legend as in Figure 2.

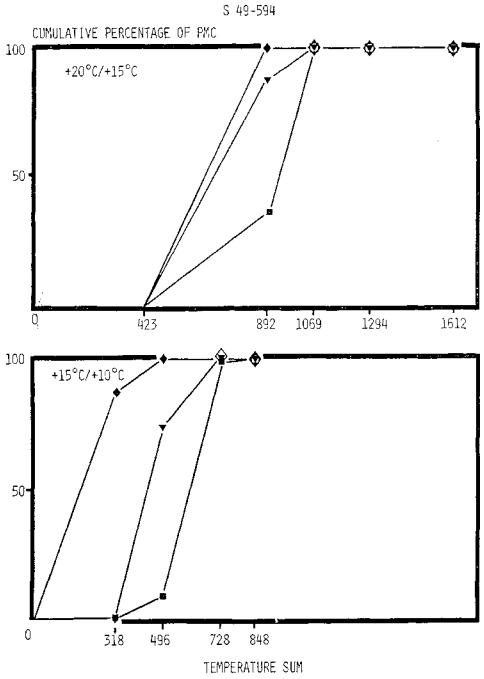

Figure 10. Graft S 49-594. Legend as in Figure 2.

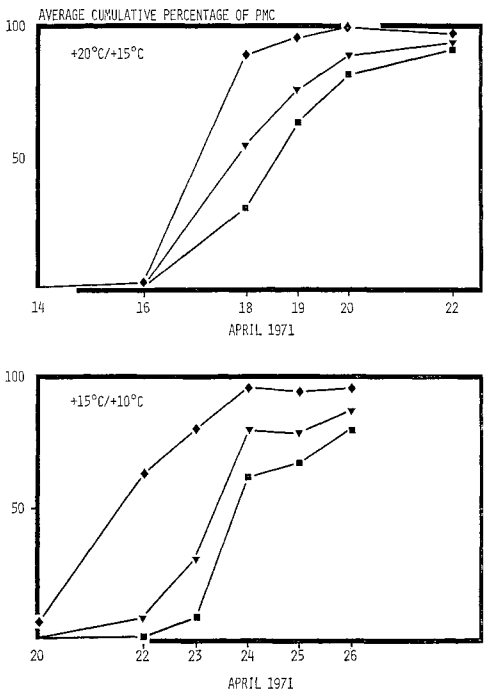

Figure 11. The average cumulative percentages of PMC at diplotene, interkinesis and tetrads plotted against time in days. Above: The first experiment (+20°C/+15°C). Below: The second experiment (+15°C/+10°C). Symbols as in Figure 2.

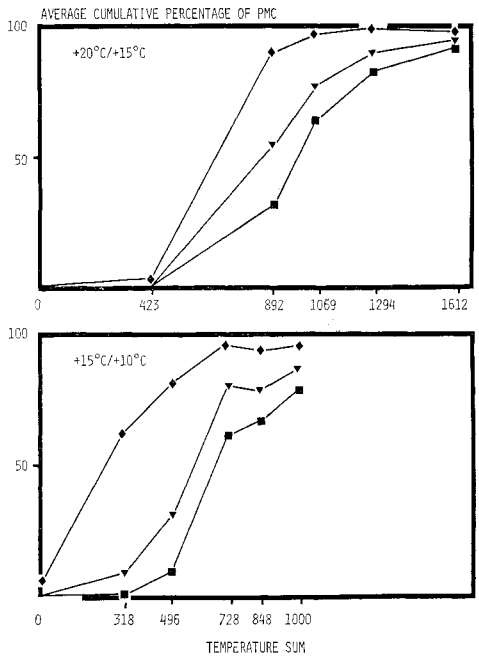

Figure 12. The average cumulative percentages of PMC at diplotene, interkinesis and tetrads plotted against the temperature sum in degree—hours. Above: The first experiment (+20°C/+15°C). Below: The second experiment (+15°C/+10°C). Symbols as in Figure 2.

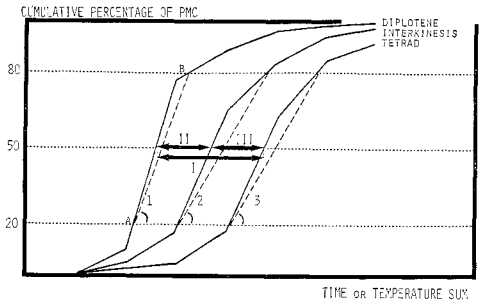

Figure 13. The model illustrates the way of obtaining data for comparisons with analysis of variance. I, II, III—the limits within which the time or the temperature sum were measured on the graphs. 1, 2, 3—the lines, for which the slopes were calculated as tangent of the angles marked (cf. the text on page 12). A and B denote the points in which the curve cuts the level of 20 or 80 per cent.

diplotene, interkinesis and tetrads were calculated and the results obtained in the two experiments were compared. In the graphs with the time on abscissa the slopes of the curves reflect the rate of the reaching of the three stages in question. In the graphs with the temperature sum on the abscissa the slopes illustrate the temperature sum requirement for reaching the stages concerned. The larger the slope, the shorter was the time needed and the lower was the temperature sum requirement for a given phase of the development.

The slope of each curve was calculated as the trigonometric tangent of the angle that the line joining points A and B makes with the line marking the 20 per cent level. A and B are the points in which the curve cuts the level of 20 per cent and 80 per cent respectively (cf. Figure 13). Only the parts of the curves lying within this interval

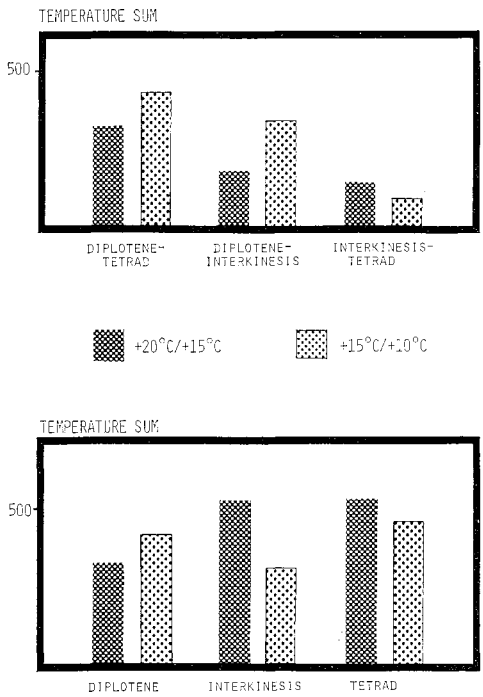

Figure 14. The average temperature sum in degree—hours required for the development within the given limits. Above: The development from 50 per cent of PMC at one stage to 50 per cent of PMC at the other stage. Below: The reaching of a stage by 20 to 80 per cent of PMC.

were taken into account, which means that exceptionally advanced as well as late PMC were excluded. (In this case rather large amounts of PMC were omitted because it was desirable to choose intervals which were comparable for as many grafts as possible.)

To give a survey of the general situation in the first and the second experiments, the average cumulative percentages of diplotene, interkinesis and tetrads based on nine grafts were plotted against the time (Figure 11) and the temperature sum (Figure 12). The situation differed somewhat between the two experiments. To illustrate this more clearly the average temperature sums needed for the development within certain limits are shown in Figure 14. It can be seen that the results of the comparison of different phases of the development were contradictory. In some cases the temperature sum requirement was higher in the experiment with the lower temperature (+15°C/+10°C). In other cases the situation was reversed.

To estimate whether the rate of development or the temperature sum requirement really differed between the two temperature conditions, analyses of variance (two way classification) were calculated on the data obtained from both types of graphs (with the time or the temperature sum on the abscissa) drawn for all grafts individually (cf. Table 2).

The entire material could not be evaluated. Because of the form of curves it was in some cases impossible (grafts 54-4684, S 3348, and S 49-437, cf. Figures 6, 8 and 9) to get data concerning the time or the temperature sum in both experiments. Only grafts in which pairs of figures could be obtained were included in the analysis of variance.

Furthermore, grafts 53-2891 and 54-4684 (Figures 4 and 6) were so late that some of the phases of the development compared fell in the first experiment into the period of April 20th to 22nd, when the temperature in the climatic cabinet had been reduced to +15°C/+10°C. These grafts were excluded from the analysis of variance con-

Table 2. Analyses of variance; the time (A) and the temperature sum (B) needed for the development within given limits as well as the slopes illustrating the rate (C) of the reaching of diplotene, interkinesis and tetrads and the temperature sum requirement (D) of this development respectively.

Marked on abscissa	Comparison	Variance ratios for the different phases of development compared		
		50 per cent diplotene — 50 per cent tetrads	50 per cent diplotene — 50 per cent interkinesis	50 per cent interkinesis — 50 per cent tetrads
A Time	Between temperatures	8.42*(L)	21.33**(L)	3.98 (H)
	Between grafts	0.48	0.82	1.18
B Temperature sum	Between temperatures	4.30 (L)	16.34**(L)	4.82 (H)
	Between grafts	0.58	1.02	1.53
		Diplotene 20—80 per cent	Interkinesis 20—80 per cent	Tetrads 20—80 per cent
C Time	Between temperatures	0.67 (H)	0.74(L)	17.29**(L)
	Between grafts	2.33	0.14	1.97
D Temperature sum	Between temperatures	6.09*(L)	4.04(L)	19.30**(L)
	Between grafts	3.91*	0.40	2.52

Symbols: * a difference significant at the five per cent level
** a difference significant at the one per cent level
H, L (high or low temperatures) denote the temperature with the higher average values of time, temperature sum and slopes obtained
Note: The high slopes indicate high rate of development and low temperature sum requirement.

cerning the phases of the development under discussion.

In the following the results of the analyses of variance are discussed (cf. Table 2).

A longer time was required in the low than in the high temperature for the development from *diplotene to the tetrad stage*. As regards the temperature sums needed for this development at the two temperature levels no significant difference was found. This implies that in this case it was the magnitude of the temperature sum which governed the rate of the development.

The most striking difference in time needed for development in the two temperatures was found in the phase *diplotene to interkinesis*. The development of this phase of meiosis required a longer time at the lower temperature level than at the higher temperature level. As regards the temperature sums needed for this development a higher value was revealed at the lower temperature than at the higher one. This indicates that the effect of temperature sum increased in this case with a rising temperature level. A given temperature sum obtained at the lower level seemed to have a less of an effect than the same temperature sum obtained at the higher level. Another explanation might be that a too low critical temperature for the calculation of the temperature sums was selected. This explanation is, however, not applicable to the other results.

The development from *interkinesis to the tetrad stage* required a somewhat longer time and larger temperature sum at the higher than at the lower temperature conditions. The difference was, however, not significant.

When comparing the slopes of the *diplotene* curves in diagrams with time on the abscissa, no significant difference was found, which means that the rate of the

development did not differ between the two temperatures. Consequently, in the diagrams with the temperature sum on the abscissa, a larger slope was obtained at the lower temperature. Thus, the temperature sum requirement was smaller at this temperature level. (It had been desirable to fix the strobili more frequently in the start of the first experiment; e.g. on April 17th. It is possible, that the reaching of diplotene proceeded faster than could be revealed on the basis of the fixings available.)

In the case of *interkinesis* no significant differences were found with regard to the slopes of the curves based on time or temperature sum. This implies that neither the rate of development nor the temperature sum requirement for the reaching of this stage differed between the two temperatures.

The slopes calculated for the *tetrad* curves in the diagrams with time on the abscissa suggested that the reaching of this stage proceeded faster (a higher slope) at the lower temperature than at the higher temperature. As regards the diagrams with the temperature sum on the abscissa, a higher slope was obtained at the low temperature. This indicates that the temperature sum requirement for the reaching of the tetrad stage was less at this temperature. In other words, the rise of temperature from +15°C/+10°C to +20°C/+15°C did not accelerate the development of this phase of meiosis; the effect was, on the contrary, a delay in the development. In this connection it may be mentioned that Chira (1965) observed that the meiotic development in the PMC of the spruce accelerated considerably at +10°—12°C, while prolonged temperatures maintained above +15°C had a negative effect on meiosis by inducing irregularities.

The results suggest that the pattern of meiotic development was not always governed by the magnitude of the temperature sum alone; the temperature level was of importance, too. Furthermore, the different phases of meiosis seemed to have had different temperature responses. The development from diplotene to interkinesis proceeded faster and had a lower temperature sum requirement at the high temperature, while at the end of meiosis the reaching of the tetrad stage proceeded faster and had a lower temperature sum requirement at the low temperature. A possible reason for this might be that the negative effect of forcing under artificial conditions, which probably increases with time, might be more pronounced at the higher temperature.

A significant difference between grafts was revealed only in the slopes of diplotene curves in diagrams with a temperature sum on the abscissa.

3.1.2 *The effect of a below zero temperature on the pattern of meiotic development*

In the third experiment the effect of the below zero temperature (−5°C) on the pattern of meiotic development was studied. The cumulative percentages of diplotene, interkinesis, and tetrads were calculated for each graft individually. The data are compiled in Figures 15—18.

The data show that the development ceases following the exposure to −5°C on April 27th. The further development starts subsequent to the cold treatment, as is reflected by the PMC analysed following fixing on May 3rd.

From Figures 15—18 it seems as if there was a decline in the curves which should not take place since the curves show cumulative percentages. This phenomenon may probably be attributed to the fact that the material analysed was too limited (ten strobili per a fixing and a graft). However, the space in the climatic cabinet did not allow a study of any large material. Another possible explanation might be a cold induction of completely damaged strobili. However, strobili completely lacking regular PMC were observed in a too low frequency to be able to explain the decline observed.

It was of particular interest to compare the meiotic development in the second and the third experiments which were carried out at the same temperature above zero,

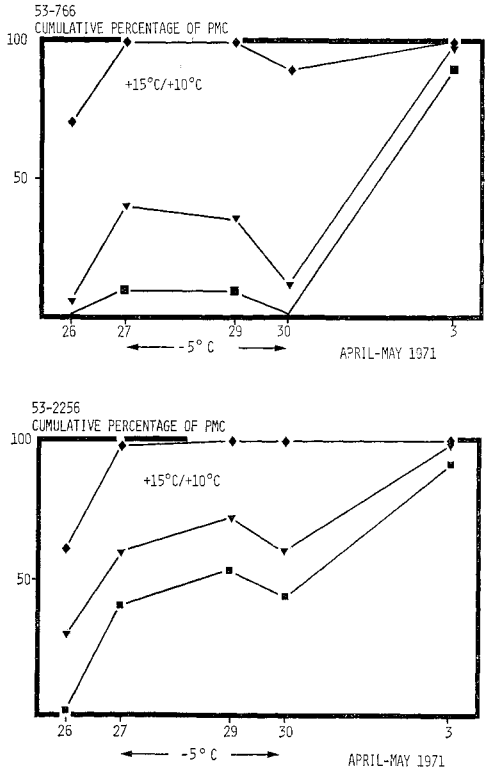

Figure 15. Grafts 53-766 and 53-2256. The cumulative percentages of PMC at diplotene, interkinesis and tetrads plotted against time in days in the third experiment (+15°C/+10°C with a three-day period at −5°C). Symbols: ◆ diplotene, ▼ interkinesis, ■ tetrad.

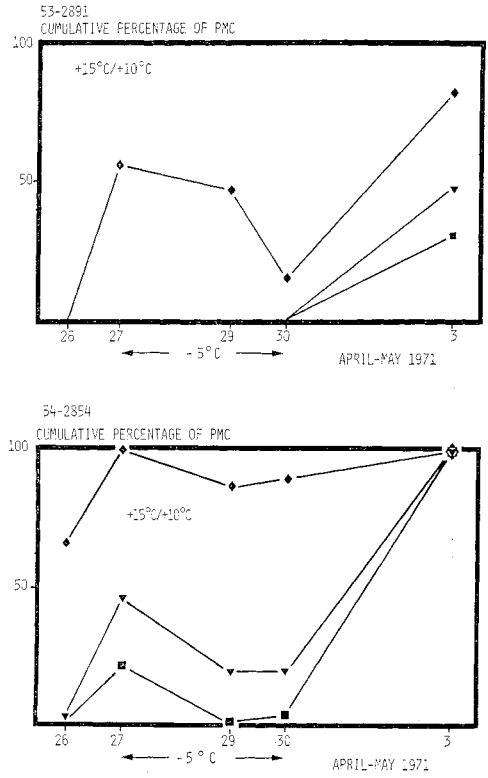

Figure 16. Grafts 53-2891 and 54-2854. Legend as in Figure 15.

namely +15°C/+10°C. If we leave out of consideration the different date for the collecting of the twigs, the three day period at −5°C constitutes the only difference between the two experiments. In other words, the difference between these two experiments in the pattern of meiotic development might be ascribed to the effect of the below zero temperature in the third experiment.

The average cumulative percentages calculated for diplotene, interkinesis and tetrads in the second and the third experiments can be compared in Figure 19. The curves for both experiments represent the same material (grafts S 3348 and S 49-437, which were not included in the third experiment, were omitted when calculating the average cumulative percentages in the second experiment). The time in days is marked on the abscissa.

It may be seen that the curves in the upper and in the lower parts of Figure 19 differ considerably. The development in the material exposed to the temperature of −5°C for three days stopped during this time and started again when the temperature was changed to +15°C/+10°C. It seemed as if the development was delayed even after the treatment with −5°C has ceased.

3.1.3 *The effect of the origin of the material on the pattern of meiotic development*

According to Sarvas (1967) the autochthonous tree populations are adapted to the climatic conditions prevailing at their growth habitats. Thus, a given process starts when a given percentage of the local average annual temperature sum—, $\Sigma(t_m - 5)$, has

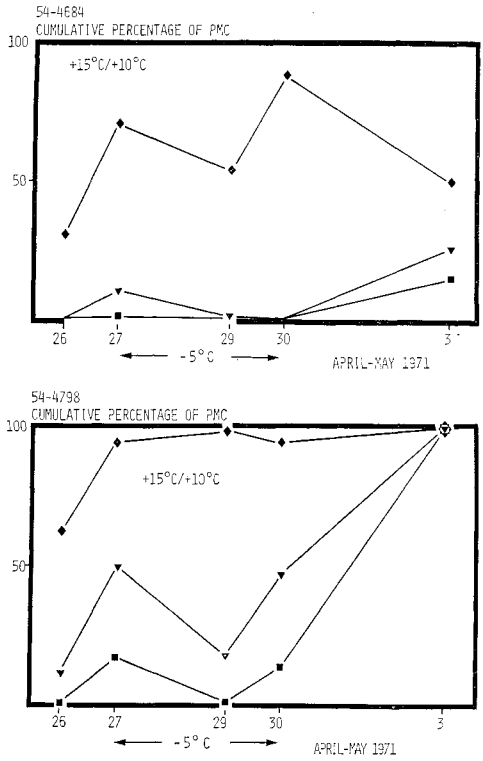

Figure 17. Grafts 54-4684 and 54-4798. Legend as in Figure 15.

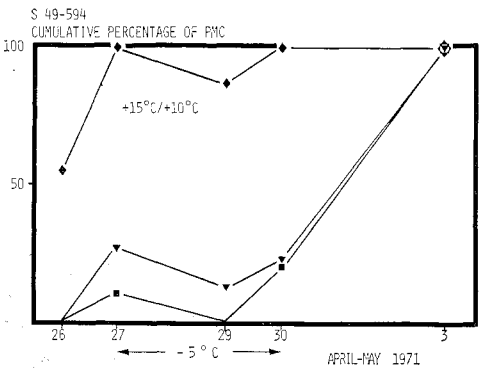

Figure 18. Graft S 49-594. Legend as in Figure 15.

been reached. Therefore, it might be expected that the populations of southern origin are adapted to their environmental conditions in such a way that the temperature sum needed for the meiotic development is larger than in populations of northern origin.

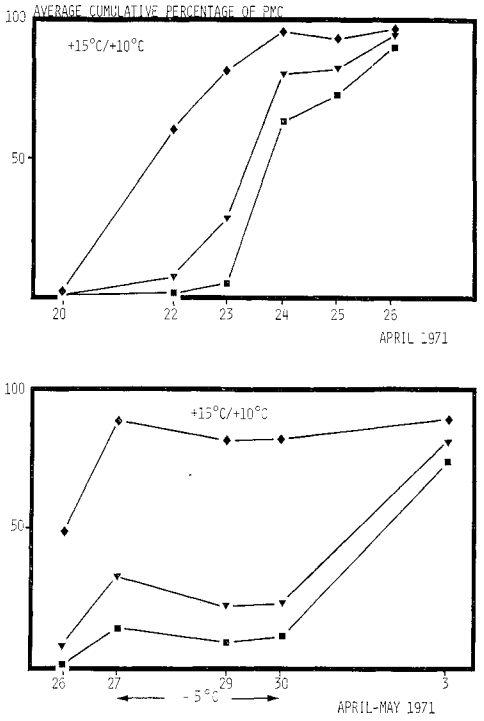

Figure 19. The average cumulative percentages of PMC at diplotene, interkinesis and tetrads plotted against time in days. Above: The second experiment (+15°C/+10°C). Below: The third experiment (+15°C/+10°C and a three-day period at −5°C). Symbols as in Figure 15.

In this connection it might be mentioned that Eriksson *et al.* (1972) observed no great differences as regards the onset and the completion of the meiotic development between autochthonous and introduced trees of *Picea abies*. This suggests that the origin does not affect the pattern of meiotic development in this species to any great extent.

The different origin of the material in the present study allowed an investigation on the relationship between the pattern of meiotic development and the origin index

$$\text{(origin index} = \text{latitude} + \frac{\text{altitude in metres}}{100}$$

cf. Ekberg *et al.* 1972). The pattern of meiotic development in individual grafts was illustrated by the temperature sum required for the development from 50 per cent of PMC in the pachytene stage to 50 per cent

of tetrads. This temperature sum was read between the descending part of the pachytene curve and the ascending part of the tetrad curve, both illustrating the changes in the percentages of the stages concerned.

This temperature sum seemed to be rather constant for individual grafts in the second experiment but it varied considerably from graft to graft in the first one. The correlation between the temperature sum and the origin index was calculated on data from the first experiment. The correlation coefficient was not significant ($r = -0.56$). The reason for this was, however, the graft 53-2891 which required a too large temperature sum with regard to its origin index. If the data for this graft were excluded from the calculation, the correlation coefficient was found to be $r = -0.68^*$. This suggests that the more southern the origin, the larger is the temperature sum needed for development within the limits given.

However, if the correlation was calculated between the origin index and the temperature sum needed for the development from diplotene to the tetrad stage, (read at the 50 per cent level on the graphs with cumulative percentages of stages concerned —cf. Figure 13), the correlation coefficient obtained was not significant, not even after excluding the graft 53-2891.

As might be seen, the data as regards the relationship between the origin of the grafts and the pattern of meiotic development are somewhat contradictory. A final elucidation of this problem has to be postponed until more extensive material has been investigated.

3.2 **Irregularities in PMC exposed to a below zero temperature**

3.2.1 *The occurrence of different types of irregularity*

The different types of irregularity found in PMC of larch have been described by Eriksson (1968). The irregularities observed in the present investigation could be classified in the same way. The most frequent types of irregular PMC in Norway spruce were illustrated by microphotographs in the paper by Eriksson *et al.* (1970 b).

As can be seen from Table 3 stickiness C and degeneration were the most common irregularities. The class "others" includes stickiness B and bridges which occurred rarely. Since bridges probably originate from stickiness it can be stated that stickiness and degeneration are the two main types of irregularity found.

The high percentage of degeneration observed in the strobili fixed on April 26th can probably be ascribed to the drop in temperature outdoors to $-7.8°C$ on April 24th (Figure 20). The percentage of stickiness C on the above mentioned fixing occasion was surprisingly low.

The relatively high percentage of stickiness C observed in the fixing on April 27th

Table 3. The frequency of different types of irregularity.

Date	Percentages of all PMC					Percentages of aberrant PMC	
	Stickiness C	Degeneration	Others	Total aberrant	N	Stickiness C	Degeneration
April 26	0.46	3.2	0.54	4.2	6900	11.0	76.2
27	7.0	0.01	0.20	7.2	6900	97.0	0.14
29[a]	7.2	4.6	0.39	12.2	6900	58.9	37.7
30[a]	6.8	4.9	0.35	12.0	6900	56.3	40.8
May 3	1.8	9.7	0.78	12.3	7000	14.5	78.9

[a] Fixings made during the treatment at $-5°C$

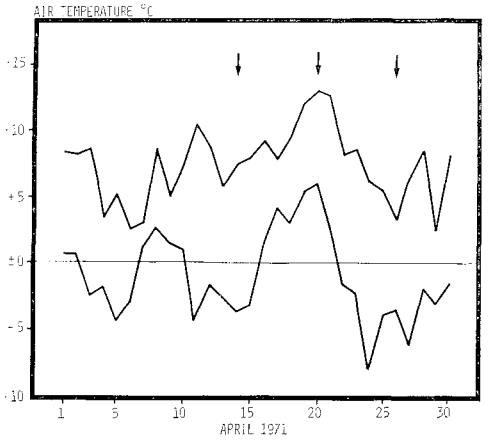

Figure 20. The curves for the maximum and the minimum temperature outdoors in April 1971. The arrows mark the dates of collection of twigs.

can probably be explained by the abrupt increase of the temperature following the placing of the twigs indoors. It might be expected that PMC are more sensitive to rapid than gradual changes of temperature. As seen from Table 3 the amount of one of the most severe irregularities—degeneration—was very low just before the start of the treatment at −5°C (April 27th). During this treatment the percentage of degeneration increased and it continued to increase even after the change of temperature to +15°C/+10°C. The percentage of stickiness C calculated on all PMC seemed not to be influenced by the temperature of −5°C when compared with the fixing before starting the cold treatment (April 27th). The decrease in the frequency of stickiness C is discernible on May 3rd perhaps owing to a partial healing of this irregularity after changing the temperature to +15°C/+10°C again. According to Eriksson 1968 stickiness C is a rather moderate irregularity which can probably heal. Another possible explanation for the decrease in the amount of stickiness C might be the decrease in the frequency of metaphase I, a stage which most often suffers from this irregularity.

In the two last columns in Table 3 the percentages of both stickiness C and degeneration of all aberrations can be seen. Stickiness C constituted the overwhelming majority of irregularities just before the exposure of the material to −5°C. Simultaneously as degeneration increased during and after the treatment at −5°C, the proportion of stickiness C decreased. The total amount of irregularities increased somewhat after placing the material in the climatic cabinet. It increased further after starting the treatment with −5°C and remained constant during and after this treatment.

3.2.2 *Irregularities observed at different meiotic stages*

The frequency of different types of irregularities discussed in the preceding section is partially dependent on the occurrence of different meiotic stages as some types of irregularity are typical for certain stages. Therefore, it was interesting to study the distribution of the irregularities among different meiotic stages. The three diagrams in Figure 21 show the situation:

A) before the start of the treatment at −5°C (April 27th)
B) during the treatment (based on fixings on April 29th and 30th)
C) three days after the end of the treatment at −5°C (May 3rd).

For the same fixing occasions a distribution of all investigated PMC among different meiotic stages was calculated (cf. Figure 21). In each diagram the sum of PMC investigated in all seven grafts on one (A, C) or two (B) fixing occasions, respectively the sum of irregularities observed in all seven grafts on the same fixing occasions constituted 100 per cent.

In diagrams A and B pachytene, diplotene, metaphase I, interkinesis and tetrads stand out as the most frequent stages or, in other words, as the stages with the longest duration. In diagram C tetrads dominate, but pachytene still exceeds ten per cent.

The columns for irregularities show that a great many damaged PMC were found at metaphase I. (The irregularity in question was stickiness C.) This is especially pro-

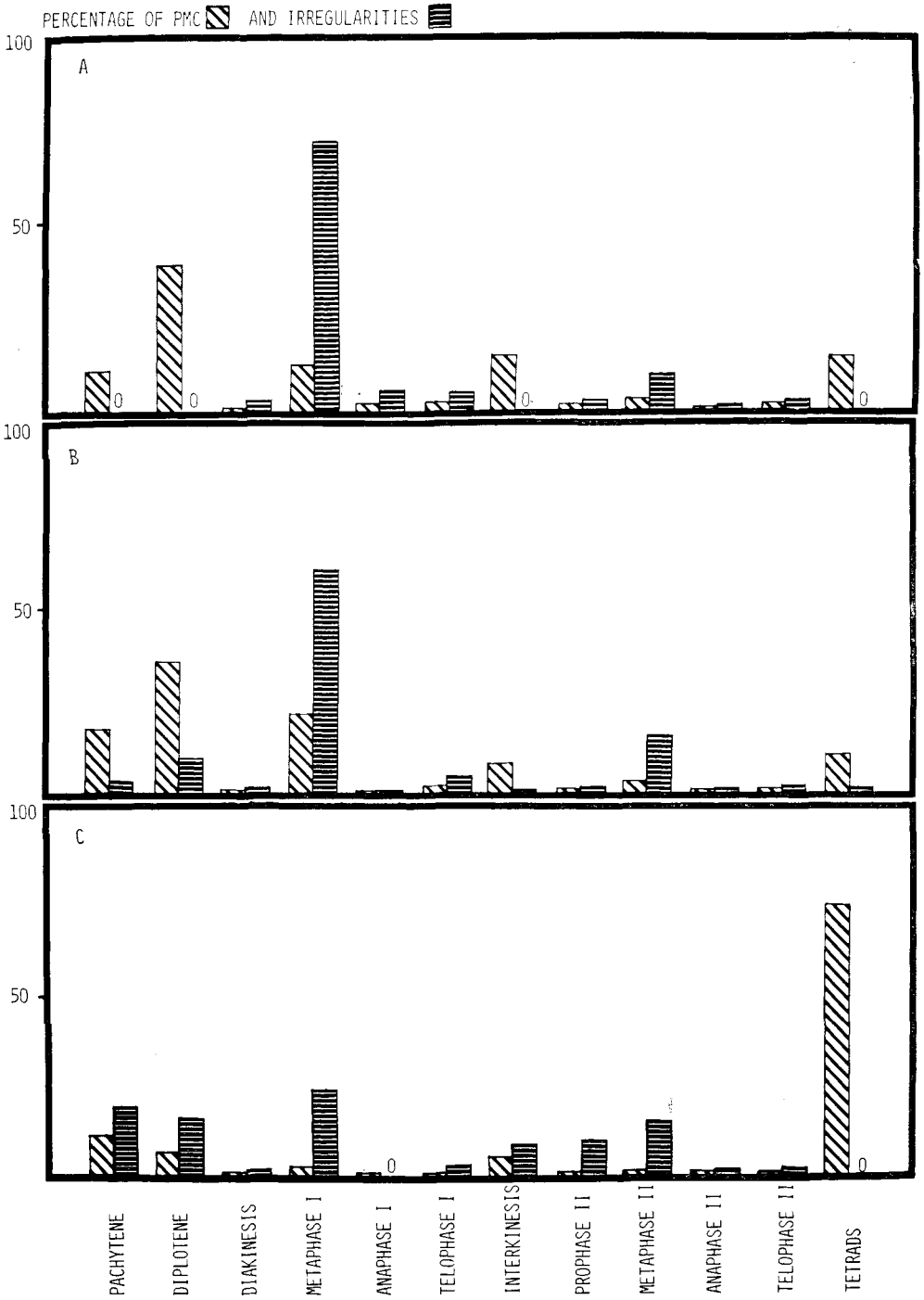

Figure 21. The distribution of PMC and irregularities among different meiotic stages before (A), during (B) and after (C) the treatment at $-5°C$ (see page 18).

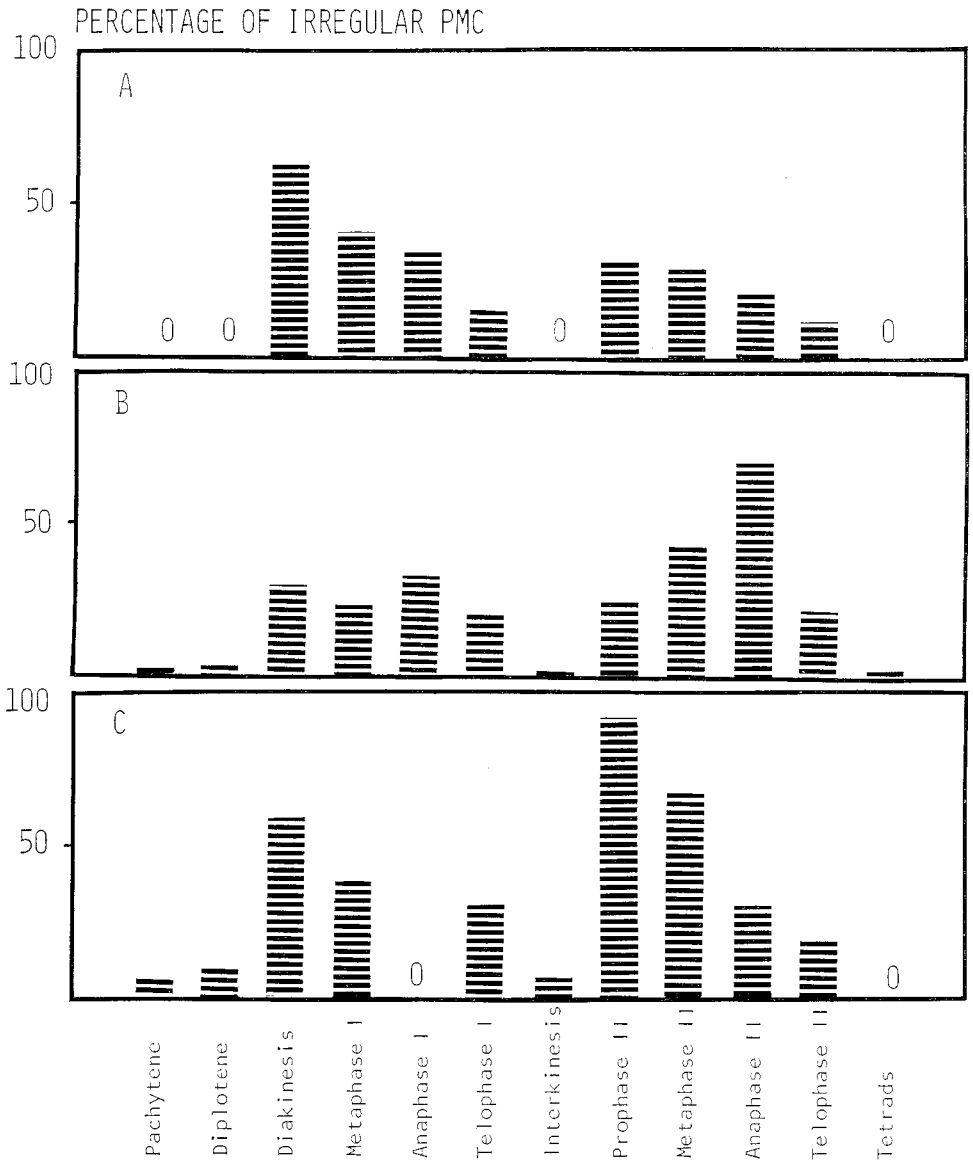

Figure 22. The percentage of irregular PMC of all PMC at particular stages before (A), during (B) and after (C) the treatment at −5°C.

nounced in diagrams A and B. In fixings evaluated in these diagrams metaphase I was the only sensitive stage reached by a large amount of PMC. As the frequency of metaphase I decreased, the irregularities were distributed more evenly among the other stages (diagram C).

The percentages of irregular PMC at different meiotic stages of all irregular PMC (Figure 21) are influenced by the frequency of individual stages. In Figure 22 three diagrams are presented in which the percentage of the irregular PMC of all PMC at each stage is calculated for the same fixing occasions as in Figure 21.

It can be seen that diakinesis—telophase I and prophase II—telophase II often suffer from irregularities, while pachytene,

diplotene, interkinesis and tetrads seem to be relatively insensitive stages. Irregularities at telophase I, metaphase II, anaphase II, and telophase II increased during the treatment at −5°C (diagram B). Surprisingly, the percentage of irregular metaphases I of all metaphases I decreased considerably during the treatment. The same was valid for diakinesis but in this case it can be explained by the fact that the percentages of irregularities varied strongly from date to date due to a sporadic occurrence of this stage.

Andersson (1974) found in PMC of Norway spruce that the meiotic stages most sensitive to low temperatures were metaphase I—anaphase I and metaphase II—anaphase II.

After changing of the temperature to +15°C/+10°C again the percentage of irregularities increased further in most stages, especially in prophase II and metaphase II (diagram C in Figure 22). The effect of the cold treatment was probably partially delayed. Another explanation might be that the abrupt change of temperature upwards has contributed to the increase in the amount of irregularities in the stages concerned. This change of temperature which amounted to 20°C, was accomplished in approximately four hours.

3.2.3 *The relationship between the occurrence of sensitive stages and irregularities*

It is known that meiosis is a very sensitive part of the generative development in conifers. However, the sensitivity of different meiotic stages varies. According to the results obtained by Eriksson (1968) in larch, some stages, for example diplotene, seemed to be insensitive to low temperatures. The frequency of irregularities induced would consequently be dependent on the frequency of sensitive stages during the exposure to low temperatures.

To study the effect of temperature on the occurrence of irregularities in PMC of Norway spruce, meiosis was divided into three parts (Eriksson *et al.* 1970 b). The first one included pachytene and diplotene which are relatively insensitive stages, the second one contained diakinesis—telophase I and prophase II—telophase II—*i.e.* all stages considered to be temperature sensitive and the third part consisted of tetrads with low sensitivity. In the present investigation the results were evaluated in a similar way except for the fact that the percentages were calculated individually for pachytene and diplotene.

The graphs illustrating the pattern of meiotic development and the percentages of irregular PMC in individual grafts are shown in Figures 23—29. With respect to the pattern of meiosis and the amount of irregularities two groups of grafts could be distinguished.

The first group consists of two grafts

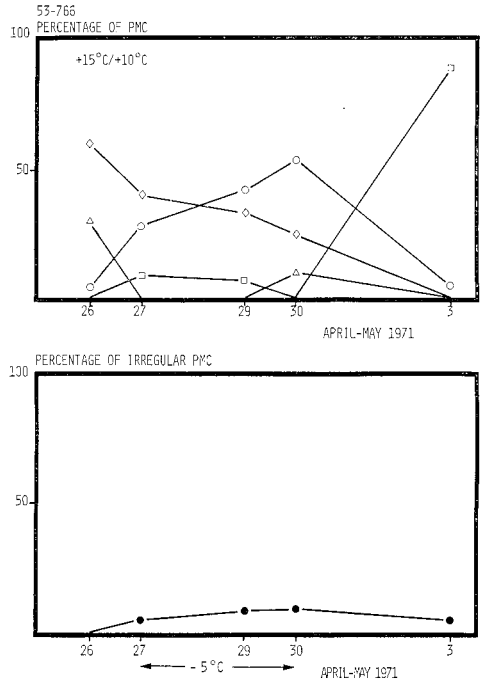

Figure 23. Graft 53-766. Above: The meiotic development during the third experiment (+15°C/+10°C with a three-day period at −5°C) illustrated by the percentage of PMC at different stages. Symbols: △ pachytene, ◇ diplotene, ○ diakinesis—telophase I and prophase II—telophase II, □ tetrad. Below: The total percentage of irregularities in approximately the same PMC.

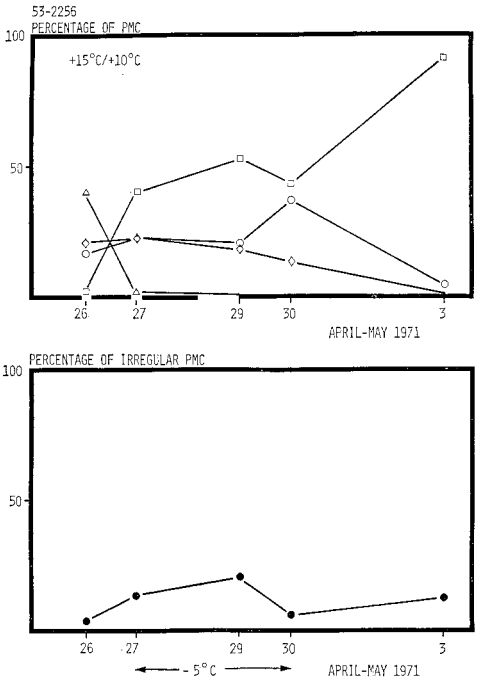

Figure 24. Graft 53-2256. Legend as in Figure 23.

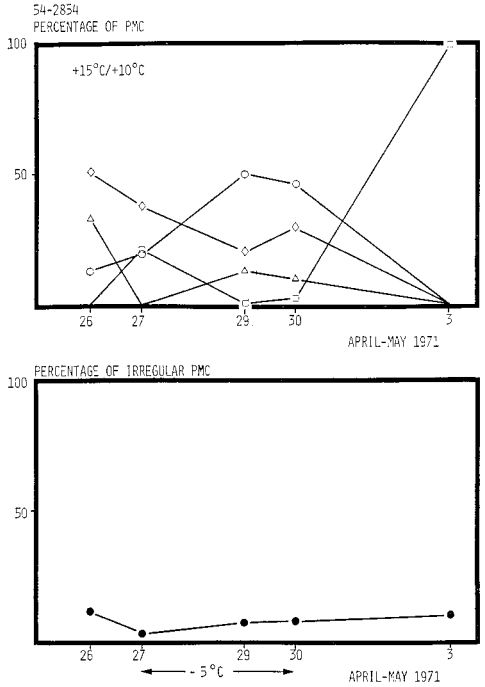

Figure 26. Graft 54-2854. Legend as in Figure 23.

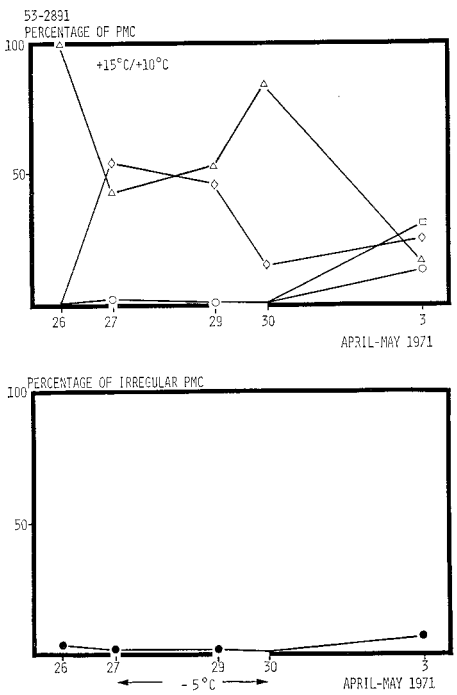

Figure 25. Graft 53-2891. Legend as in Figure 23.

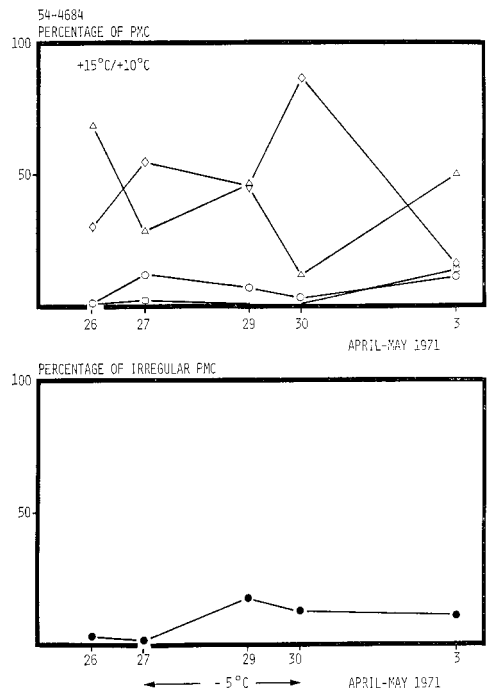

Figure 27. Graft 54-4684. Legend as in Figure 23.

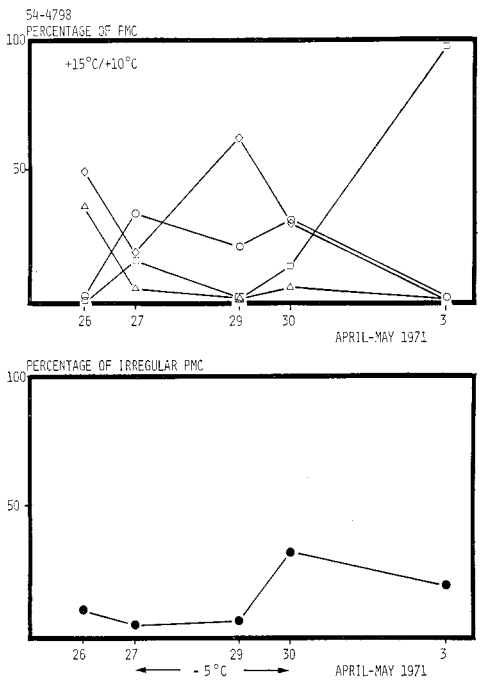

Figure 28. Graft 54-4798. Legend as in Figure 23.

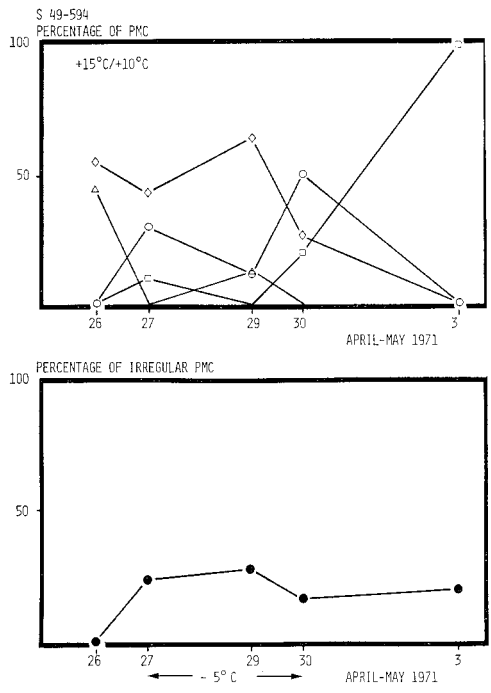

Figure 29. Graft S 49-594. Legend as in Figure 23.

(53-2891 and 54-4684) that differed from the others with respect to the rate of development. On April 30th almost all PMC in these grafts were still at pachytene or diplotene, in other words, very low percentages of PMC at sensitive stages were exposed to temperatures below zero (Figures 25 and 27). This was especially pronounced in graft 53-2891, in which hardly any sensitive stages were found during the cold treatment. As was expected the amount of irregular PMC was very low. In graft 54-4684 a somewhat higher percentage of irregularities (on April 29th and 30th) were partially caused by the occurrence of completely damaged strobili, i.e. strobili lacking regular PMC.

Following the start of the cold treatment the frequencies of pachytene and diplotene seemed to vary strongly between the fixing occasions. One gets the impression that in these grafts the cold treatment affected the pattern of meiotic development but hardly induced any irregularities. The grafts in the second group were more advanced than those in the first one. It means that rather high percentages of the sensitive stages were exposed to the temperature of $-5°C$. The response varied somewhat from graft to graft. For example, grafts 53-766 and 54-2854 (Figures 23 and 26) have conspicuously low percentages of irregularities despite the high amount of the sensitive stages exposed. A slightly higher frequency of irregularities was found in the graft 53-2256 (Figure 24). However, a rather large proportion (ten per cent) of irregularities found on April 29 was due to one completely damaged strobilus, i.e. it could not be related to the sensitive stages shown. (It was not possible to classify meiotic stages in completely damaged PMC.) The highest percentages of irregularities were obtained for grafts 54-4798 and S 49-594 (Figures 28 and 29). However, even in these grafts the frequency of irregular PMC would decrease if the completely damaged strobili were omitted in the calculation. Apparently the frequency of irregularities which could be related to the occurrence of PMC at sensitive stages was not very high.

3.2.4 *The frequency of irregularities in the grafts of different origin*

The percentages or irregular PMC in individual grafts are shown in Table 4. Since the material had been exposed to a low temperature outdoors (−7.8°C on April 24th, cf. Figure 20) some initial irregularities were found already in the first fixing.

As a measure of temperature sensitivity the increase of the percentage of irregularities after a cold treatment can be used. For each graft the average can be calculated on percentages of irregular PMC in fixings made during the treatment at −5°C (April 29th and 30th). The obtained figure should be compared with the percentage of irregularities found before the start of this treatment (April 27th). The problem is that an effect of the cold treatment can sometimes probably be delayed; in this way causing an increase of irregularities also after placing the material at an above zero temperature. Considering this the average percentage of irregularities induced at −5°C should be based on three fixing occasions (i.e. April 29th, 30th and May 3rd). On the other hand some irregularities can probably heal after exposure to a more favourable temperature (cf. Eriksson 1968). To use even the fixing on May 3rd for the calculation would consequently lead to the receiving of too low average percentages of irregularities induced by treatment.

Taking the above mentioned facts into account, two ways of calculating the increase of irregularities were used:

a) $\frac{\text{April 29th} + \text{April 30th}}{2} - \text{April 27th}$

b) $\frac{\text{April 29th} + \text{April 30th} + \text{May 3rd}}{3} - \text{April 27th}$

The dates indicate the fixings used for the calculation.

The data on the increase of the percentage of irregularities are presented in the last two columns in Table 4. The figures obtained in the two different ways of calculation differ somewhat. Evidently, the average percentage of irregularities during (and after) the treatment at −5°C is in most instances higher than the percentage of irregularities before the treatment. In other words, the amount of irregularities has increased in most instances following the treatment with −5°C. Especially high increases could be seen in the grafts 54-4684 and 54-4798 which are of the most southern origin. The latter already has a high amount of irregularities in the first fixing from the material outdoors. In one instance (graft 53-2256) the percentage of irregular PMC did not change, in another instance (graft

Table 4. The percentages of irregularities observed on different fixing occasions in individual grafts and the difference between the frequency of irregularities before and after the treatment at −5°C, calculated in two ways (a, b, cf. page 24).

Graft No.	Percentage of irregularities					Difference between the frequency of irregularities before and after treatment	
	April 26	April 27	April 29[a]	April 30[a]	May 3	a	b
53-766	0	5.0	8.6	9.0	5.2	3.8	2.6
53-2256	2.4	12.4	19.6	5.1	13.4	0	0.3
53-2891	3.2	0.50	0.40	0	6.6	—0.3	1.8
54-2854	11.1	2.5	6.7	7.9	10.0	4.8	5.7
54-4684	2.4	1.5	17.6	11.6	10.4	13.1	11.7
54-4798	10.0	5.4	6.0	32.7	20.0	14.0	14.2
S 49-594	0.10	23.3	28.0	16.4	20.0	—1.1	—1.8

[a] Fixings made during the treatment at −5°C

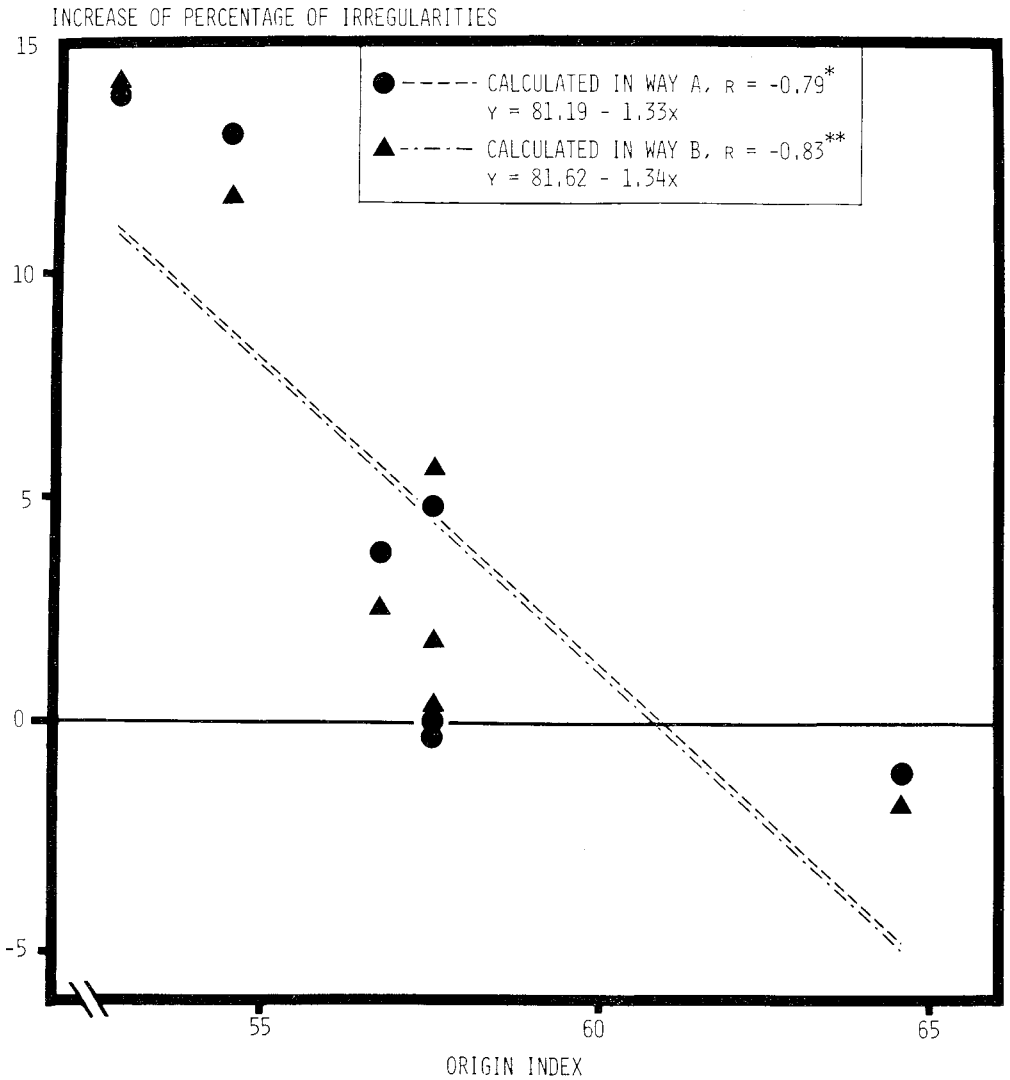

Figure 30. The regression of the increase in the frequency of irregularities after the treatment at −5°C (calculated in two ways—cf. page 24) on the origin index of grafts.

S 49-594) the percentage decreased slightly after the cold treatment. This might be due to the high amounts of irregular PMC found in the above mentioned grafts (53-2256 and S 49-594) just before the start of the treatment at −5°C.

To decide if a relationship exists between the increase of irregularities following the treatment at −5°C and the origin of the grafts, correlation coefficients were calculated on the basis of the figures obtained in the two ways (a, b). The regression lines were declining (cf. Figure 30), and the correlation coefficients were $r = -0.79^*$ and $r = -0.83^{**}$.

To sum up, the grafts with a high origin index (severe climatic conditions at their origin habitats) were characterized by a lesser increase of irregularities following the cold treatment than grafts with a low origin index.

4 Concluding remarks

4.1 The effect of the temperature on the pattern of meiotic development in pollen mother cells (PMC) of Norway spruce

The PMC were exposed to two different temperatures (thermoperiods +20°C/+15°C and +15°C/+10°C). The cumulative percentages of PMC at diplotene, interkinesis and the tetrad stage were calculated for various fixing occasions and plotted against time and temperature sum respectively.

As illustrated in Figure 13, both time and temperature sums needed for the development of three phases of meiosis (I—III) were determined, and the slopes of the diplotene, interkinesis and tetrad curves (1—3) were computed. The results obtained in the two different temperature conditions were compared with analyses of variance (cf. Table 2).

With one exception no significant differences between the grafts were obtained (cf. Table 2).

When comparing different phases of meiosis it was found that the temperature response of PMC varied from phase to phase. The early phases were accelerated by higher temperature which is evident from the left side of Figure 31. This tendency was slight as regards reaching the *diplotene* stage and very strong during the development from *diplotene to interkinesis*. The latter phase seemed to be the most readily accelerated by the higher temperature.

The development from *diplotene to tetrads* was also found to proceed faster at the higher temperature. The difference in the time needed for this development at the two temperatures was, however, smaller than for the *diplotene—interkinesis* development. This might be explained by the fact that the temperature response of PMC seemed to be changed during the second meiotic division.

The development of the three remaining phases studied (the reaching of *interkinesis, interkinesis—tetrads,* the reaching of *tetrads*) was not accelerated by the higher temperature. On the contrary, the development seemed to proceed faster at the lower temperature. This tendency became most pronounced at the end of the meiosis. Thus, the difference in the rate of the reaching of the tetrad stage was highly significant.

It seems as if the ability of PMC to respond to a rise of temperature by accelerating the meiotic development was large at first but declined with time. Approximately at the *interkinesis* stage the development started to proceed faster at the lower temperature. One explanation for this might be that the negative effect of forcing the twigs under artificial conditions probably increases with time and is greater at the higher temperature. In such a case the temperature response of PMC would change with time irrespective of the phase. Another explanation, based on the fact that some phases differed in temperature response despite overlapping each other to a great extent might be that the temperature response of PMC is specific to particular phases.

On the right side of the diagram in Figure 31 it can be seen that the phases of meiosis which were accelerated significantly by the higher temperature (*diplotene—interkinesis, diplotene—tetrads*) also showed a lower temperature sum requirement at this temperature. Thus, the reason for the acceleration seemed to be not only that a larger temperature sum was obtained per time unit but also a larger effect of a temperature sum at the higher temperature. For the phase *diplotene—tetrads* the best agreement was

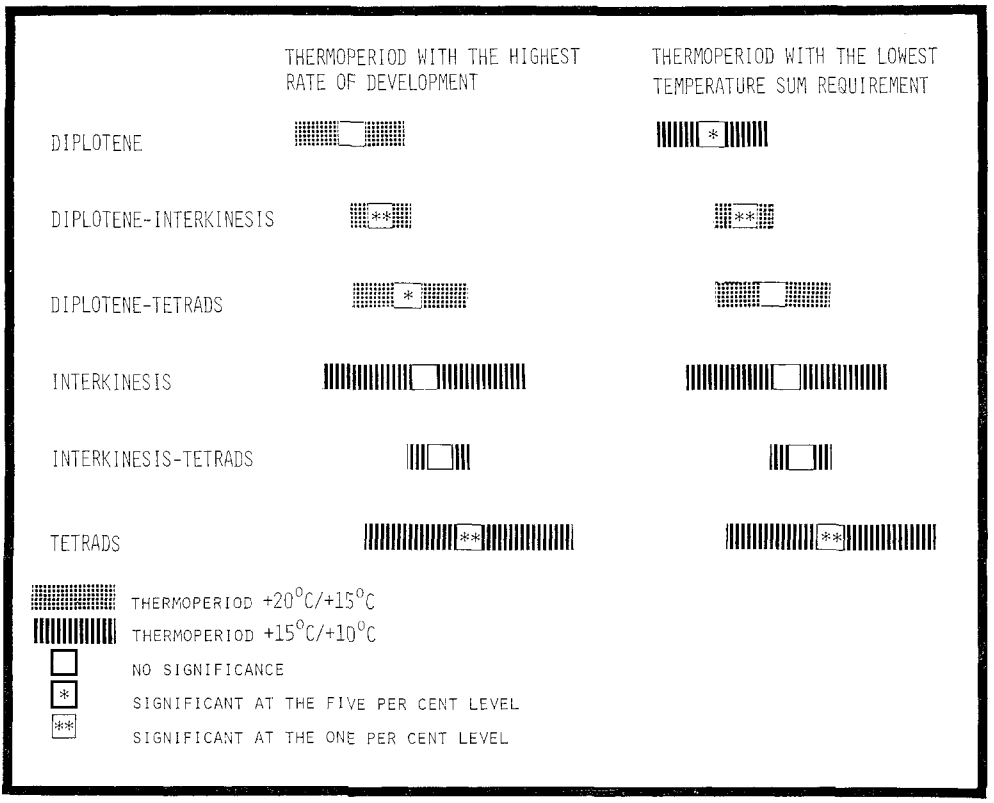

Figure 31. An outline showing thermoperiods with the highest rate of development and the lowest temperature sum requirement respectively. The columns illustrate in a schematic manner the duration and relative position of the different phases of development studied.

obtained with the expected situation, namely a higher rate of development at the higher temperature, but the same temperature sum required for the development in both thermoperiods.

The exposure of the material to −5°C for three days caused a delay in the meiotic development.

4.2 Irregularities in PMC induced by cold treatment

The most common types of irregularity revealed were stickiness C and degeneration (cf. Eriksson 1968). The irregularities observed before the exposure of the PMC to −5°C, were mainly stickiness of the C type. At the same time as the more severe damage—degeneration—increased in frequency due to the cold treatment, the proportion of stickiness C decreased. However, the total amount of irregular PMC increased following exposure to −5°C.

The meiotic stages showing the largest increase of irregularities during treatment at −5°C were telophase I, metaphase II, anaphase II and telophase II. The percentage of irregular metaphases I of all PMC at this stage decreased during treatment.

The amount of PMC at the sensitive stages was often rather high during the cold treatment. In spite of this, the frequency of irregularities was relatively low. Some irregularities were attributed to completely damaged strobili, in which the classification of meiotic stages could not be carried out.

A negative correlation between the origin

index (cf. page 16) of the grafts and the frequency of irregularities induced by cold treatment was revealed. In other words, the larger the origin index of the grafts (the more severe climatic conditions at their origin growth habitats) the lesser was the increase of irregularities following cold treatment.

5 Acknowledgements

Finally, I wish to express my thanks to all who have helped me during the course of preparing this paper.

My most sincere thanks are due to Associate Professor Gösta Eriksson for his encouragement and valuable advice. I am also grateful to Inger Ekberg, Fil. Lic., for stimulating discussions. I would also like to thank Professors Enar Andersson and Åke Gustafsson for the critical reading of the manuscript. For technical assistance I thank Mr Kjell Lännerholm and Mr Savvas Kymmas.

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6 Sammanfattning

Kvistar från granympar av olika provenienser intogs för drivning i klimatskåp. Syftet med försöken var att under kontrollerade betingelser studera temperaturens inverkan på meiosförloppet och uppkomst av oregelbundenheter i pollenmoderceller (PMC) hos gran.

6.1 Temperaturens inverkan på meiosförloppet i PMC

Meiosförloppet studerades vid två olika temperaturer (termoperioder +20°C/+15°C och +15°C/+10°C, se Figur 1). Utvecklingsförloppet hos de enskilda ymparna illustrerades med hjälp av kumulativa procent av PMC i diploten, interkines och tetradstadiet, avsatta mot tiden respektive temperatursumman (Figur 2—10). Enligt de principer som åskådliggjorts i Figur 13 avlästes tiden respektive temperatursumman som behövdes för utvecklingen inom de angivna intervallen (I—III), och kurvornas lutningskoefficienter (1—3) beräknades. Uppgifter erhållna för olika temperaturförhållanden jämfördes med hjälp av variansanalyser (jfr Tabell 2).

Med ett undantag har inga signifikanta skillnader mellan ympar erhållits (Tabell 2).

PMC's reaktion på en höjning av temperaturen varierade under meiosförloppet. Under de tidiga delarna av meiosen accelererade utvecklingen vid den högre temperaturen (se vänstra sidan av Figur 31).

Denna tendens var märkbar redan i fråga om uppnåendet av *diploten* men starkt utpräglad blev den först under utvecklingen *diploten—interkines*. Utvecklingshastigheten tycktes vara lättast att påverka med den högre temperaturen under denna del av meiosen.

Även utvecklingen från *diploten till tetradstadiet* gick snabbare vid den högre temperaturen men skillnaden i tiden som behövdes för denna utveckling i de två temperaturerna var mindre än för utvecklingen från *diploten till interkines*. Detta kunde ha orsakats av att PMC's reaktion på temperaturhöjning blev förändrad under andra meiosdelningen.

Utvecklingen under de tre återstående delar av meiosen som studerades (uppnåendet av *interkines, interkines—tetradstadiet* och uppnåendet av *tetrader)* accelererades inte av den högre temperaturen. Tvärtom, tycktes utvecklingen gå snabbare i den lägre temperaturen. Denna tendens blev mest uttalad mot slutet av meiosen; sålunda var skillnaderna i utvecklingshastigheten under uppnåendet av tetradstadiet starkt signifikanta.

Det verkar som om PMC's förmåga att reagera på ökad temperatur genom en högre utvecklingshastighet är stor i början men avtar med tiden. En kontinuerlig förändring synes föreligga. Omslagspunkten inträder ungefär under *interkinesstadiet* där utvecklingen börjar att gå något fortare vid den lägre temperaturen. En förklaring till detta kan vara att de negativa verkningarna av drivningen under artificiella förhållanden som antagligen förstärks med tiden gjorde sig mera gällande vid den högre temperaturen. Om detta är fallet skulle PMC's reaktion på temperatur förändras under drivning i klimatskåpet med tiden, oberoende av vilken del av meiosen det är fråga om. En annan förklaring, grundad på det faktum att PMC reagerar olika under delar av meiosen som till en hög grad överlappar varandra, kan vara att PMC's reaktion är karakteristisk för enskilda delar av meiosen.

På högra sidan av diagrammet i Figur 31 kan man se att de delar av meiosförloppet som accelererades signifikant vid den högre temperaturen (*diploten—interkines, diplo-*

ten—tetrader) hade även lägsta behov av temperatursumma vid denna temperatur. Orsaken till den konstaterade högre utvecklingshastigheten tycks alltså vara inte enbart större temperatursumma erhållen per tidsenhet utan också större effekt av temperatursumman vid den högre temperaturen.

Förhållandena under utvecklingen från *diploten till tetradstadiet* liknade mest de väntade, nämligen en högre utvecklingshastighet vid den högre temperaturen och behovet av samma temperatursumma för utvecklingen i båda temperaturer.

Exponering av materialet till −5°C under tre dagar förorsakade en fördröjning av meiosförloppet.

6.2 Temperaturbetingade oregelbundenheter i PMC

De oftast förekommande typerna av oregelbundenheter var stickiness av C-typ och degeneration (jfr Eriksson 1968). Den vanligaste skadetypen, som registrerades strax innan exponeringen till −5°C började, utgjordes av stickiness C. Samtidigt som en mera grav skada — degeneration — ökade i frekvens till följd av köldbehandlingen, minskade proportionen av stickiness C. Det totala antalet oregelbundna PMC ökade under köldbehandlingen. Denna åstadkom den största ökningen av oregelbundenheter i följande stadier: telofas I, metafas II, anafas II och telofas II. Däremot minskade procenttalet oregelbundna PMC i metafas I.

Antalet PMC i känsliga stadier var ofta högt under köldbehandlingen. Trots detta var frekvensen av de oregelbundenheter som kunde tillskrivas den redovisade förekomsten av känsliga stadier relativt låg.

En negativ korrelation har erhållits mellan ymparnas ursprungsindex (se s. 16) och frekvensen oregelbundenheter orsakade av behandling vid −5°C. Med andra ord, ju högre ursprungsindex (ju hårdare klimatförhållanden på ursprungslokalen) desto lägre var frekvensen av de temperaturbetingade oregelbundenheterna.

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