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Low-temperature induced irregularities in pollen mother cells of *Larix leptolepis*

Temperaturinducerade oregelbundenheter hos pollenmoderceller av japansk lärk

by

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ABSTRACT

The communication constitutes a complement to a preceding one in which the relationship between low temperature and sensitive PMC on one hand and induction of irregularities on the other hand was presented for *Larix decidua* and *L. sibirica*. Such an investigation has now been undertaken for *Larix leptolepis*. Stickiness of C-type was the most common irregularity followed by stickiness of B-type and A-type. Several different relationships between prediction areas and aberration areas were tested. It was observed that the best agreement was obtained when diakinesis, metaphase I and II and anaphase I were regarded as the temperature sensitive stages. The exclusion of the completely damaged buds from the aberration areas increased the agreements between aberration areas and prediction areas.

Different mechanisms of the temperature action on the PMC are discussed. Based on the data presented, the design of an experiment which could reveal the mechanism of the temperature action on the PMC is suggested.

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1. Introduction

In a previous paper (Eriksson, 1968) the relationship between irregularities on one hand and sensitive pollen mother cells (PMC) and low temperatures on the other was demonstrated for *Larix decidua* and *Larix sibirica*. In that paper different modes of action of the temperature were discussed. To evaluate in which way the temperature influences the induction of irregularities it is necessary to have data collected from a well designed experiment under strictly controlled conditions. Eriksson (1968) pointed out that the design of the experiments performed under controlled conditions should be preceded by less expensive experiments carried out on material growing outdoors. Therefore, it would be valuable to make a preliminary investigation of the relationship between low temperature and sensitive cells in out-door cultivated *Larix leptolepis* as a complement to the corresponding investigation presented for *Larix decidua* and *L. sibirica* by Eriksson (1968). In the present communication the data from an investigation of the meiotic development and the occurrence of irregularities in 11 Japanese larch grafts growing at Röskär outside Stockholm will be presented and discussed.

2. Material and methods

The Japanese larch clones included in the present investigation were the same as listed in Table 5 in the paper by Eriksson (1968). The origin of these clones was also presented in this table. The fixation intervals were selected according to the suggestions derived from the temperature data. At least 100 buds from each graft were collected. The buds were collected from different growth positions in the grafts. Acetic alcohol (1:3) served as a fixative. The fixation of pollen in 70 per cent alcohol took place just before the anthers were ready to dehisce.

From each male bud, PMC from at least three stamens were dissected in acetic orcein. The PMC were mixed carefully and 100 PMC were classified according to stage of development and type of irregularity.

The pollen sterility was estimated by staining the pollen grains in a solution of methylene blue. The buds from each graft were tested with respect to pollen sterility, from each bud 100 pollen grains were examined.

The temperature data was obtained from the meteorological station at Röskär situated 500 m from the growth locality of the Japanese larch grafts.

3. Results and discussion

The pattern of development from diplotene to tetrads in the different grafts is demonstrated in Fig. 1—11. In these diagrams the percentage of PMC in the stages diakinesis—anaphase I and metaphase II is shown. These stages were the most frost sensitive ones according to the data presented below. Besides this the percentage of PMC in diplotene and the tetrad stage is demonstrated. A discussion of the pattern of the meiotic development in these grafts was carried out by Eriksson *et al.* (1970).

The total percentage of irregularities is also demonstrated in Fig. 1—11. Besides this the percentage of completely damaged buds is shown. More than 150,000 PMC were examined with respect to occurrence of irregularities. The irregularities were classified according to the scheme suggested by Eriksson (1968).

The maximum and minimum temperature curves during the time for the appearance of sensitive PMC are demonstrated in Fig. 12.

To get a general information about the relative occurrence of various types of irregularity the number of PMC belonging to different categories of irregularity has been summed for each graft separately (cf. Table 1). This table reveals that stickiness of C-type was the dominating irregularity. Stickiness of A- and B-type were also of a frequent occurrence whereas polyspory and univalents were almost completely lacking. Therefore, it could be stated that most of the irregularities was of a mild character which suggests the possibility of healing of them to a great extent.

If the curves for the total percentage of irregularities are examined it is seen that the irregularities mostly disappeared when the tetrad stage was reached by all PMC. This could be due to healing to a great extent of the cells which showed stickiness of C-type. Another explanation might be that the irregularities could not be detected during the tetrad stage. This is contradicted by the fact that the pollen

Table 1. The total number of normal PMC and irregular PMC belonging to various types of irregularity in the different grafts.

Graft	Normal PMC	Irregular PMC									
		Stickiness				Fragmentation	Degeneration	Univalents	Micronuclei	Spindle abnormalities	Polyspory
		A	B	C	Bridges						
AZU R7	10,804	300	780	1948	35	4	28	1			
AZU R8	14,058		972	1268	14	1	87				
NAR 18	10,351	100	38	963	33	9	6				
NAR 19	12,791		335	1413	41	8	9		3		
NAR J16	11,686		439	1235	43	7	89		1		
NAR J20	12,080	100	65	2002	36	1	16				
REN L7	15,094	100	64	777	49	16					
TAK P4	9,932	800	700	1306	50	6	61	1	2	36	6
TAK P7	16,854	200	687	1988	38	5	26		1	1	
TAK Q19	13,217	529	1239	994	24		5		90	1	1
YATSU S4	9,464	400	858	1358	17	3					

sterility was low mostly not exceeding five per cent. On the other hand it might be suggested that the irregularities were hidden in the pollen grains as well.

Still another explanation for the disappearance of the irregularities could be that the stickiness of C-type was due to artefacts owing to fixing of the buds in a fixative of low temperature. However, the fixing solution was not of the same low temperature as the air temperature since the solutions were kept in a car until the time for the fixation. Mostly the fixing solutions were not exposed to the air temperature for more than 15 minutes. Furthermore, it might be mentioned that buds taken directly to the laboratory for analysis revealed the same types of irregularity as the fixed buds. Therefore, the probability that stickiness of C-type originates from artefacts must be regarded as low.

The concepts of aberration area and prediction area were discussed in detail in the paper by Eriksson (1968). In similarity with the situation in *Larix decidua* and *L. leptolepis* an evaluation of the relationship between irregularities on one hand and sensitive cells and low temperature on the other hand must be analysed by plotting aberration areas against prediction areas (cf. Eriksson, 1968).

Two different aberration areas were calculated for each graft, the first comprising all types of irregularity whereas the second comprised all types of irregularity except for the completely damaged buds.

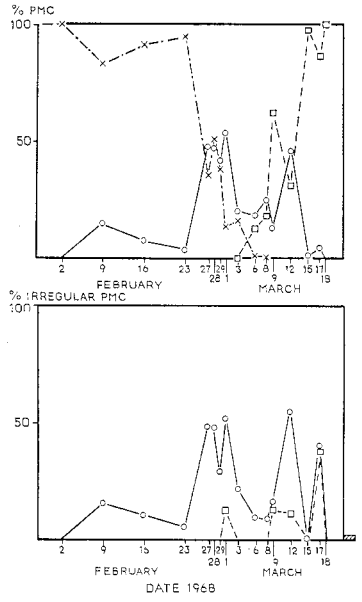

Fig. 1. Clone Azu R 7, *L. leptolepis*.

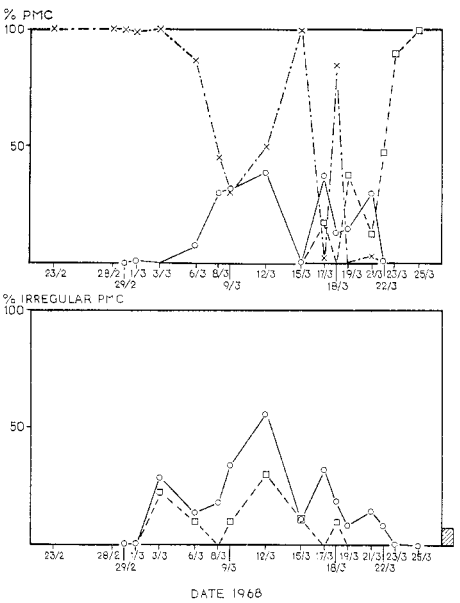

Fig. 2. Clone Azu R 8, *L. leptolepis*.

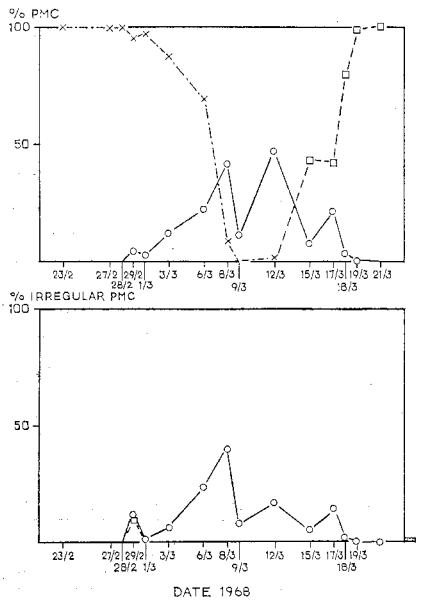

Fig. 3. Clone Nar I 8, *L. leptolepis*.

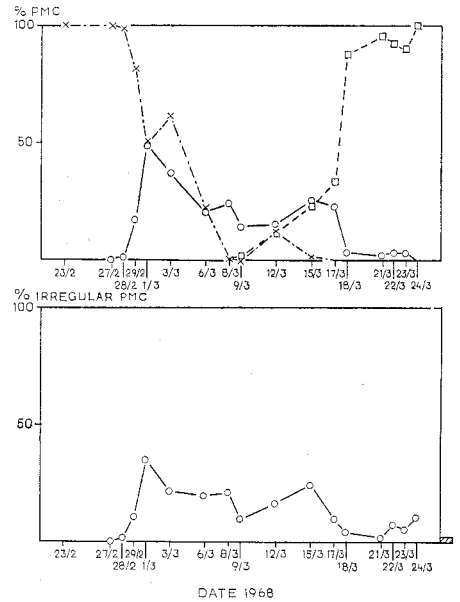

Fig. 4. Clone Nar I 9, *L. leptolepis*.

Fig. 1—4. *Above:* The meiotic development of the PMC. × = % PMC in diplotene, ○ = % PMC in diakinesis — anaphase I + metaphase II, □ = % PMC which has reached the tetrad stage.
Below: The total percentage of irregularities (○) and the percentage of completely damaged buds (□). The hatched columns refer to the pollen sterility.

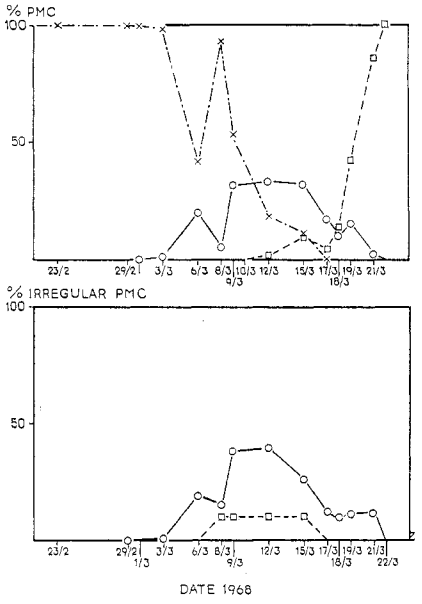

Fig. 5. Clone Nar J 16, *L. leptolepis*.

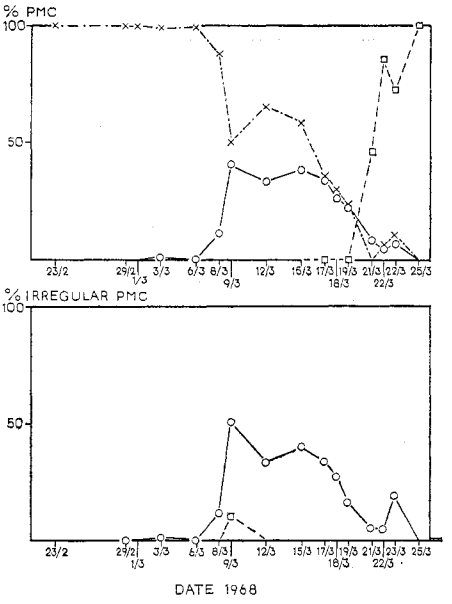

Fig. 6. Clone Nar J 20, *L. leptolepis*.

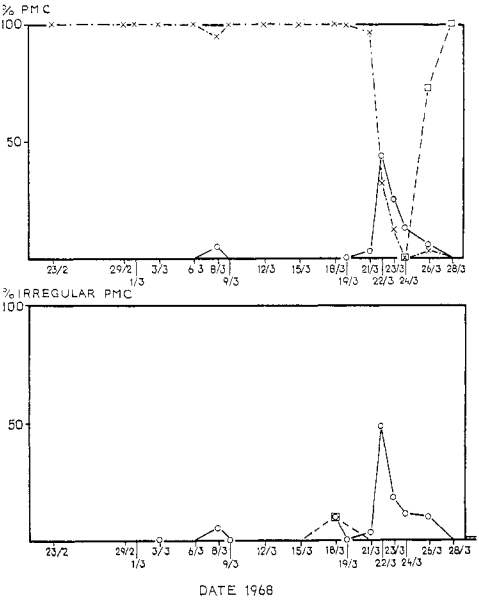

Fig. 7. Clone Ren L 7, *L. leptolepis*.

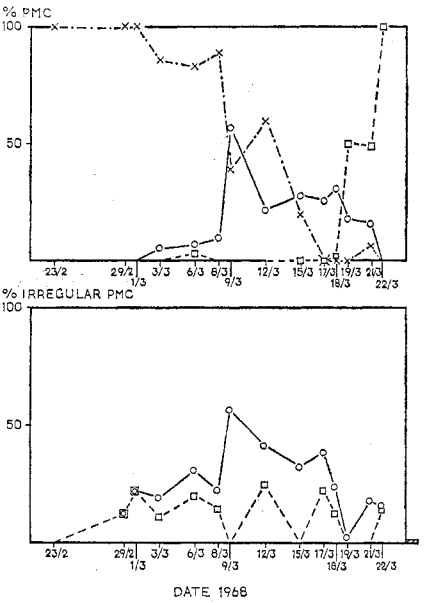

Fig. 8. Clone Tak P 4, *L. leptolepis*.

Fig. 5—8. *Above:* The meiotic development of the PMC. × = % PMC in diplotene, ○ = % PMC in diakinesis — anaphase I + metaphase II, □ = % PMC which has reached the tetrad stage.
Below: The total percentage of irregularities (○) and the percentage of completely damaged buds (□). The hatched columns refer to the pollen sterility.

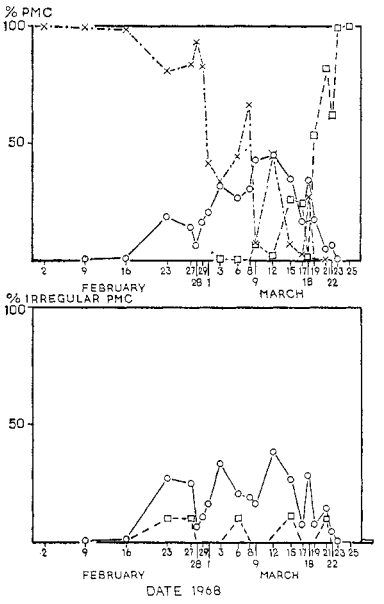

Fig. 9. Clone Tak P 7, *L. leptolepis.*

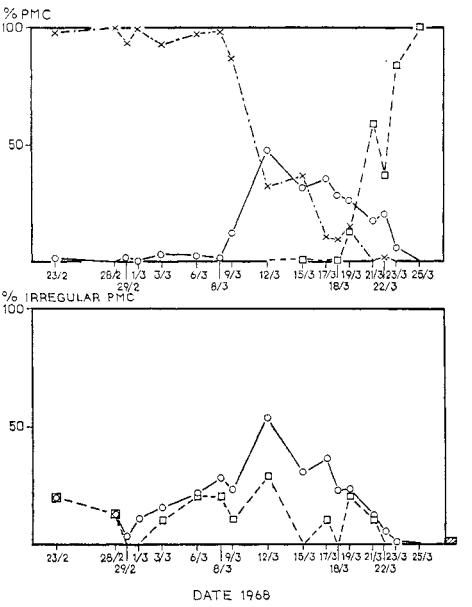

Fig. 10. Clone Tak Q 19, *L. leptolepis.*

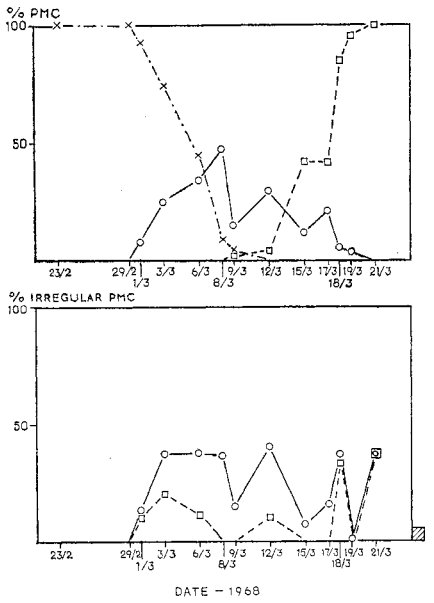

Fig. 11. Clone Yatsu S 4, *L. leptolepis.*

Fig. 9—11. *Above:* The meiotic development of the PMC. × = % PMC in diplotene, ○ = % PMC in diakinesis — anaphase I + metaphase II, □ = % PMC which has reached the tetrad stage.
Below: The total percentage of irregularities (○) and the percentage of completely damaged buds (□). The hatched columns refer to the pollen sterility.

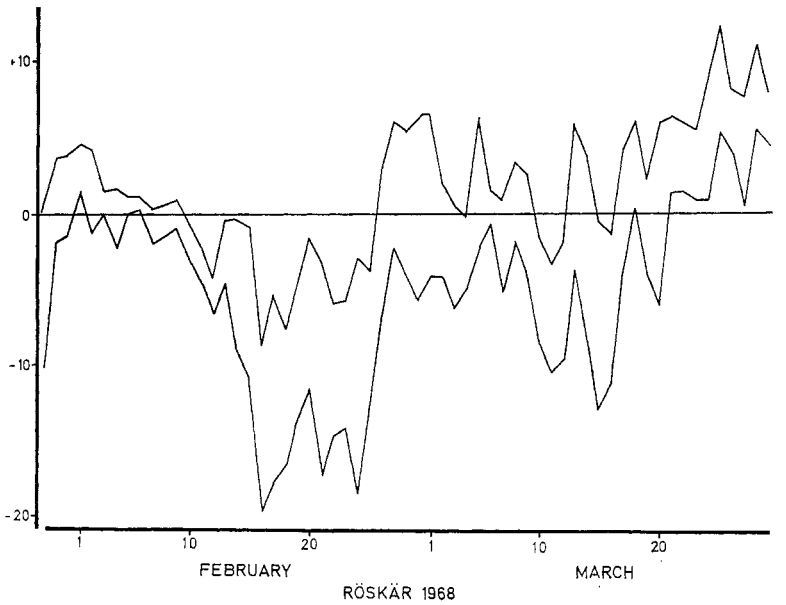

Fig. 12. The maximum and minimum temperature curves at Röskär from 29 January to 28 March 1968.

The reason for excluding those buds from the aberration area was that their origin is not yet completely understood (cf. Eriksson *et al.*, 1970). Furthermore, it is probable that the induction mechanism is different in the PMC of the completely damaged buds on one hand and the PMC showing irregularities like stickiness of C-type on the other hand. Thus, completely damaged buds sometimes occurred before any sensitive PMC had been detected (cf. Fig. 8 and 10).

Several different relationships between prediction areas and aberration areas were tested graphically, some of which were of such a poor appearance that no numerical calculations were needed to disclose an agreement between prediction area and aberration area. In this connection it is worth mentioning that all relationships, where the completely damaged buds were tested separately were of this type.

In Table 2 the relationships tested numerically have been compiled. The data in this table reveal that the variance ratio varied considerably. The relationships 1 (the poorest one) and 6 (the best one) in Table 2 are graphically demonstrated in Fig. 13—14. From Table 2 it may be seen that the best agreement is obtained when the aberration

Table 2. Regressions in *L. leptolepis* of aberration area on sensitive cells × low temperatures as well as regressions of aberration area on areas of sensitive cells.

No.	Relationship studied		Variance ratio (F) for testing regression
1	Total aberration area	— % cells (diak. — A I) × minus degrees below — 2°C	8.74*
2	Aberration area except for completely damaged buds	— % cells (diak. — A I) × minus degrees below — 2° C	11.4 **
3	Total aberration area	— % cells (diak., MI, AI, MII) × minus degrees below — 2°C	15.5 **
4	Aberration area except for completely damaged buds	— ditto	30.2 ***
5	Total aberration area	— % cells (diak., MI, AI, MII) at minus degrees below — 2°C	18.8 **
6	Aberration area except for completely damaged buds	— ditto	53.8 ***
7	Total aberration area	— % cells (diak. — AI) at minus degrees below — 2°C	15.0 **
8	Aberration area except for completely damaged buds	— ditto	32.8 ***

area is tested against prediction areas comprising diakinesis, M I, A I and M II as the sensitive stages. The high sensitivity of these stages agrees with the observations of the frost sensitivity in PMC of Norway spruce (Andersson, 1970; Ekberg *et al.*, 1970). The inclusion of the completely damaged buds into the aberration area resulted in poorer relationships than when the completely damaged buds were excluded from the aberration area.

A few hypothetical examples of the amount of irregularity expected following different mechanisms of the temperature action on the PMC are illustrated in Fig. 15. The mechanisms are rather simple in these examples, a linear (A) or an exponential increase (D) of the amount of irregularity by the temperature or (B and C) a linear increase in a certain temperature range and a temperature independent induction of irregularities in another temperature range. Still more complicated mechanisms of the temperature action on the PMC can be suggested by combining the schemes followed by the different types of irregularity in Fig. 15.

As pointed out previously (Eriksson, 1968) it is not believed that an exact temperature response of the PMC can be revealed in

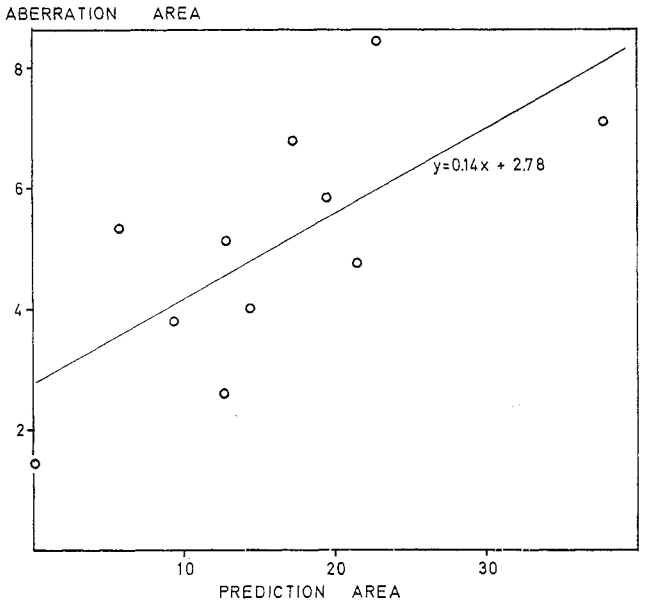

Fig. 13. The relationship between aberration area and prediction area. The aberration area comprises all irregularities observed. The prediction area was calculated from the percentage of cells in diakinesis — anaphase I at temperatures below —2°C. The linear regression demonstrated refers to No. 1 in Table 2.

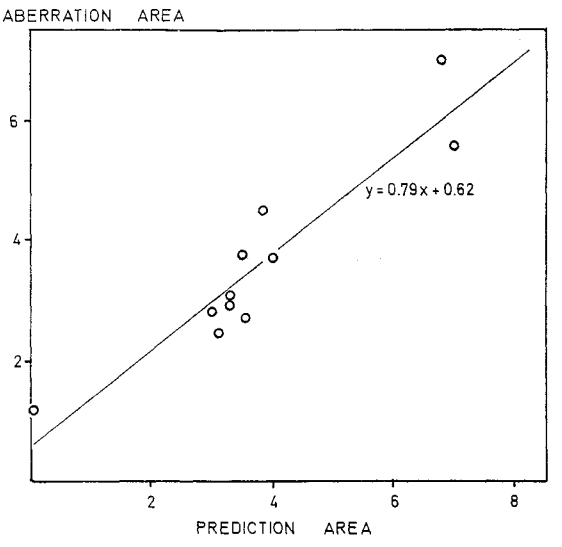

Fig. 14. The relationship between aberration area and prediction area. The aberration area does not comprise the irregularities observed in the completely damaged buds. The prediction area was calculated from the percentage of cells in diakinesis — anaphase I + metaphase II at temperatures below —2°C. The linear regression demonstrated refers to No. 6 in Table 2.

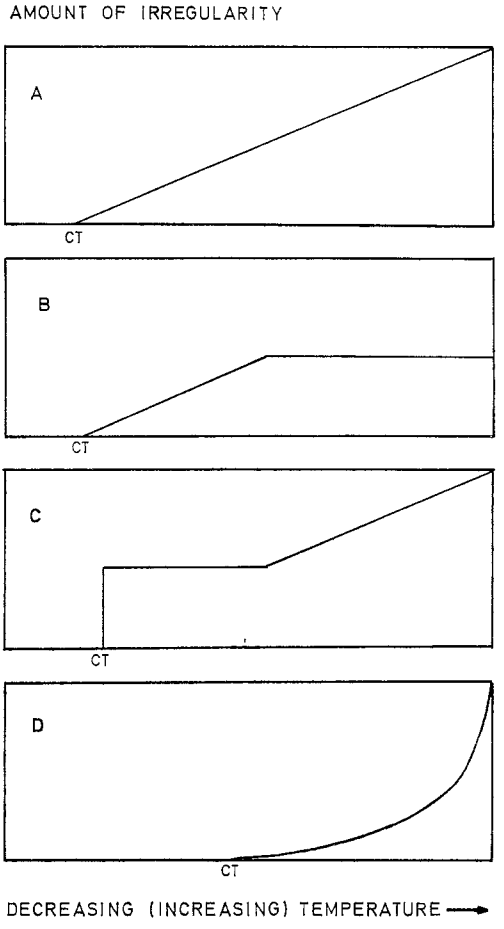

Fig. 15. In the diagram the temperature influence upon the induction of irregularities is demonstrated. In this connection it is worth pointing out that not only low but also high temperatures might provoke irregularities in the PMC (cf. Andersson, 1970; Chira, 1965). CT = critical temperature, which means the temperature below (above) which irregularities are induced. A—D could be regarded as different types of irregularity. For types A and B the critical temperature was assumed to be the same. For A there is a linear increase of the temperature effect throughout the whole temperature range whereas there is a linear increase within a certain temperature range for type B. For type B there is a threshold level, below (above) this level all temperatures provoke an effect independent of how low (high) the temperature is. For type C there is a temperature independent induction of irregularities just below (above) the critical temperature followed by a linear increase at lower (higher) temperatures. For type D it was assumed that the temperature effect increases exponentially.

experiments of the present design. The exact temperature response of the PMC can only be obtained from experiments carried out during well defined temperature conditions. At the moment the information needed for the design of experiments which could reveal the mechanism of the temperature action (cf. Fig. 15) have become available. The following procedure of such an experiment is suggested:

1. PMC in diplotene are exposed to low temperature in order to break the dormancy.

2. Subsequently these PMC are exposed to + 5° C to provoke an initiation of further development from diplotene, thereby sensitive PMC appear.

3. The sensitive PMC should be exposed to − 1° C, − 2° C, − 3° C, − 4° C, − 5° C, − 6° C for 1, 2, 3 or 4 days at each temperature level.

From such an experiment it will be possible to obtain information concerning:

A. the critical temperature for induction of various types of irregularity,

B. the exposure time needed for induction of various types of irregularity,

C. the action mechanism of the temperature, whether the temperature effect is linear or exponential or if there is a threshold effect (cf. B and C in Fig. 15).

Based on the data from the experiment outlined above another experiment could be planned to obtain a complete understanding of the temperature response of the PMC.

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Sammanfattning

Temperaturinducerade oregelbundenheter hos pollenmoderceller av japansk lärk

Den presenterade undersökningen utgör ett komplement till en tidigare studie av sambandet mellan låga temperaturer och känsliga celler å ena sidan samt induktion av oregelbundenheter å den andra sidan hos *Larix decidua* och *L. sibirica*. Denna undersökning har nu utsträckts till att även omfatta *Larix leptolepis*. Stickiness av C-typ var den vanligast förekommande oregelbundenheten följd av stickiness av B-typ och A-typ. Flera olika samband mellan »prediction areas» och »aberration areas» har testats (Tab. 2 och Fig. 13—14). (Begreppen »prediction area» och »aberration area» har utvecklats i en tidigare uppsats, Eriksson, 1968.) De bästa sambanden erhölls när diakines, metafas I och II samt anafas I betraktades som de temperaturkänsliga stadierna. Då de fullständigt skadade knopparna uteslöts ur aberrationsytan erhölls bättre samband mellan »prediction areas» och »aberration areas».

Olika mekanismer hos temperaturverkan på pollenmodercellerna diskuterades med utgångspunkt från Fig. 15. Med ledning av erhållna data framlades ett förslag till utformning av ett experiment för en fullständig förståelse av temperaturens inverkan på pollenmodercellerna. Dylika experiment kan givetvis endast genomföras på material odlat under noggrann temperaturkontroll.