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Temperature response of pollen mother cells in *Larix* and its importance for pollen formation

Temperaturens betydelse för pollenbildningen hos lärk

by

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Introduction

Owing to the large size of the chromosomes in the conifers they have constituted good research objects for cytologists. This is verified in the review by KHOSHOO (1961) where numerous references to investigations of diversified design will be found. Karyotype analysis has been one of the main interests in these investigations. SIMAK (1962, 1964) studying the karyotype in *Larix*, revealed a difference between *L. decidua* and *L. sibirica*. Thus, chromosome IV in *L. decidua* has a hardly visible tertiary constriction whereas this chromosome contains a secondary constriction in *L. sibirica*. On the other hand, *L. decidua* has a secondary constriction in chromosome VII which is not present in *L. sibirica*. Both species are identical with respect to chromosome length and centromere index.

Of special interest in the study of the evolution of the karyotype are the studies by HAIR and BEUZENBERG (1958) of the genus *Podocarpus*. The haploid chromosome number within this genus varies from 10 to 19. Some species contain mainly telocentric chromosomes, whereas metacentric chromosomes predominate in others. However, the number of chromosomal arms seems to be constant (= 20), which suggests that the telocentric chromosomes have arisen by splitting of the metacentric chromosomes or *vice versa*, i.e. the metacentric have arisen by fusion of the telocentric chromosomes. If any of these hypotheses were true, trivalents would be expected to occur during meiosis in the hybrids between species having different numbers of meta- and telocentric chromosomes. This was actually observed by HAIR and BEUZENBERG in the cross between *Podocarpus nivalis* and *Podocarpus hallii*. The first species contains 18 telocentric and 1 metacentric chromosome and the second one contains 14 telocentric and 3 metacentric ones.

Meiosis in PMC (pollen mother cells) of established or presumed species hybrids has frequently been described in the literature (SAX 1932; HIRAYOSHI *et al.* 1943; ROSS and DUNCAN 1949; VIDAKOVIĆ 1958; SAX 1960; SAYLOR and SMITH 1966; SAYLOR 1967). Related to these investigations is the study of the chromosome pairing in a triploid *Larix* by KNABEN (1953). According to SYRACH LARSEN and WESTERGAARD (1938), this triploid had arisen due to fertilization of *L. decidua* by diploid pollen of *L. occidentalis*. Both KNABEN, SYRACH LARSEN and WESTERGAARD found irregularities during meiosis of the PMC.

Meiosis in conifers has been studied for other purposes as well. In the early investigations of meiosis performed by HOFMEISTER (1848), BELAJEFF (1894), STRASBURGER (1895), JUEL (1900), TIMBERLAKE (1900), ISHIKAWA (1902), ALLEN (1903), FERGUSON (1904), NORÉN (1907), LEWIS (1908), NĚMEC (1910), the division in itself as well as the spindle formation might be regarded as the main objects of study.

Studies of spontaneous chromosomal irregularities (MATSUURA and SUTÔ 1935; SUGIHARA 1940; ANDERSSON 1947 A and B, 1954, 1965; CHRISTIANSEN 1950; IWAKAWA and CHIBA 1952; CHIBA and WATANABE 1952; KNABEN 1953; AASS 1957; KHOSHOO 1957 A and B; BARNER and CHRISTIANSEN 1960; CHRISTIANSEN 1960; MANŽOS and POZDNJAKOV 1960; MERGEN and LESTER 1961; CHIRA 1964, 1965 A, 1967; KANTOR and CHIRA 1965; CHANDLER and MAVRODINEAU 1965; ERIKSSON *et al.* 1967; EKBERG and ERIKSSON 1967; EKBERG *et al.* 1968; RUNQUIST 1968) or induced (MERGEN 1963; MERGEN and JOHANSEN 1963; ERIKSSON *et al.* 1966) constitute another line of research followed in the investigations of meiosis.

In Scandinavian literature (from Finland, Norway and Sweden) it has long been pointed out that the environmental conditions during flowering are of great importance for proper seed formation (cf. SCHOTTE 1911; RENVALL 1912; EIDE 1923, 1931, 1932; TIRÉN 1935; ROBAK 1948). This was frequently discussed in connection with the problems of good seed setting in the trees growing close to the limit of natural distribution of the species. The weather conditions during flowering must be regarded as important, although it might be assumed that the trees have regulatory mechanisms which prevent a large pollen dispersal under unfavourable conditions, e.g. during rain or snow. However, it does not seem to have been realized that the first potential risk for low seed setting appears during meiosis of the PMC and MMC (megaspore mother cells) (cf. BERGMAN 1960). Since the hazards of frost during flowering have been pointed out so frequently, it is somewhat astonishing that no investigations on meiosis in the conifers were performed in Fennoscandia until the forties when ANDERSSON began investigations on Norway spruce. The likelihood of frost's occurring during the meiotic divisions clearly depends upon the time of the year when this sensitive phase of development takes place. An elucidation of this point would therefore be of fundamental interest for an evaluation of the potential hazards for individual genera to be damaged by frost during meiosis.

The situation in the genus *Larix* seems to be the best known in this respect. In the following reports it is more or less clearly stated that meiosis starts during the autumn and is completed during the winter, BELAJEFF (1894), STRASBURGER (1895), ALLEN (1903), NĚMEC (1910), DEVISÉ (1922), SAXTON (1929), SAX and SAX (1933), KNABEN (1953), EKBERG and

ERIKSSON (1967). Suggestions of another pattern of development, such as those given by BARNER and CHRISTIANSEN (1960), CHRISTIANSEN (1960), CHANDLER and MAVRODINEAU (1965) are probably erroneous and due to the contamination of the diffuse diplotene (cf. EKBERG *et al.* 1968) and the prepachytene stages. It might be added that the entire meiotic division was observed to take place during the autumn in some grafts of *Larix sibirica* by ERIKSSON *et al.* (1967). This last observation connects *Larix* with those genera in which the entire meiotic division takes place during the autumn. This is the case for *Taxus* growing in England or the United States (cf. DUPLER 1917; HAWKER 1930; DARK 1932; SAX and SAX 1933; KEEN 1958) where the meiotic division was observed in September—November. Another time was given by CHIRA (1964) for *Taxus* growing in Slovakia. CHIRA distinguished between three different types of developmental pattern. In the first group meiosis started during the first day of January and was finished after one week. The second group started on 7 January and meiosis was not completed until the end of March. In the third group the entire meiotic division took place in April. The development was shown to be dependent upon the temperature during the course of meiosis.

Certain *Juniperus* species complete meiosis during summer or early autumn, as for instance, *J. chinensis* (SAX and SAX 1933), *J. horizontalis* (ROSS and DUNCAN 1949) and *J. virginiana* (SAX and SAX 1933; ROSS and DUNCAN 1949) whereas others start and complete meiosis during spring: *J. communis* (NORÉN 1907; OTTLEY 1909; NICHOLS 1910; SAX and SAX 1933) and *J. rigida* (SAX and SAX 1933). Finally in *Cedrus* (SAX and SAX 1933) and *Cryptomeria* (CHIBA and WATANABE 1952), meiosis was reported to take place during the autumn.

In most of the genera investigated by SAX and SAX (1933) meiosis in PMC was observed to take place during the spring, *viz.* in *Abies*, *Picea*, *Pinus*, *Pseudolarix*, *Thuja*, and *Tsuga*. This has been confirmed for *Abies* by MERGEN and LESTER (1961), for *Picea* by ANDERSSON (1947 A and B, 1966), for *Pinus* by COULTER and CHAMBERLAIN (1901), VOGEL (1936), AASS (1957), CHIRA (1963, 1967), MERGEN *et al.* (1963) and ANDERSSON (1966). This type of development has also been reported for *Pseudotsuga* by ALLEN (1943) and for *Cunninghamia* by MIYAKE (1911). In *Keeteleria* growing in the province Yunnan, China, meiosis appeared during January (WANG 1948). The only report concerning the time of meiosis in a conifer growing in the southern hemispher emanates from GULLINE (1952), who observed that meiosis in *Athrotaxis* growing in Tasmania took place in July.

Although the investigations presented above were carried out in various

countries with quite different climatic conditions, three main types of meiotic development might be distinguished:

1. Meiosis is started and completed during the autumn.
2. Meiosis is started during autumn and finished during spring.
3. Meiosis is started and completed during spring.

Transitions occur, as in *Larix sibirica* (cf. ERIKSSON *et al.* 1967; EKBERG *et al.* 1968), which might be attributed to the influence of the climatic conditions on this species growing in Sweden outside its native range of distribution. Similar observations were made during autumn 1967 by KARTEL (personal communication) for *Larix sibirica* growing in the neighbourhood of Minsk, in the Soviet Union. This observation suggests that the meiotic development in one species is not completely tied to a certain time of the year but might be changed.

It is expected that the time of meiosis within one species varies from year to year, depending upon the temperature conditions of the different years. Besides the temperature, ANDERSSON (1947 A) has pointed out the influence of light upon the rate of division (experimental values from greenhouses).

The meiotic divisions in the PMC of the conifers which are of some practical value for Swedish forestry appear in the sequence *Larix—Picea—Pinus* (ANDERSSON 1966). If the sensitivity of the PMC to frost is the same in the three genera, the probability that frost damage will occur will increase in the sequence: *Pinus — Picea — Larix.*

Although more than 70 reports of meiosis in PMC of conifers have been published, the information about climatically conditioned disturbances during this development is rather limited.

In the Arboretum at Mlynany, Slovakia, CHIRA investigated the meiosis of the PMC of about 30 species of conifers (CHIRA 1963, 1964, 1965 A and B, 1967; KANTOR and CHIRA 1965). He has shown the duration of different phases of pollen development, and the temperature during this period. From his studies it is evident that damage in the PMC of the individual species appears at different temperature levels. Thus + 2—4° C caused disturbances in 20 per cent of the PMC of *Pinus edulis* (CHIRA 1967), whereas no aberrations were observed in the PMC of *Picea excelsa* exposed to − 2° C. On the other hand such a temperature provoked complete pollen sterility in *Abies koreana.* The aberrations in *Pinus edulis* were induced to the same extent during metaphase I—anaphase I and metaphase II—anaphase II. For *Taxus baccata* CHIRA (1964) reported that three days at − 4° C caused damage during prophase I—telophase I but to a lesser extent during the second meiotic division. For *Pinus nigra* and *Pinus silvestris* CHIRA (1963) obtained pollen sterility amounting to 100 and 83 per cent respectively,

which he interpreted as being due to a 15-day period of temperatures below 0° C during the sensitive part of the meiotic division.

ANDERSSON (1954) reported that no stickiness occurred at temperatures above −4° C in *Picea abies*.

Although CHRISTIANSEN (1960) observed various types of irregularity during meiosis, such as stickiness, fragmentation, micronuclei, bridges, etc., he did not discuss the temperatures responsible for the different types of abnormality. From his paper it appears that the lowest temperature during the stages diakinesis—telophase II amounted to −2.3° C. Therefore, at least such a temperature may be responsible for inducing damage in *Larix decidua*.

The first investigation with the purpose of studying whether climatic conditions during meiosis were responsible for a reduction in the seed setting was reported by VOGEL (1936). However, during the two years' observation he did not notice any abnormalities during meiosis, which may probably be explained by the presence of favourable temperature conditions during the course of meiosis. In this connection it is necessary to point out that this observation by VOGEL (1936) does not rule out the possibility that climatically conditioned disturbances might occur in another year.

Finally, it should be added that some of the most severely damaged PMC observed were those shown by EKBERG and ERIKSSON (1967). In their investigation, meiosis in the PMC of *Larix leptolepis* was reported to have collapsed completely, as no pollen formation at all took place. During development from diplotene to telophase II, temperatures down to −15° C occurred. Therefore, it was impossible to estimate at which temperature the irregularities were induced.

Not only low temperatures, but high ones, too, might be responsible for irregularities during meiosis. Thus ANDERSSON (1966 and personal communication) reported that high temperatures caused too fast a rate of division in spruce, which provoked desynapsis of the bivalents, and fragmentations.

IWAKAWA and CHIBA (1952) suggested that high temperatures were responsible for the irregularities observed by them in the pollen of *Cryptomeria*. This hypothesis was confirmed in the investigation by CHIBA and WATANABE (1952), who observed the same type of aberrant cells to occur more frequently following heat treatment (+ 30— + 40° C) during meiosis.

These observations of irregularities, provoked by unfavourable temperature conditions during meiosis are not only of purely theoretical interest, but might also be of practical importance. The irregularities will in most cases cause sterility of the gametes carrying chromosomal abnormalities. This means that such gametes cannot take part in fertilization. Therefore,

from this reasoning it is a short step to the idea that irregularities induced during meiosis are indirectly responsible for the frequently observed poor seed-setting in *Larix*, a suggestion which has previously been discussed for *Pinus silvestris* and *Picea abies* by VOGEL (1936) and ANDERSSON (1954, 1965, 1966) respectively. The poor seed-setting in *Larix* has been reported on several occasions. Thus NILSSON (1959) stated that the percentage of empty seeds usually amounted to 50 per cent or more. According to KIELLANDER (1966 B) a germinability of 30 per cent must be regarded as normal, even following abundant flowering. Still less promising data were reported by EDLUND (1966). Thus, the Siberian larch seeds harvested in the seed orchard at Domsjöänget (cf. Fig. 1) 1958—1960 showed a germinability between 0 and 5.4 per cent. HEIKINHEIMO (1937) reported that the average percentage of empty seeds of *Larix sibirica* amounted to 70.9 per cent. For *Larix decidua* MESSER (1956) reported different percentages of empty seeds, depending on the crown types of the trees. The percentages varied between 59.8—80.1 and increased with the size of the crown.

Climatically conditioned irregularities during meiosis are certainly not the only cause of the poor seed setting in *Larix*, but they may under certain circumstances be of great importance. Thus, no seed development can take place when pollen formation has broken down, as was the case for *Larix leptolepis* in the spring of 1966 at Stockholm (EKBERG and ERIKSSON 1967). In this connection it might be questioned to what extent the seed setting is influenced by the presence of sterile pollen at percentages somewhat below 100 per cent or considerably lower than 100 per cent.

According to SARVAS (1952, 1955, 1957, 1958), the main reason for the formation of empty seeds in birch and spruce is the lack of pollination. He has theoretically calculated the expected amount of empty seeds on the basis of the amount of pollen available. Furthermore, he has obtained good agreement between the calculated and the experimentally observed curves. The necessity of having pollen in sufficient amount is also apparent from the investigations of HAGEM (1917) and CALLAHAM (1967). CALLAHAM carried out species crossings within the genus *Pinus* using pollen samples containing sterile pollen grains in various percentages. When the amount of sterile pollen amounted to 80—90 per cent the production of seeds was considerably reduced.

It might therefore be expected that pollen sterilities of the level observed for *Larix decidua* and *Larix sibirica* 1966 (EKBERG and ERIKSSON 1967), amounting to around 85—90 per cent, will prevent good seed setting. However, this might not be of any significance if the sterile pollen grains were compensated for by fertile pollen grains in great amounts from surrounding stands where the climatic conditions had allowed proper pollen

formation. Therefore, the importance of high pollen sterilities is also intimately connected with questions of pollen dispersal.

From the reports of DYAKOWSKA (1936) and DYLIS (1948, cited from EDLUND 1966) it is evident that the pollen dispersal in *Larix* is rather limited compared to that in other conifers. DYLIS reported that 61 per cent of the pollen had fallen below the crown of the tree at a wind velocity of 7—9 m/sec, whereas no less than 93 per cent were observed at the velocity of 3—3.5 m/sec. About 96 per cent of the pollen was observed within a distance of 50 m from the tree at the highest wind velocity. This means that the pollen source for an individual larch tree is limited to its neighbours growing within a short distance from the tree and that inbreeding is probably of considerable frequency.

Therefore, in *Larix* a locally occurring environmentally caused pollen sterility cannot be compensated for by remote pollen, which increases the importance of having high pollen fertility within each locality if good seed setting is to be obtained. This problem is of less importance in Norway spruce, in which the range of pollen dispersal is considerably larger than that in *Larix* (cf. DYAKOWSKA 1936, ANDERSSON 1955).

Owing to the expected high degree of inbreeding, the question of self-sterility is of dominating interest. Low self-fertility has frequently been regarded as one reason for the poor seed setting in *Larix* (cf. DYLIS 1948; NILSSON 1959; DIEKERT 1964). LANGNER (1951—1952) suggested that *L. decidua* and *L. leptolepis* are highly self-sterile but great individual variability exists in this respect. The same conclusion might be arrived from the investigations of GOTHE (1952) and DIEKERT (1964). In his study of the embryogeny in *L. decidua*, *L. leptolepis* and *L. sibirica* HÅKANSSON (1960) observed degenerating embryos, especially in *L. sibirica*. Whether this is the same phenomenon as the self-sterility reported above, or whether it is a separate phenomenon, is not possible to determine. However, SARVAS (1962) and DOGRA (1967) reported that the self-sterility in *Pinus* was due to degeneration of embryos, which suggests that the same might be the case in *Larix*, too. It is probable that in nature there is in larch a basic percentage of empty seeds resulting from partial self-sterility in the same way as in spruce (SARVAS 1955, 1958; ANDERSSON 1965). It is obvious that we cannot wholly compensate for the basic sterility by trying to get a pollen fertility of 100 percent. It must, however, be emphasized that a good pollen fertility might help to keep the percentage of empty seeds in *Larix* on the basic level and not at a higher level.

The purpose of the present investigation was

1. to study the entire meiotic development in the PMC of *L. decidua*, *L. leptolepis*, and *L. sibirica*,

2. to test the universality of the earlier observation that the further development from diplotene was initiated during the autumn in *L. sibirica*,

3. to study and describe various types of meiotic irregularity,

4. to investigate the relationship between the induction of damage, the meiotic stage and temperature,

5. to estimate the pollen sterility and to investigate the relation of pollen sterility to meiotic irregularities.

It might be pointed out that no exact temperature relations can be obtained under natural conditions but I am convinced that the study will give the information needed for a well-designed investigation under controlled environmental conditions such as are available in a phytotron.

If the problems behind the five points presented above are elucidated, the first step for an understanding of the factors responsible for the low seed setting in *Larix* will have been taken. The next step will be to study the meiosis in the MMC and examine the embryo development following cross- and self-pollinations with pollen of known fertility. According to SCHOTTE (1917) and KIELLANDER (1958, 1964, 1966 A and B) the three species included in the investigation have been regarded as the most promising ones for cultivation in Sweden.

Materials and methods

Grafts investigated 1966—67. The trees used during this season were 8—10 year old grafts which were potted during the autumn of 1964. They were all placed in the garden of the Royal College of Forestry during the period of observation. The number of grafts of the individual clones can be seen in the list below.

Species	Clone	Number of grafts
Larix decidua	Punkaharju SF-E 412	6
	" SF-E 655	7
	" SF-E 657	6
Larix leptolepis	Skärsnäs L 1003	25
Larix sibirica	Bispgården Z 1001	7
	" Z 1002	7
	" Z 1003	6

The two species *L. decidua* and *L. sibirica* show great similarities. Therefore, a proper classification might in some cases be difficult to carry out (cf. EDLUND 1966). The SF-E clones were all of Finnish provenance and designated as *L. decidua.* However, SF-E 655 showed the cone characteristics of *L. sibirica.* Furthermore, the needles of SF-E 655 were quite yellow on 10 October 1966, as was the case in the Siberian larches, whereas the needles of the grafts belonging to SF-E 412 and 657 were still green. However, this criterion is not definite, as there is a great variation within a species in this respect. Since there are difficulties in distinguishing *L. decidua* from *L. sibirica,* it might be debated whether or not a further division of the Siberian larch into different species, *viz. L. sibirica* (*sensu stricto*) and *L. Sucaczewii* is justifiable. The name *L. sibirica* will throughout this communication be used *sensu lato.* In polymorphic species such as the larches, Scots pine and Norway spruce, it would be more relevant to designate trees by their origin as is done in the OECD-scheme for certification of forest reproductive material moving in international trade. This is, however, not possible for most of the grafts used in the present investigation, as their true origin is not known.

Grafts investigated during 1967—68. Besides the grafts studied during 1966—67 several other grafts growing at different localities (cf. Fig. 1) were tested

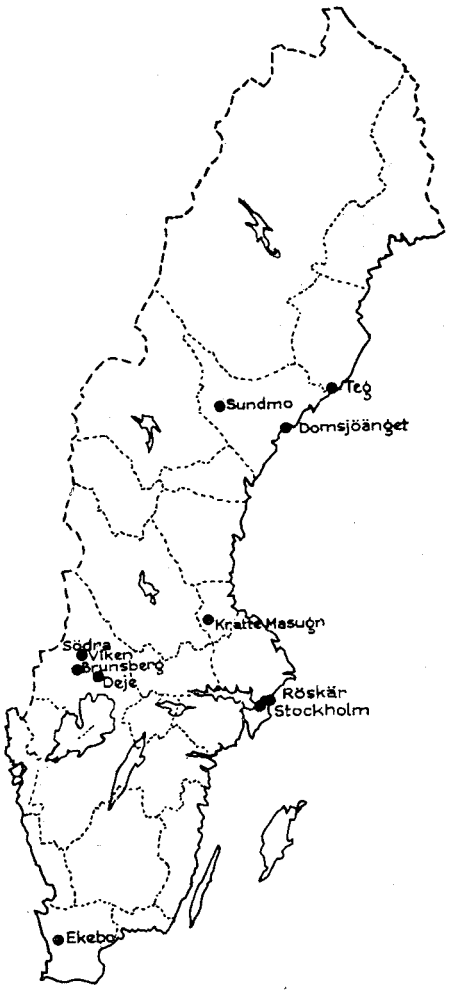

Fig. 1. The planting sites for the grafts of *Larix decidua*, *L. leptolepis* and *L. sibirica* studied during 1967—68.

this season. Mostly the grafts were growing in seed orchards. The latitudes, longitudes and altitudes of these localities are summarized in Table 1. It would be most interesting to study the behaviour of the same clone grown at different localities. However, this was possible only to a limited extent, as may be seen from Tables 2—4, where the location of the individual clones is indicated. Only at Grabbtorp was the origin of the grafts of *L. decidua* and *L. leptolepis* accurately known. A list of these grafts is given in Table 5. The main interest in this material was focused on the passing of diplotene.

Fixations. During the season 1966—67 it was planned to make fixed preparations from each clone every 14 days until the further development from

Table 1. The latitudes, the longitudes and the altitudes of the localities included in the investigation during the season 1967—1968. The localities for temperature measurements are also indicated.

Locality	Latitude	Longitude	Altitude	Temperature data obtained from
Ekebo	55°56′	13°07′	80 m	Svalöv
Stockholm[1]	59°22′	18°04′	3 »	
Broknäs	59°25′	18°17′	20 »	Röskär
Ernvik	59°25′	18°12′	10 »	”
Grabbtorp	59°25′	18°11′	20 »	”
Brunsberg	59°37′	12°59′	83 »	Arvika
Deje	59°35′	13°31′	61 »	Karlstad
Södra Viken	59°52′	13°02′	130 »	Arvika
Kratte Masugn	60°27′	16°24′	135 »	
Domsjöänget	63°15′	18°43′	15 »	Kasa
Teg	63°48′	20°17′	7 »	
Sundmo	63°30′	16°42′	200 »	
Temperature stations:				
Svalöv	55°55′	13°07′	72 m	
Röskär	59°25′	18°10′	15 »	
Karlstad	59°22′	13°28′	47 »	
Arvika	59°40′	12°37′	58 »	
Kasa	63°20′	19°04′	30 »	

[1]College of Forestry

diplotene was assumed to take place. This should happen in late February or early March, according to previous results (Ekberg and Eriksson 1967). Owing to the limited number of generative buds, all grafts belonging to the same clone were not included on all occasions. However, when it was detected that the diplotene stage had been passed during October—December in some grafts of one clone, but not in others, it was realized that fixed preparations should involve all grafts under examination. This was done from 2 January 1967. From that date fixing was done on occasions when it was suspected that the temperature conditions might have provoked some development. When the development from diplotene to tetrads took place in most of the grafts of *L. decidua*, preparations were made at intervals of two to four days. Usually 5—6 buds were collected, which usually guaranteed the presence of 3—4 male buds. Female and male buds are relatively alike during the autumn and early winter, which made the fixation unsuccessful in a few cases. From each graft an attempt was made to fix buds at various growth positions.

During the autumn of 1967 the first fixed preparation could not be made simultaneously at all localities but fixing went on from the middle to the end of October. From 3 November fixing was carried out at three-week

Table 2. Compilation of the clones of L. decidua investigated at different localities 1967—68.

L. decidua	Broknäs	Deje	Domsjö-änget	Ekebo	Kratte Masugn	Sundmo
E 1001		×[1]				×
E 1009		×		×		
E 2002	×	×		×		
E 2004	×					
E 2005	×					
E 2006	×					
E 2009		×[1]				
E 2012		×		×		
E 2013		×		×		
L 2001		×		×		
L 2002		×				
M 3005		×				
N 2001		×		×		
R 1001		×		×		
R 1002		×		×		
R 1003		×		×		
S 2002	×					
S 6204	×					
X 1002					×	
X 1003					×	
X 2001					×	
X 2002					×	
X 2003					×	
DK 7013				×		
IS 6						×
IS 180			×			
IS 272						×
IS 520						×
IS 521						×

[1]Brunsberg

intervals. Unfortunately, flowering in several grafts was poor, which caused a lack of male buds on some occasions. Also, during this season some extra preparations were made when the temperature conditions suggested that further development from diplotene might have taken place.

The fixing of pollen took place just before the anthers were ready to dehisce.

Table 3. Compilation of the clones of L. leptolepis investigated at different localities 1967—68.

L. leptolepis	Broknäs	Brunsberg	Ekebo	Ernvik	Kratte Masugn	Sundmo
F 1001			×			
L 8		×				
L 1001			×			
L 1002	×					
L 1004	×					
L 1006	×					
L 1007	×					
L 2005			×			
L 2006			×			
M 2001			×			
M 2002		×[1]	×			
M 2003		×[2]	×			
M 3018		×				
N 2005			×			
X 4600	×			×		
X 4601					×	
X 4602					×	
IS 526						×
UP 1					×	

[1]Södra Viken. [2]Deje.

Temperature measurements. In most of the seed orchards it was not possible to arrange continuous temperature readings. Therefore, only approximate information concerning the local temperature conditions could be obtained by the aid of the meteorological stations listed in Table 1. In Stockholm the temperatures during the season 1966—67 were measured by an ordinary maximum and minimum thermometer hanging in one of the grafts. By this arrangement only the minimum temperatures can be regarded as reliable. Temperature data were also obtained from the meteorological station in Stockholm, situated about 4 km from the Royal College of Forestry. The agreement between the minimum temperature observations of the two localities within Stockholm was not complete. The temperatures at the College of Forestry were somewhat lower, which was expected, owing to the exposure of the garden as compared with the meteorological station in Stockholm.

Analysis of the meiotic development. From each male bud, PMC from at least three stamens were dissected in acetic orcein. The PMC were mixed

Table 4. Compilation of the clones of L. sibirica investigated at different localities 1967—68.

L. sibirica	Domsjö-änget	Ekebo	Ernvik	Grabb-torp	Kratte Masugn	Södra Viken	Sundmo	Teg
E 1002			×					
E 1004			×					
E 1005		×	×			×		
E 1006						×		
E 2011		×				×		
U 1001		×				×		
W 2001		×			×	×		
W 2002					×	×		
W 2003					×	×		
W 2004						×		
W 2005					×			
W 2006					×			
W 5001		×				×		×
W 5002		×	×			×		×
W 5005								×
W 5006			×					
W 5007			×					
W 5008		×				×		
Y 1001								×
Y 4002	×							×
Y 4005							×	
Y 4006	×					×	×	
AC 1001	×							×
AC 1002	×							×
AC 1003	×							×
AC 1004	×							×
AC 2003	×	×					×	×
AC 2006	×	×						
AC 3003	×							×
AC 3004	×							
IS 85							×	
IS 181							×	
SF 7011					×			
SF 7012					×			
SF 7014					×			
SF 7015					×			
SF 7017					×			
50-401				×				
50-402				×				
50-403				×				
50-425				×				
50-427				×				
50-428				×				
50-434				×				
50-435				×				
51-606				×				

Table 5. The native latitudes, longitudes and altitudes of the grafts investigated at Grabbtorp 1967—68.

Provenance with individual tree number	Latitude	Longitude	Altitude
L. decidua:			
Bělidlo 3 I 11	49°38′	18°25′	610 m
Ormea 8	44°12′	7°54′	1350 "
Ormea 10	44°12′	7°54′	1350 "
Pergine 1	46°05′	11°15′	1000 "
Pergine 4	46°05′	11°15′	1000 "
Pergine 6	46°05′	11°15′	600 "
Pergine 10	46°05′	11°15′	600 "
Pragelato 14	45°01′	6°55′	1530 "
Staré Hory 114	48°52′	19°14′	850 "
L. leptolepis:[1]			
Azu R 7	35°57′	138°43′	1500 m
Azu R 8	35°57′	138°43′	1500 "
Nar I 8	35°26′	138°41′	1320 "
Nar I 9	35°26′	138°41′	1320 "
Nar J 16	35°23′	138°42′	2500 "
Nar J 20	35°23′	138°42′	2500 "
Ren L 7	36°48′	137°48′	2200 "
Tak P 4	36°25′	137°43′	2700 "
Tak P 7	36°25′	137°43′	2700 "
Tak Q 19	36°26′	137°41′	1400 "
Yatsu S 4	36°03′	138°24′	1800 "

[1] The same abbreviations as used by LANGNER (1958) are given for the grafts of L. leptolepis.

carefully and 100 PMC were classified according to stage of development. To avoid bias when both meiotic divisions were present simultaneously all classifications were carried out on a PMC basis, which means, for example, that one M I is equivalent to two M II. Usually there were no problems concerning the classification, since the first meiotic division was followed only exceptionally by wall formation. Severely damaged cells could not be classified with respect to meiotic stage. Various numbers of buds were analysed on the different occasions, depending on the number of buds available. When it was realized that there was a great variation within a graft as well as between grafts of the same clone, the number of buds investigated was increased. During the season 1967—68 all PMC of a slide (1000—2000) were examined in order to try to detect where the diplotene stage was passed only by a few cells from individual buds. For the most part, no estimation of the percentages of cells in different stages was carried out in this material.

Analysis of irregularities. The quantitative estimation of irregularities was only performed during 1966—67 in the grafts growing in Stockholm. The

grafts involved in this analysis were selected either on the basis of their pattern of development; late, early, or intermediate passing of diplotene, or on the basis of the observed sterility; high, intermediate or low. This analysis was carried out independently of the previous analysis of the pattern of development, although the same buds were tested in both cases. At first 100 PMC were classified with respect to meiotic stage. Subsequently, an attempt was made to classify exactly the same cells with respect to various categories of irregularity. Where there was a high percentage of damaged cells, it was technically impossible to evaluate exactly the same cells as were examined for stage of meiosis. A description of the different types of irregularity observed is presented in a separate chapter below.

When only microspores (individual or still within a tetrad) were present in a slide, 400 microspores were examined. This was done to facilitate a comparison between percentage of aberrations at the end of meiosis and the pollen sterility. One example will illustrate this. If one of the four microspores of a tetrad was degenerating, this should contribute 1 per cent to the percentage of irregularities calculated on a PMC basis, whereas it would contribute only 0.25 per cent to the percentage calculated per 400 microspores. Conversely, an octad consisting of eight abnormal microspores would on a PMC basis give one per cent, whereas on a microspore basis it constitutes two per cent. Obviously, the calculation on a microspore basis should be more related to the pollen sterility than the calculation on a PMC basis. The average percentage of irregularities of one fixing day was determined from all buds of the individual grafts examined on this particular fixing day.

Pollen sterility. The classification of pollen grains as sterile or fertile ones is difficult. In accordance with previous investigations (Eriksson *et al.* 1966; Ekberg and Eriksson 1967) the pollen sterility was estimated by staining the pollen grains in a solution of methylene blue. Usually ten buds from each graft were tested with respect to pollen sterility; from each bud 100 pollen grains were examined.

Christiansen (1960) reported that the pollen of *Larix* did not germinate artificially. Similarly, Illies (1956) failed to obtain germination in larch pollen following colchicine treatment of the male buds. Nor did she observe any pollen germination in the control material, although the appearance of the pollen grains suggested a fertility of about 95 per cent.

According to Jensen (personal communication) the *Larix* pollen grains germinate within 2—4 weeks following their application on to a nutrient medium. To avoid bacterial infection of the medium, the pollen grains must be disinfected before application, which is a laborious and time-consuming procedure. When large amounts of pollen grains from various localities must be examined it is evident that tests of pollen germinability in *Larix* cannot

conveniently be made. Furthermore, nothing is known about the fertilization capacity of the pollen grains which have germinated. It might be recalled that one of the ways of inducing haploidy in diploid species is to irradiate pollen and use it in crossings, in the hope that it will be genetically dead but physiologically alive. Hence, it should be able to germinate and stimulate the development of the egg to an embryo without a preceding fertilization. The working out of a convenient method for estimation of pollen sterility in *Larix* must be regarded as of utmost importance for the future research concerning seed setting in *Larix*.

Pollen measurements. To obtain information concerning the development of pollen grains and about the disappearance of certain degenerating microspores, microspores fixed on 28 March, and mature pollen grains, fixed on 15 April, were measured. The pollen grains were stained in methylene blue. From each graft 100 PMC from three different buds were measured. In some of the grafts no fixation was made on 28 March or the microspores or pollen grains were shrivelled to such an extent that accurate measuring was impossible. These were the reasons for the lower number of pollen measured in a few cases.

Pattern of development

Breaking of dormancy. The denotation of diplotene as a sort of resting stage has previously been pointed out (EKBERG and ERIKSSON 1967; ERIKSSON *et al.* 1967). The diffuse appearance of the chromosomes (cf. EKBERG *et al.* 1968) during this stage lends further support to this denotation. During diplotene the PMC seem to be rather insensitive to temperatures down to −30° C (EKBERG and ERIKSSON 1967). When the further development from the diplotene stage is initiated the cells are sensitive to external agents such as frost (cf. below). Therefore, it is of particular interest to investigate what conditions are responsible for the breaking of the dormancy.

First it is necessary to establish the date on which all PMC had reached diplotene. The data obtained from autumn 1967 have been summarized in Table 6 for the three species under investigation, growing at different localities. Although the species are represented by different numbers of clones at the various localities (cf. Tables 2—4) the tendency for diplotene in the PMC to be reached in the sequence *L. sibirica—L. decidua—L. leptolepis* seems to be evident. This is also supported by the observations before the complete reaching of the diplotene stage. This tendency was also observed during autumn 1966 when *L. sibirica* and *L. decidua* had reached diplotene completely on 10 and 24 October, respectively, whereas the maximum for

Table 6. The first fixing date 1967 when all PMC of the species L. decidua, L. leptolepis and L. sibirica had reached the diplotene stage at different localities.

	L. decidua	*L. leptolepis*	*L. sibirica*
Ekebo	3.11	3.11	13.10[1]
Broknäs	3.11	3.11	—
Ernvik	—	3.11	23.10[1]
Grabbtorp	3.11	24.11	11.10[1]
Brunsberg	26.10[1]	3.11	—
Deje	3.11	24.11	—
Södra Viken	—	3.11	26.10[1]
Kratte Masugn	3.11	3.11	16.10[1]
Domsjöänget	3.11	—	19.10[1]
Teg	—	—	19.10[1]
Sundmo	24.11	Not reached on 15.12	24.11

[1] First fixing occasion.

diplotene in *L. leptolepis* was not observed until the end of the year or even the beginning of the next year (cf. Table 14 and Fig. 15).

The extremely late reaching of diplotene in clone L 1003 1966—67 (cf. Table 14 and Fig. 15) was probably due to unfavourable temperature conditions during that season, as a few tests in beginning of November 1967 revealed that diplotene had been reached by all PMC at that time. The relatively mild weather during October 1967 (cf. Fig. 5) probably favoured the reaching of diplotene before the occurrence of frost. In this connection the observations made by SIMAK (personal communication) in his phytotron experiments concerning lignification in *Larix* are worth mentioning. His experiments revealed that lignification was established more easily at high temperature than at low ones. Earlier, chilling has been believed to provoke this process. Similarly it is assumed that development to diplotene in the PMC is enhanced by relatively high temperatures during the autumn.

As has been pointed out elsewhere (ERIKSSON *et al.* 1967) the most conspicuous difference between the pattern of development during the two seasons 1965—66 and 1966—67 was the early initiation of the further development from diplotene in *L. sibirica* during autumn 1966. The first observation of this phenomenon was made in PMC fixed as early as 24 October 1966 (graft Z 1002—14).

This difference in pattern of development in the same grafts during two consecutive seasons must be attributed to the climatic conditions prevailing during the two seasons. During 1965—66, when the grafts were growing at Röskär, the minimum temperature from the beginning of November until the beginning of March was almost constantly below or close to 0° C (cf. Fig. 2). On the other hand, temperature fluctuations around 0° C were frequent during September—November 1966 (cf. Fig. 3). It is probable that the periods with minus temperatures were low enough and long enough to be able to break the assumed dormancy. Once the dormancy of the PMC has been broken they will start further development if temperatures sufficiently high (+ 2— + 5° C) are present for a certain time (cf. CHRISTIANSEN 1960; EKBERG and ERIKSSON 1967). During the autumn of 1965 this possibility did not exist, as may be seen from Fig. 2.

During autumn 1966 the most advanced development was observed in the grafts belonging to clone Z 1002 (cf. Fig. 17 below). Therefore, it was remarkable that clone Z 1001 showed at the end of 1967 the most advanced development, estimated as the percentages of buds in which diplotene had partly been passed. These percentages amounted to 36, 10 and 11 for Z 1001, Z 1002, and Z 1003 respectively. The observations from 1966 suggest that a period of low temperatures like the one occurring from 6 Dec.—12 Dec. 1967 (Fig. 5) should have been able to break the dormancy in all three clones.

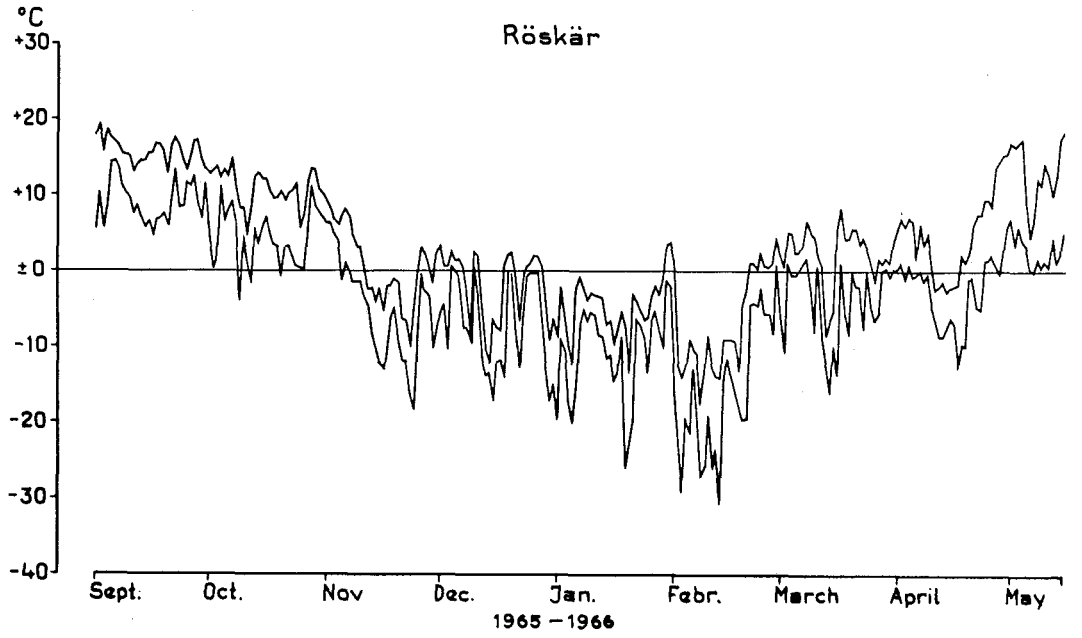

Fig. 2. Maximum and minimum temperature curves for the time of meiosis in PMC at Röskär 1965—66.

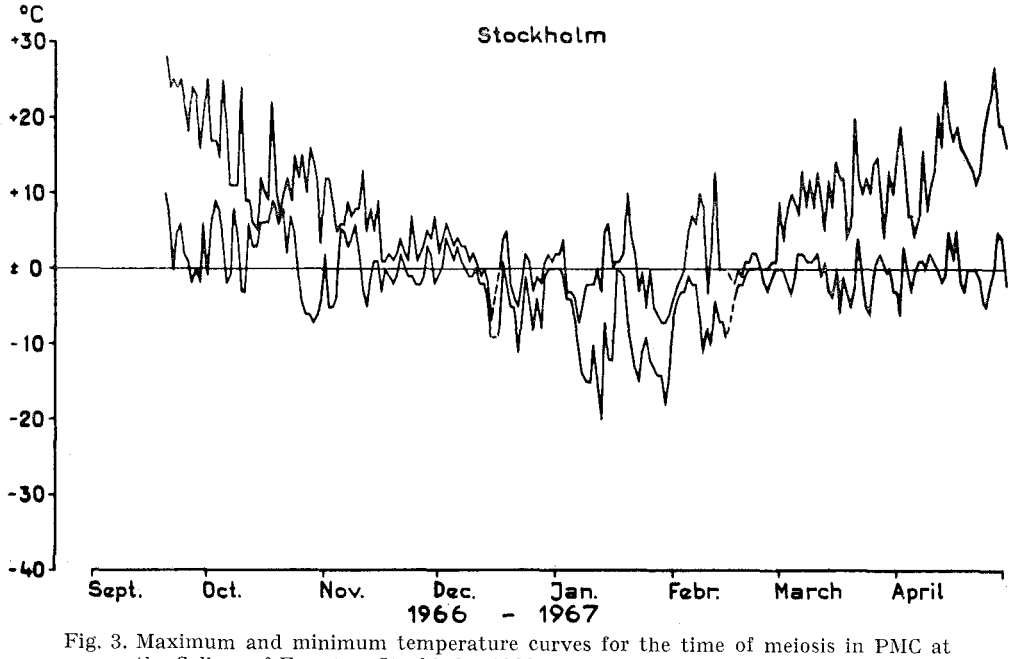

Fig. 3. Maximum and minimum temperature curves for the time of meiosis in PMC at the College of Forestry, Stockholm 1966—67.

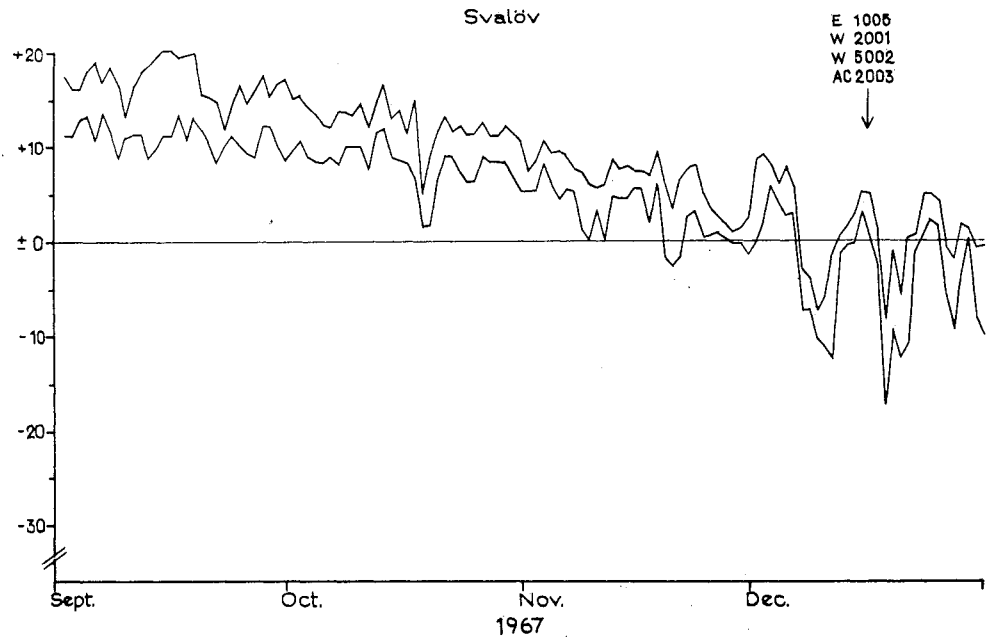

Fig. 4. Maximum and minimum temperature curves during September—December 1967 at Svalöv. The first date of observation for passing of diplotene in individual grafts of *L. sibirica* growing at Ekebo is indicated.

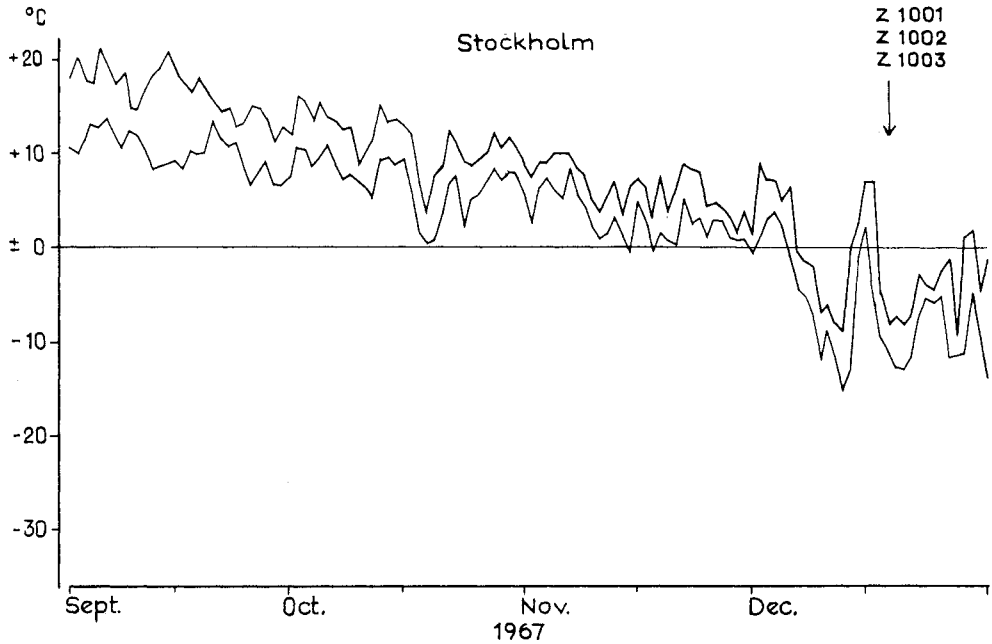

Fig. 5. Maximum and minimum temperature curves during September—December 1967 at Stockholm. The first date of observation for passing of diplotene in individual grafts of *L. sibirica* is indicated.

It might be suggested that the difference in the pattern of development between the two seasons is due to the fact that dormancy is easier to break in Z 1002 than in Z 1001. However, the temperature needed to initiate further development should be lower in Z 1001 than in Z 1002; but a final evaluation of this cannot be made under outdoor conditions.

The possibility that fluctuations around 0° C are needed to break dormancy cannot be completely excluded. The data from Stockholm 1967 contradict this, as the minimum curve was hardly below 0° C until 5 December (Fig. 5) whereafter the temperature was below 0° C until a short period of mild weather appeared around 15 December. This period of cold weather must be responsible for the breaking of dormancy and the period of mild weather must be responsible for the initiation of the further development from diplotene which was observed on 19 December in all three clones growing in Stockholm.

In *L. leptolepis* and *L. decidua* no passing of the diplotene stage was observed during autumn 1967. The total number of buds of *L. decidua* and *L. leptolepis* investigated from the fixings carried out on 15—18 December amounted to 227 and 149 respectively. This means that altogether 350,000—700,000 PMC were examined without the detection of any later stages than diplotene.

A detailed presentation of the data from 1968 concerning breaking of dormancy and the further development from diplotene in *L. decidua* and *L. leptolepis* will be given elsewhere. Only one example will be presented to illustrate the earlier initiation of the development from diplotene in *L. leptolepis* than in *L. decidua*. Thus no passing of diplotene was observed among the 69 European larch buds fixed on 3 March at Grabbtorp, whereas 54 of the 93 Japanese larch buds showed passing of diplotene to some extent on that date.

In *L. sibirica* an initiation of the further development from diplotene had taken place on 19 December, 1967, in several grafts at all localities except Ernvik and Grabbtorp. This has been demonstrated in Figs. 4—11, where the first fixing date for passing of diplotene of individual grafts is indicated. The maximum and minimum temperatures for the time of observation are also shown in these figures.

It might be of interest to investigate to what extent meiosis had proceeded following the passing of diplotene. The percentages of buds in which diplotene had been passed to some extent are shown in Table 7; in no case did they exceed 30 per cent. Furthermore, the number of PMC which had passed was mostly low, but amounted to as much as about 50 per cent in some cases. Even microspores occurred frequently in the fixings from Ekebo and Stockholm on 16 and 19 December. It can be stated that the passing of

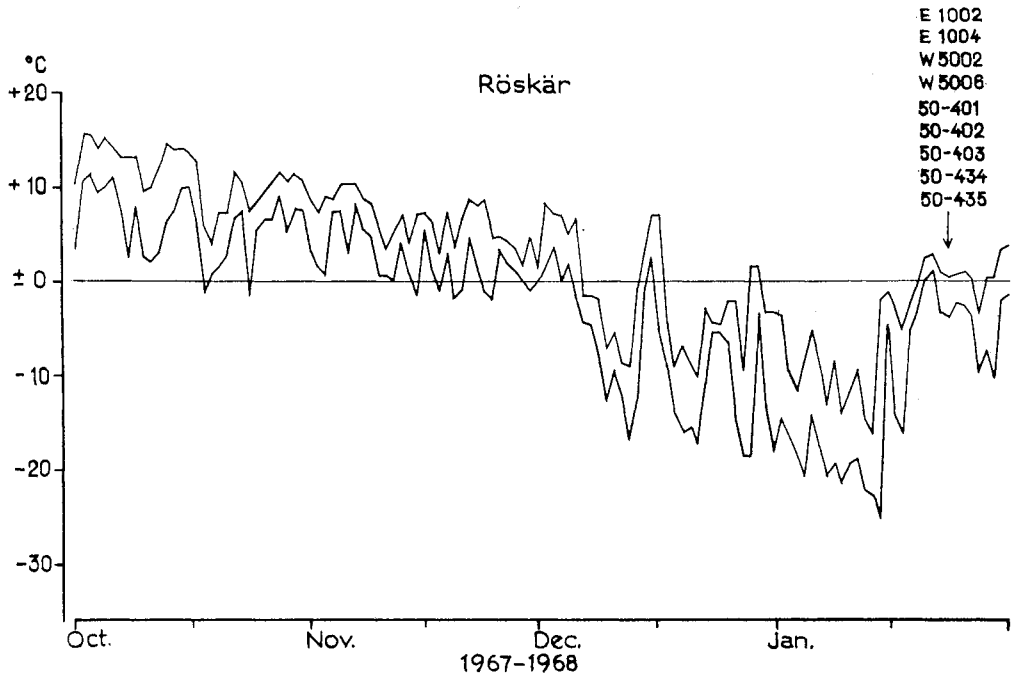

Fig. 6. Maximum and minimum temperature curves October 1967—January 1968 at Röskär. The first date of observation for passing of diplotene in individual grafts of *L. sibirica* growing at Ernvik or Grabbtorp is indicated.

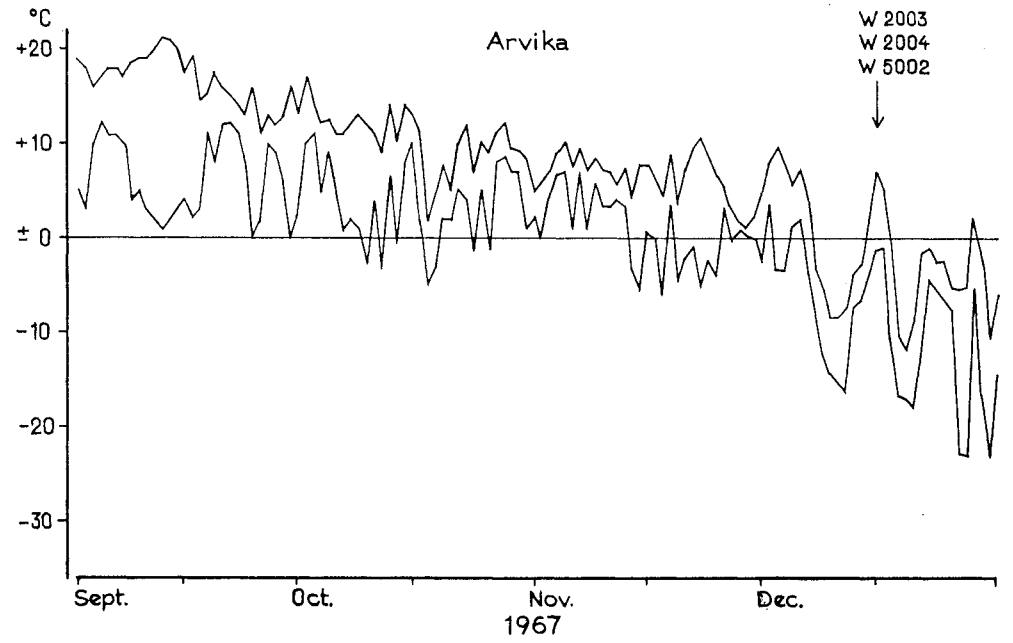

Fig. 7. Maximum and minimum temperature curves during September—December 1967 at Arvika. The first date of observation for passing of diplotene in individual grafts of *L. sibirica* growing at Södra Viken is indicated.

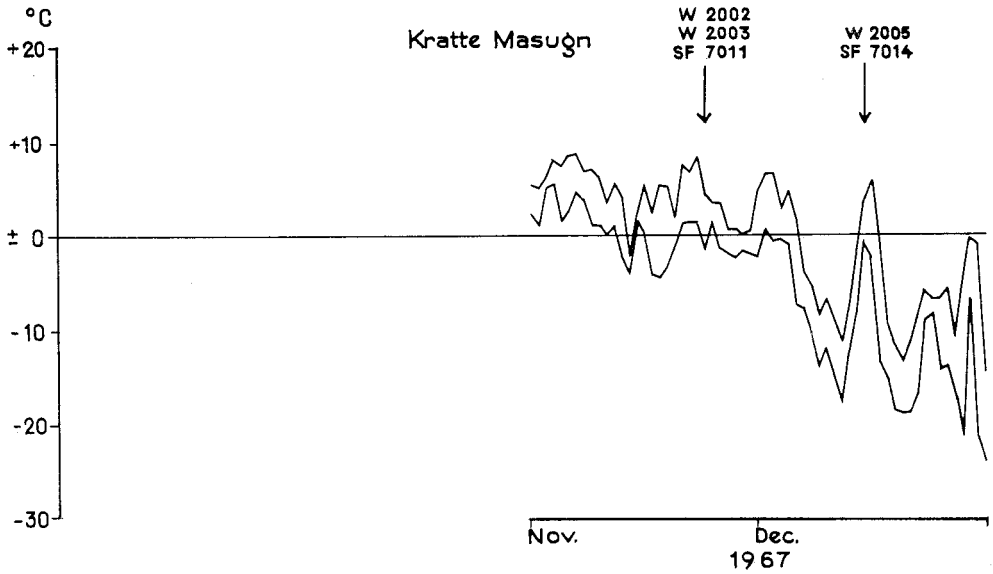

Fig. 8. Maximum and minimum temperature curves during November—December 1967 at Kratte Masugn. The first date of observation for passing of diplotene in individual grafts of *L. sibirica* is indicated.

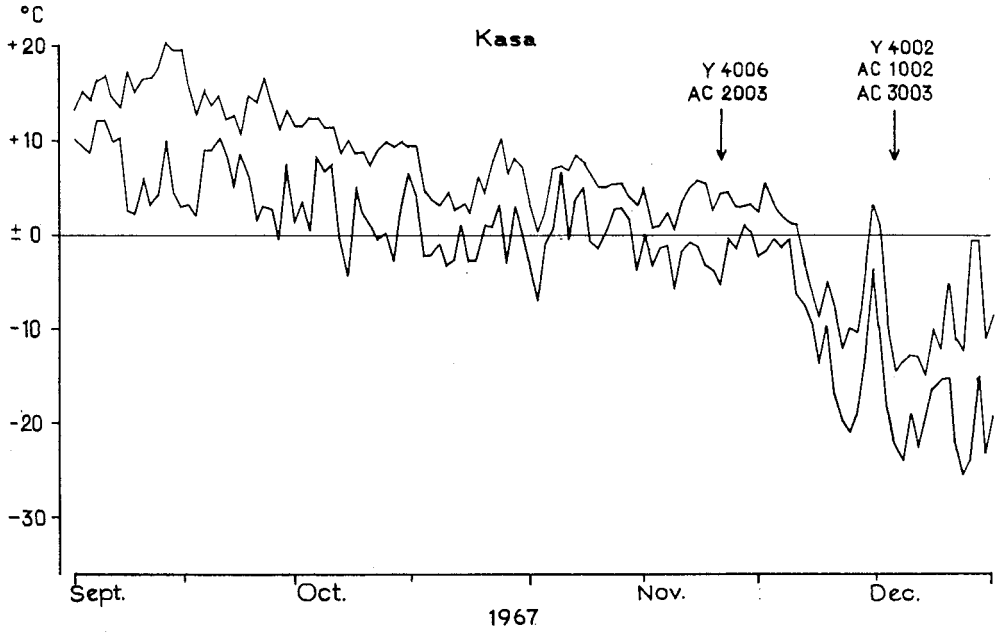

Fig. 9. Maximum and minimum temperature curves during September—December 1967 at Kasa. The first date of observation for passing of diplotene in individual grafts of *L. sibirica* growing at Domsjöänget is indicated.

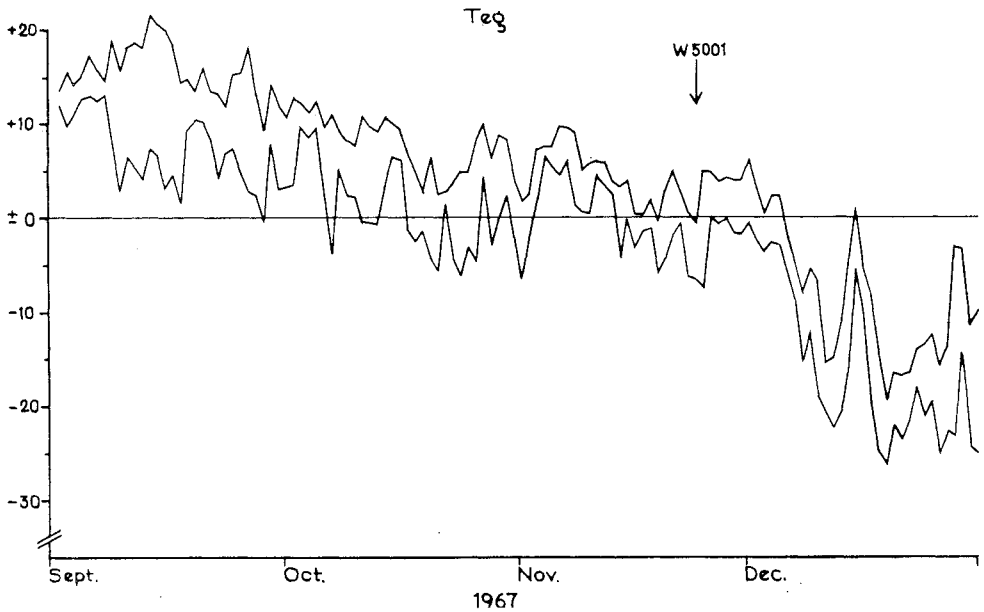

Fig. 10. Maximum and minimum temperature curves during September—December 1967 at Teg. The first date of observation for passing of diplotene in individual grafts of *L. sibirica* is indicated.

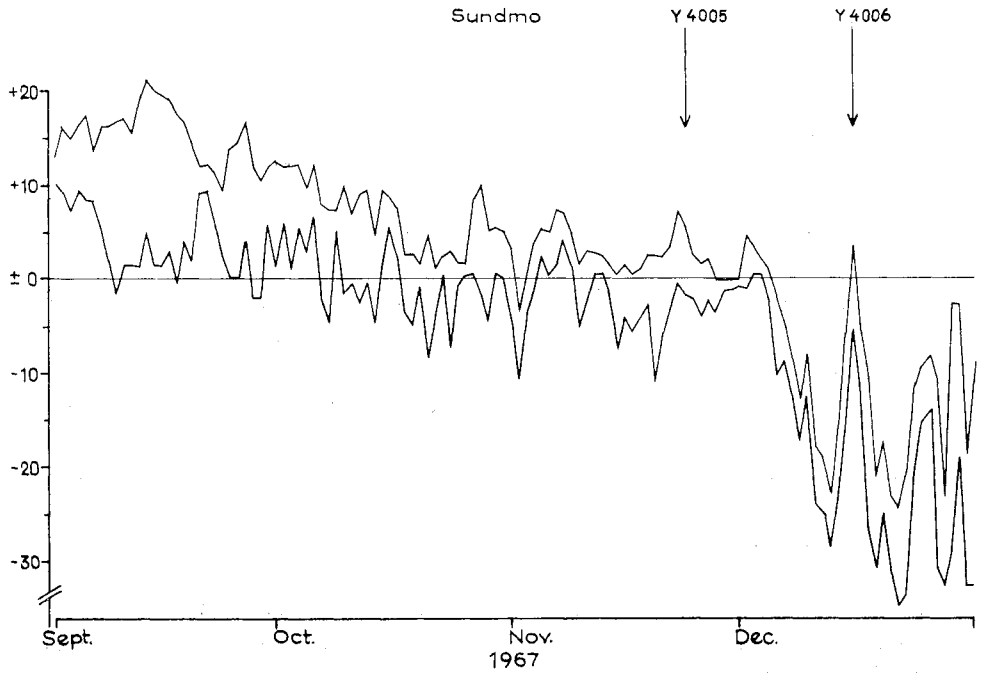

Fig. 11. Maximum and minimum temperature curves during September—December 1967 at Sundmo. The first date of observation for passing of diplotene in individual grafts of *L. sibirica* is indicated.

Table 7. The number of grafts and buds as well as the percentages of buds of L. sibirica where diplotene was partly passed at different dates and localities during the season 1967—68. The total numbers of grafts and buds examined are shown within brackets.

Locality	Fixing date					
	24.11			15.12—19.12		
	Grafts	Buds	% buds	Grafts	Buds	% buds
Ekebo.....	0 (8)	0 (45)	0	4 (8)	14 (52)	27
Stockholm....				3 (3)	14 (74)	19
Ernvik.....				0 (7)	0 (41)	0
Grabbtorp....				0 (6)	0 (33)	0
Södra Viken..	0 (12)	0 (65)	0	3 (12)	5 (95)	5
Kratte Masugn	3 (10)	3 (69)	4	5 (10)	12 (68)	18
Domsjöänget .	2 (10)	2 (100)	2	5 (10)	8 (100)	8
Sundmo.....	1 (5)	4 (50)	8	2 (5)	7 (50)	14
Teg.....	1 (6)	1 (29)	3	0 (6)	0 (22)	0

diplotene was limited to a low percentage of PMC. The differences in percentage between the various localities can only partly be attributed to the fact that different grafts were tested at the various localities. This will partly be evident from the observations of clones growing at more than one locality. The situation in these clones is shown in Table 8.

As may be seen from this table, diplotene was frequently passed at one locality but not at another. However, the limitations of the temperature measurements, as well as the number of buds tested from each graft, makes a final evaluation of the temperature response of the individual grafts impossible. Thus chance can in most cases not be excluded as the reason for the difference in stage of development of the same clone growing at two or more localities. However, it is hard to believe that all of the differences listed in the table should be due to chance alone.

Only one clone will be discussed in detail. Clone E 1005 was selected for this discussion, as diplotene was passed to a great extent at Ekebo but not at all at Ernvik and Södra Viken. Out of the ten buds tested at Ekebo, three contained only diplotene. In two of the buds a few PMC had passed diplotene whereas numerous PMC had passed diplotene in the five other buds. The dormancy is evidently broken at Ekebo and is probably also broken at Ernvik and Södra Viken, as the temperature curves were lower at these two localities than at Ekebo. Thus, the difference in passing of diplotene must be attributed to differences in the heat quantity available following the breakage of dormancy. These differences are small, as may be seen from the temperature curves in Figs. 4, 6, and 7, which suggests that the observed difference should be due to chance alone. However, a χ^2-test revealed that the differences in distribution of buds containing only diplotene and buds

Table 8. The stage of meiotic development in L. sibirica clones growing at more than one locality. The test was performed on December 15—18th 1967.

Clone	Diplotene partly passed	No passing observed
E 1005	Ekebo	Ernvik, Södra Viken
E 2011		Ekebo, Södra Viken
U 1001		Ekebo, Södra Viken
W 2001	Ekebo	Södra Viken, Kratte Masugn
W 2002	Kratte Masugn	Södra Viken
W 2003	Södra Viken, Kratte Masugn	
W 5001	Teg	Ekebo, Södra Viken
W 5002	Södra Viken , Ekebo	Ernvik, Teg
Y 4006	Domsjöänget, Sundmo	Södra Viken
AC 1004		Domsjöänget, Teg
AC 2003	Ekebo, Domsjöänget	Sundmo
AC 2006		Ekebo, Domsjöänget
AC 3003	Domsjöänget	Teg

containing later stages as well, was significant at the 1 per cent level for the comparison between Ekebo and Södra Viken and at the 5 per cent level for Ekebo—Ernvik.

Following a period of a few days with a maximum temperature of around + 3.0° C at Röskär (cf. Fig. 42), a fixing was carried out at Ernvik on 23 January. At that date four out of six grafts showed a passing of the diplotene stage. Especially good information about the temperature requirements for the initiation of the further development from diplotene was obtained in graft W 5006. From this graft five buds were investigated on 19 December. All contained only diplotene, whereas eight out of 11 buds fixed on 23 January showed a passing of diplotene.

These distributions differed at the 5 per cent level, as revealed by a χ^2-test. Thus, it is highly probable that the period of mild weather from 19—23 January was responsible for the initiation of the further development from diplotene. Similarly, low temperatures during this period must be responsible for the frost damage observed in the PMC fixed on 23 January. This will be discussed later on.

From the observations made during the season 1967—68 it might be concluded that the dormancy in most of the Siberian larch clones examined is broken by relatively short periods during which the temperature is only a few degrees below 0° C. Similarly, the further development from diplotene is initiated by relatively moderate temperatures amounting to a few degrees above 0° C. This constitutes a confirmation of earlier observations in *L. sibirica* by Ekberg and Eriksson (1967) and Ekberg *et al.* (1968).

The data (compare also Ekberg *et al.* 1968) concerning the further development from diplotene can be summed up as follows:

1. The initiation takes place early in *Larix sibirica.*
2. The initiation takes place late in *Larix decidua.*
3. The initiation takes plac atively late in *Larix leptolepis* (partly based on unpublished data from the winter 1968).

This suggests that there is a great difference in temperature response of the diplotene stage in *L. sibirica* on the one hand and the same stage in *L. decidua* and *L. leptolepis* on the other hand. The most plausible explanation for this difference is the presence of genetic factors which regulate the temperature response.

The temperature conditions in the native distribution area of *L. sibirica* are quite different from those in Sweden. Thus, in Siberia as well as in the European part of the Soviet Union, the continental type of climate prevails, which means stable low temperature conditions during the winter. In contrast to this, the Swedish climate is characterized by frequent fluctuations about 0° C, especially in southern Sweden. Therefore in the Soviet Union there have been few possibilities for selection in favour of Siberian larch trees which showed a temperature response like that of the European larch. Rather, it might be advantageous to respond quickly once the period of low temperature was passed. As the Siberian larch has been cultivated in Sweden for a few generations only, selection has not yet been able to eliminate biotypes showing this temperature response which is detrimental for proper pollen formation (cf. page 118). This is especially probable since no selection or only mild selection has been able to act on the female germ line (see below).

Quite another situation is met within *L. decidua* and *L. leptolepis* where the temperature conditions of the native distribution areas are different from those to which *L. sibirica* is exposed. However, WACHTER (1962) characterized the common features of the native distribution areas of *L. decidua* as follows: "Diese Gemeinsamkeiten bestehen vor allem darin, dass die Lärchengebiete gegenüber direktem Westwettereinfluss weitgehend abgeschirmt sind", thus suggesting a continental type of climate of these regions. On the other hand MAYER (1964) stated that the climatic conditions in the natural distribution area of *L. decidua* in the East Alps varied considerably from a subcontinental climate to one of maritime character. It is probable that temperature fluctuations about 0° C occur to some extent. Therefore, it is also probable that selection has acted against biotypes in which the dormancy was broken within a few days or in which the further development from diplotene took place following short exposures (hours—day) to temperatures a few degrees above 0° C. Owing to the frequent occurrence of temperature fluctuations in the native distribution area of Japanese larch, the resistance of this species to such conditions is easily explicable.

The influence of the day length on the time of initiation in *L. decidua* and

L. leptolepis cannot be neglected. The fact that the further development from diplotene mostly starts at the end of February or during March, following exposures to temperatures of + 2— + 5° C for some time, does not rule out this assumed influence of day length. It might merely mean that the cells at that time of the year are prepared to respond to a favourable temperature condition. An influence of day length in *L. sibirica* can be ruled out, as initiations have been observed from October to March in this species. The final evaluation of this question cannot be made until phytotron experiments (which are planned) have been carried out.

The reasons for the difference in time of initiation of the further development within a clone cannot be given with certainty. Owing to the small size of the grafts, shadowing was of limited importance, which reduces the possibilities for occurrence of temperature differences between the buds. However, the position of the buds on the grafts might be of importance for the physiological status of the PMC or surrounding cells. The water supply could be expected to be of importance for the physiological activity of the PMC, which obviously takes place when the meiotic procedure again is started following the rest during diplotene. Therefore, the availability of water to the individual buds might be suggested as one of the reasons for the observed differences in stage of development.

Completion of meiosis. The development from diplotene to microspore formation was studied in detail during the season 1966—67. Some difficulties in evaluating the pattern of development may be pointed out here. To obtain accurate information it is necessary to study the variation in development within stamens and within buds as well as between buds of the same graft. Furthermore, the variation between buds of different grafts belonging to the same clone is of interest.

The meiotic stages diakinesis-telophase II appear hardly alone in the individual stamens. This is demonstrated for the second meiotic division in Table 9, where the percentages of PMC in various stages were determined for all stamens in one bud from graft Z 1003-9 fixed on 8 March 1966. The stamens are arranged helically. In this particular bud the whorls, counted from the base of the bud, contained the followind numbers of stamens, 16, 13, 8, and 6 respectively. From Table 9 it may be seen that the development starts at the base of the bud and proceeds upwards. This Type of development (within a bud) has been confirmed for other buds.

On the examination of the PMC during the season 1966—67 it was apparent that great variability existed in stage of development between the buds from the same graft. To illustrate this variability the results from one fixing of nine buds from graft Z 1002-8 (*L. sibirica*) carried out on 29 December, 1966 will be presented. The buds were growing within a distance

Table 9. Distribution of meiotic stages in the individual stamens of one male strobilus from graft Z 1003-9 fixed on 8 March 1966. The stamen No. indicates the position in the strobilus starting from the periphery.

Stamen No.	Interphase	Prophase II	Metaphase II	Anaphase II	Telophase II	Tetrad
1					2	98
2			8	14	8	70
3		2	6	14	8	70
4	4	14	24	14	8	36
5	2	4	16	6	6	66
6			8	4	6	82
7	10	8	20	6	4	52
8	2	4	12	6	10	66
9	12	4	26	10	22	26
10			8	10	4	78
11	2	2	14	12	4	66
12	4	8	14	6	14	54
13		4	8	10	14	64
14		2	6	10	2	80
15			2	2	2	94
16			2	2	6	90
17		6	24	6	10	54
18	2	8	16	14	10	50
19	2	4	16	14	10	54
20		2	12	6	8	72
21	8	2	6	6	16	62
22	2	6	44	22	16	10
23	2	2	38	20	8	30
24	22	24	18	10	16	10
25	28	20	36	8	8	
26	2	6	24	8	20	40
27	8	12	40	6	14	20
28	12	16	44	14	6	8
29	12	4	26	14	26	18
30	8	12	18	8	24	30
31	8	8	20	10	20	34
32	4	4	28	10	34	20
33	22	16	26	6	14	16
34	22	22	24	10	10	12
35	22	12	26	14	10	16
36	23	13	18	13	26	7
37	6	6	10	22	22	34
38	12	10	16	14	14	34
39	10	10	27	7	19	27
40	24	16	24	10	12	14
41	14	10	24	12	16	24
42	16	8	28	10	22	16
43	24	16	22	8	14	16
	8.2 ± 1.3	7.6 ± 1.0	19.3 ± 1.7	9.9 ± 0.8	12.7 ± 1.1	42.3 ± 4.2

of 8 cm from one another on the same branch. The distribution of PMC in different meiotic stages is shown in Table 10, where the buds are numbered from the base to the apex according to their position on the branch.

Table 10. The number of PMC of different meiotic stages from 9 buds growing within a distance of 8 cm on the same branch in graft Z 1002-8 of L. sibirica. The fixation was performed on 29 December 1966.

Bud No.	Number of PMC in the stages							
	Diplotene	Metaphase I	Anaphase I	Interphase	Anaphase II	Telophase II	Tetrad	Microspores
1	100							
2								100
3	30	9	1	10	1	1	10	38
4	21	13		3			13	50
5	65	8		3			2	22
6	96	3		1				
7	100							
8	84	10		6				
9	98	2						
Average ± standard error	66.0 ± 13.0	5.0 ± 1.7	0.11 ± 0.11	2.6 ± 1.2	0.11 ± 0.11	0.11 ± 0.11	2.8 ± 1.7	23.3 ± 11.5

The data of Table 10 clearly indicate that the stage of development in two buds growing close to each other can be quite different. Buds Nos. 1 and 2, for instance, were growing within a distance of 1 cm from each other. This means that relatively large numbers of buds must be analysed to obtain appropriate information about the stage of development within the individual grafts. This is in agreement with the statement of ILLIES (1956) that the stage of development varies considerably from bud to bud. It might be added that the case presented constitutes an extreme one.

The data covering the course of meiosis are shown separately for the individual clones in Tables 11—17. In these tables the percentages of PMC in the various meiotic stages on different occasions, as well as the total number of PMC analysed, are presented.

In Figs. 12—18 the pattern of development of the individual clones is demonstrated graphically. In these diagrams the percentages of cells in the diplotene stage and the percentages of cells which have reached the tetrad stage are shown. Besides this, the summarized percentage of cells in the stages diakinesis—anaphase I is shown separately, as these stages according to radiogenetic investigations constitute the most radiosensitive stages for induction of various types of chromosomal aberration (cf. ERIKSSON and TAVRIN 1965).

Apparent differences in the pattern of development between various clones are present. Thus the diplotene stage was passed by 50 per cent of the PMC of the Siberian larch clone Z 1002 (cf. Fig. 17) before the maximum percentage of the diplotene stage was reached in the Japanese larch clone L 1003 (cf. Fig. 15). However, the initiation of further development from diplo-

Table 11. The percentage of cells of different stages and the total number of PMC analysed on different fixing occasions. Clone SF-E 412 L. decidua.

Fixing date	Diplo-tene	Dia-kinesis	Meta-phase I	Ana-phase I	Telo-phase I	Inter-phase	Pro-phase II	Meta-phase II	Ana-phase II	Telo-phase II	Tet-rads	Micro-spores	No. of PMC analysed
8 Nov. 66	100												600
5 Dec. 66	100												600
12 Dec. 66	100												200
19 Dec. 66	100												200
26 Dec. 66	100												700
2 Jan. 67	99.2	0.12	0.29			0.29		0.06					1700
17 Jan. 67	99.9				0.06								1700
7 Feb. 67	98.6	0.04	0.17			0.70		0.04			0.48		2300
21 Feb. 67	90.8		0.31		0.06	2.4	0.88	2.1	0.12	0.12	3.1		1600
27 Feb. 67	92.6	0.30	3.2	0.38	0.15	1.1	0.77	0.77	0.15		2.0		1300
2 Mar. 67	66.6	0.45	12.5	1.0	0.55	16.1	0.91	1.3	0.36		0.27		1100
6 Mar. 67	34.5		15.3	0.77	2.0	23.8	2.1	3.0	0.50	2.0	7.6	8.2	2200
8 Mar. 67	7.1		15.4	1.4	4.4	21.3	0.95	1.3	0.16	0.95	35.3	11.8	1900
10 Mar. 67					0.20	9.3	5.1	6.1	0.33	3.9	26.6	48.5	1500
14 Mar. 67												100	1400

Table 12. The percentage of cells of different stages and the total number of PMC analysed on different fixing occasions. Clone SF-E 655 L. decidua.

Fixing date	Diplo-tene	Dia-kinesis	Meta-phase I	Ana-phase I	Telo-phase I	Inter-phase	Pro-phase II	Meta-phase II	Ana-phase II	Telo-phase II	Tet-rads	Micro-spores	No. of PMC analysed
8 Nov. 66	100												600
14 Nov. 66	100												200
21 Nov. 66	100												200
28 Nov. 66	100												600
5 Dec. 66	100												300
12 Dec. 66	100												100
19 Dec. 66	85.4		1.6		0.13	2.1	0.38	1.3		0.2	8.9		800
26 Dec. 66	100												300
2 Jan. 67	73.0	0.28	7.2	0.43	0.28	7.3	0.28	1.3	0.28	0.14	1.4	8.2	780
17 Jan. 67	92.8		1.4	0.30	1.4	2.7	0.18	0.74	0.08		1.1		2700
7 Feb. 67	64.4	0.63	19.4	0.59	1.1	11.3	0.67	0.78	0.26	0.03	0.85		2700
21 Feb. 67	22.2		15.9	1.6	2.7	28.2	4.1	5.5	0.92	2.0	1.6	5.2	2500
27 Feb. 67	2.0	0.13	1.3	0.75	1.1	24.3	5.0	7.2	2.6	4.8	38.7	12.1	2400
2 Mar. 67	0.06					0.44	0.06	0.61	0.06	0.78	88.0	10.0	1800
6 Mar. 67						0.25					79.1	20.7	2800
8 Mar. 67											92.9	7.1	2800
10 Mar. 67											0.95	99.1	2100
14 Mar. 67												100	1400

Table 13. The percentage of cells of different stages and the total number of PMC analysed on different fixing occasions. Clone SF-E 657 L. decidua.

Fixing date	Diplo-tene	Dia-kinesis	Meta-phase I	Ana-phase I	Telo-phase I	Inter-phase	Pro-phase II	Meta-phase II	Ana-phase II	Telo-phase II	Tet-rads	Micro-spores	No. of PMC analysed
8 Nov. 66	100												500
14 Nov. 66	100												300
21 Nov. 66	100												300
28 Nov. 66	100												100
5 Dec. 66	100												500
12 Dec. 66	100												200
26 Dec. 66	100												600
2 Jan. 67	99.4		0.45	0.05							0.10		2000
17 Jan. 67	100												2200
7 Feb. 67	100												2300
21 Feb. 67	100												2100
27 Feb. 67	95.8		3.7	0.31		0.25							1600
2 Mar. 67	74.1		16.3	0.27	0.80	7.4	0.33	0.53			0.27		1500
6 Mar. 67	17.5		15.1	2.1	4.1	36.4	2.8	7.0	0.86	1.3	6.9	5.9	2200
8 Mar. 67				0.42	0.33	8.3	3.1	3.5	0.29	2.2	71.7	10.1	2400
10 Mar. 67						0.27	0.07	0.07			3.5	96.1	1500
14 Mar. 67												100	300

Table 14. The percentage of cells of different stages and the total number of PMC analysed on different fixing occasions. Clone L 1003 L. leptolepis.

Fixing date	Pachy-tene	Diplo-tene	Dia-kinesis	Meta-phase I	Ana-phase I	Telo-phase I	Inter-phase	Pro-phase II	Meta-phase II	Ana-phase II	Telo-phase II	Tet-rads	Mi-cros-pores	No. of PMC analysed
8 Nov. 66	47.0	53.0												1700
14 Nov. 66	10.3	88.7			0.67	0.33								600
23 Nov. 66	40.0	60.0												500
28 Nov. 66	37.5	62.5												800
5 Dec. 66	50.0	50.0												1000
12 Dec. 66	50.0	50.0												1200
19 Dec. 66	19.2	80.8												1300
26 Dec. 66	4.5	91.4		4.1										1100
2 Jan. 67		97.6		2.4		0.03	0.08							3800
17 Jan. 67	5.5	78.0		15.9	0.07	0.18	0.33		0.02					4500
7 Feb. 67	0.02	77.2	0.13	14.0	0.16	0.37	4.5	0.11	0.22	0.09	0.09	0.80	2.2	4500
21 Feb. 67	14.3	55.6		13.3	0.57	0.36	10.0	0.46	1.3	0.21	0.21	2.7	1.0	2900
27 Feb. 67	18.9	23.5		10.6	0.65	2.0	21.4	1.3	1.4	0.54	0.23	12.9	6.6	2600
2 Mar. 67		23.3		12.7	0.19	0.74	15.5	2.0	3.6	1.2	5.5	31.4	3.8	1900
6 Mar. 67	5.2	18.8		1.9	0.07	0.10	2.9	1.1	1.4	0.70	0.73	30.9	36.1	3000
8 Mar. 67	1.4	5.7		2.6	0.27		0.13					76.7	13.3	3000

Table 15. The percentage of cells of different stages and the total number of PMC analysed on different fixing occasions. Clone Z 1001 L. sibirica.

Fixing date	Diplo-tene	Dia-kinesis	Meta-phase I	Ana-phase I	Telo-phase I	Inter-phase	Pro-phase II	Meta-phase II	Ana-phase II	Telo-phase II	Tet-rads	Micro-spores	No. of PMC analysed
10 Oct. 66	100												400
17 Oct. 66	100												500
31 Oct. 66	100												700
7 Nov. 66	100												200
15 Nov. 66	100												2400
21 Nov. 66	98.4		0.70		0.20	0.50					0.20		1000
5 Dec. 66	94.2	0.28	3.8	0.14	0.57								700
12 Dec. 66	98.0		0.36	0.18		1.3					0.09		1100
19 Dec. 66	80.9	0.06	1.6	0.24	0.18	2.0	0.24	0.18	0.29	0.06	4.5	9.8	1700
26 Dec. 66	87.2	0.05	5.2		0.94	2.1	0.15	0.41			1.2	2.8	1700
2 Jan. 67	84.8	0.29	5.3	0.17	0.50	2.9	0.08	0.04		0.17	5.9		2400
17 Jan. 67	69.9	0.07	4.9	2.8	2.8	5.3	1.1	0.88	0.04	0.23	3.4	8.5	2600
7 Feb. 67	57.9	0.36	25.7	0.93	1.4	12.9	0.43	0.07			0.28		700
21 Feb. 67	13.9		16.9	2.1	2.8	17.9	0.75	5.3	0.12	1.1	21.8	17.4	1600
27 Feb. 67	3.0		1.5	0.65	1.1	13.8	5.8	5.0	1.8	2.2	31.6	33.7	2600
2 Mar. 67				0.07	0.20	4.4	0.33	1.4	0.33	4.0	51.9	37.3	1500
6 Mar. 67					0.04			0.08		0.19	15.4	84 3	2600
8 Mar. 67											22.7	77.3	900

Table 16. The percentage of cells of different stages and the total number of PMC analysed on different fixing occasions. Clone Z 1002 L. sibirica.

Fixing date	Diplo-tene	Dia-kinesis	Meta-phase I	Ana-phase I	Telo-phase I	Inter-phase	Pro-phase II	Meta-phase II	Ana-phase II	Telo-phase II	Tet-rads	Micro-spores	No. of PMC analysed
10 Oct. 66	100												200
17 Oct. 66	100												300
31 Oct. 66	92.0		0.84	0.36	0.10	3.6	0.05	0.15	0.05	0.10	2.8		1900
15 Nov. 66	73.0		0.94		0.22	1.2		0.33	0.56	0.17	2.6	21.3	1800
21 Nov. 66	48.6	0.38	0.38	0.19	0.14	3.8	0.05	0.52	0.38	0.05	43.0	2.5	2100
28 Nov. 66	44.6		0.67	0.22		0.72	0.11	0.56	0.05	0.05	49.1	4.0	1800
5 Dec. 66	54.0		0.70	0.10	0.25	1.2	0.25	0.25	0.40	0.10	21.2	21.5	2000
12 Dec. 66	26.5		4.3	0.24	0.24	1.4	0.35	0.18	0.29	0.12	0.47	65.9	1700
19 Dec. 66	30.2		3.4	0.03	0.50	1.5	0.22				1.5	62.8	3200
26 Dec. 66	31.7	0.15	5.4	0.20	0.75	7.3	0.60	0.95	0.05	0.50	4.8	47.6	2000
2 Jan. 67	22.9	0.04	5.9	0.38	0.73	5.7	0.35	0.85	0.35	0.35	56.6	5.9	2600
17 Jan. 67	9.8		3.6	1.2	2.3	7.6	1.5	0.61	0.10	0.29	39.0	33.8	3100
7 Feb. 67	3.0		3.8	0.27	0.70	5.7	0.16	3.2	0.08	0.22	78.6	4.2	3700
21 Feb. 67	0.05		0.73	0.18	0.14	4.8	0.64	0.41	0.05	0.14	76.9	16.1	2200
27 Feb. 67											34.5	65.5	1900
2 Mar. 67											81.2	18.8	2300
6 Mar. 67												100	2800

Table 17. The percentage of cells of different stages and the total number of PMC analysed on different fixing occasions. Clone Z 1003 L. sibirica.

Fixing date	Diplo-tene	Dia-kinesis	Meta-phase I	Ana-phase I	Telo-phase I	Inter-phase	Pro-phase II	Meta-phase II	Ana-phase II	Telo-phase II	Tet-rads	Micro-spores	No. of PMC analysed
10 Oct. 66	100												900
15 Nov. 66	100												1700
12 Dec. 66	99.0	0.67				0.33							300
19 Dec. 66	97.4	0.22	0.88			1.2	0.09				0.09		900
2 Jan. 67	88.1		2.8	0.36	0.57	7.5	0.14	0.29			0.21		1400
17 Jan. 67	90.9		2.4	1.3	1.1	3.9		0.27			0.13		1500
7 Feb. 67	63.0		14.1	0.27	0.73	17.7	0.27	1.9			2.0		1500
21 Feb. 67	28.0		41.4	2.5	3.6	8.6	0.13	1.9		0.50	0.88	12.5	800
27 Feb. 67					0.25	18.6	4.6	10.9	1.8	2.6	47.5	13.8	800
2 Mar. 67						0.45	0.09	2.6	0.73	1.1	76.5	18.6	1100
6 Mar. 67	4.8		0.72									94.5	1800
10 Mar. 67												100	1000

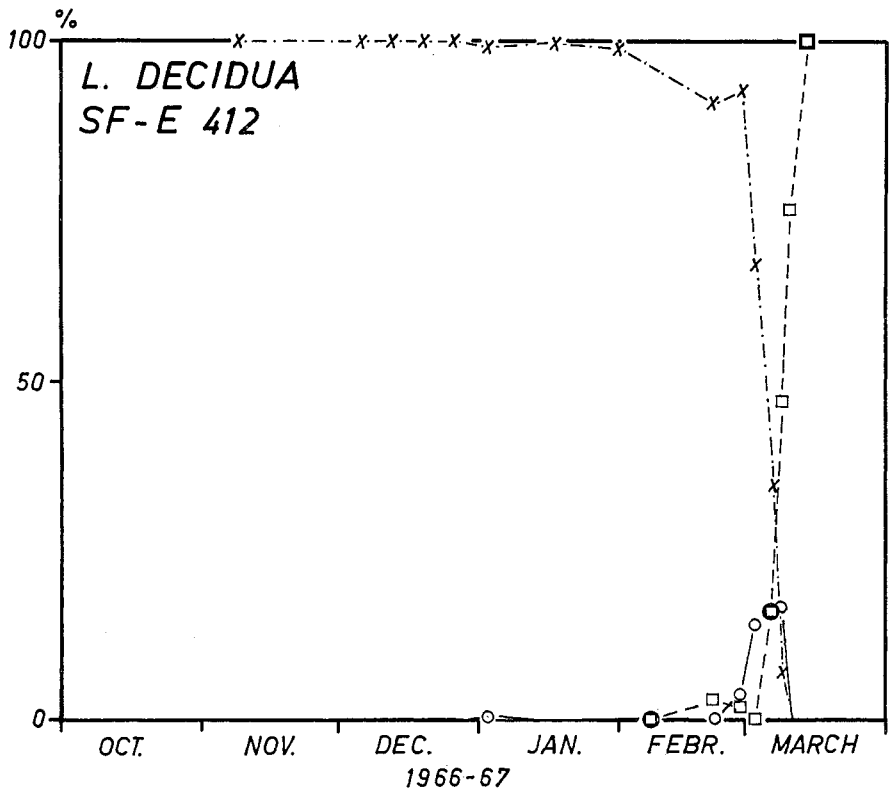

Fig. 12. The percentages of cells in diplotene (×), diakinesis—anaphase I (○), and tetrads—microspores (□) in clone SF-E 412 of *Larix decidua* on different occasions.

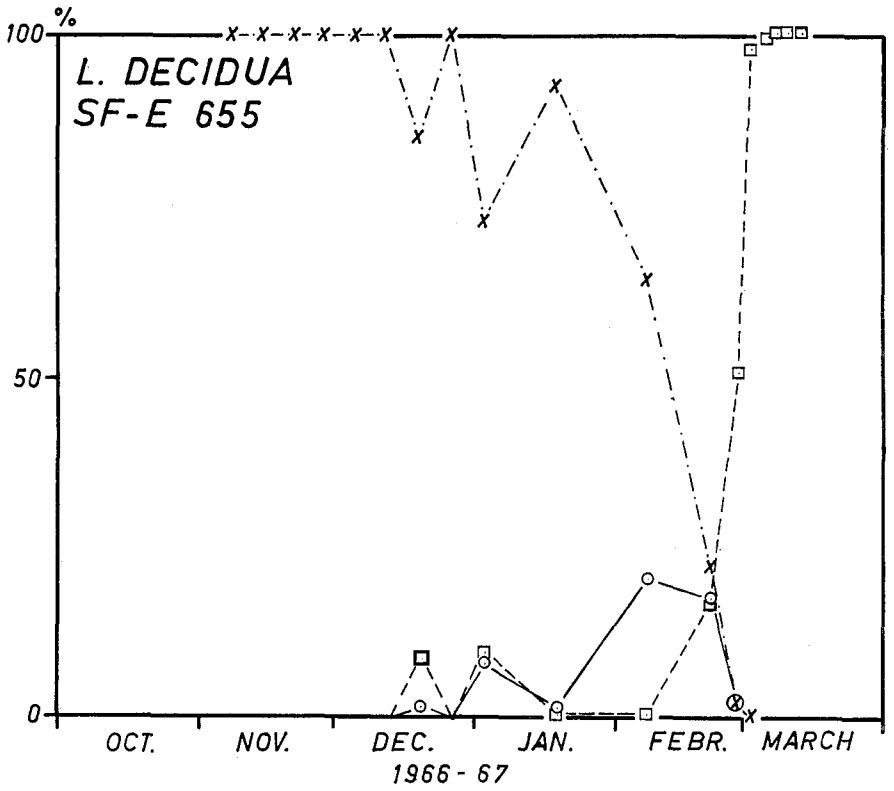

Fig. 13. The percentages of cells in diplotene (×), diakinesis—anaphase I (○), and tetrads—microspores (□) in clone SF-E 655 of *Larix decidua* on different occasions.

tene took place almost immediately following this reaching of the maximum in L 1003. The occurrence of pachytene in January—March in some of the buds (Table 14) is probably due to a complete cessation of the development in those pachytene PMC. Thus they will never complete the meiotic division.

Of particular interest was the observation that only two of seven clones tested showed the same pattern of development as was observed both for *L. decidua* and *L. sibirica* during the season 1965—66. Both clones belonged to *L. decidua*. The reason for this difference in pattern of development from season to season must be attributed to the difference in temperature conditions during the two seasons, as was pointed out above.

The four different types of developmental pattern might be characterized in the following way:

1. Diplotene is of comparatively long duration, about four months. The

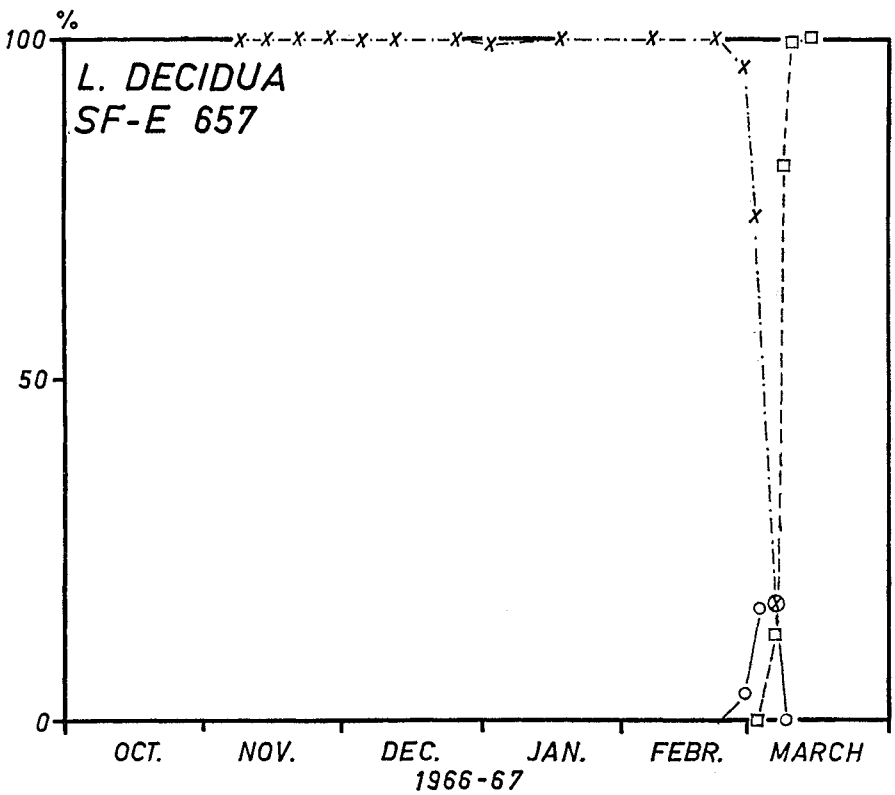

Fig. 14. The percentages of cells in diplotene (×), diakinesis—anaphase I (○), and tetrads—microspores (□) in clone SF-E 657 of *Larix decidua* on different occasions.

development, diakinesis—microspores proceeds rapidly within a period of circa ten days. Clones SF-E 412 and SF-E 657 of *L. decidua* (Figs. 12 and 14).

2. Diplotene is of short duration. The completion of meiosis proceeds relatively rapidly. Clone Z 1002, *L. sibirica* (Fig. 17).

3. This group is intermediate to the preceding ones. Thus, the initiation of the further development took place later than in (2) and the disappearance of diplotene proceeded relatively slowly in the beginning. Clones Z 1001 and Z 1003, *L. sibirica*, and clone SF-E 655 of *L. decidua* (Figs. 13, 16 and 18).

4. Diplotene was reached late and passed early although relatively slowly as in group (3). Clone L 1003, *L. leptolepis* (Fig. 15).

The rate of division following the initiation from diplotene is probably dependent on the temperature conditions following the initiation. Differences between groups cannot be regarded as proved, since the temperature

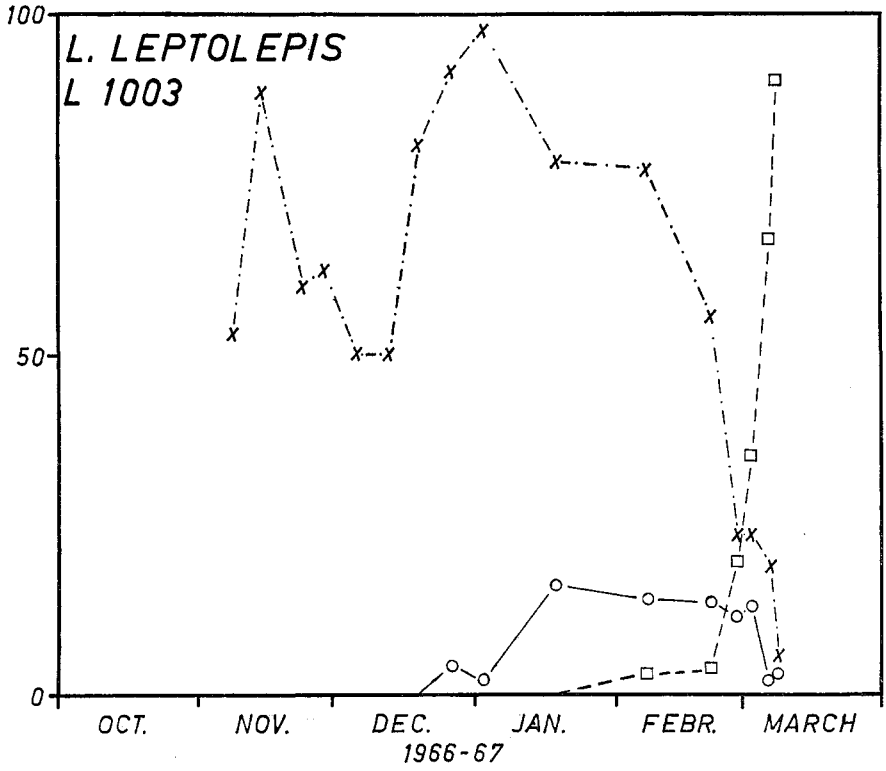

Fig. 15. The percentages of cells in diplotene (×), diakinesis—anaphase I (○), and tetrads—microspores (□) in clone L 1003 of *Larix leptolepis* on different occasions.

conditions varied during the time of this further development of the individual clones.

The differences between these groups with regard to pattern of development are so apparent that no numerical calculations are needed to verify this statement. The standard errors of the percentages reach their maxima during the phase of the highest activity in division, i.e. close to the point for 50 per cent of PMC in active division. This occurred at various times in the different clones. For one fixing day, selected according to the requirement for 50 per cent of PMC in active division of the individual clones, standard errors were calculated. The results from this calculation are summarized in Table 18. From this table it is evident that the errors are relatively large. This is also expected, since during this phase of active division buds might appear which are retarded in development and still contain only

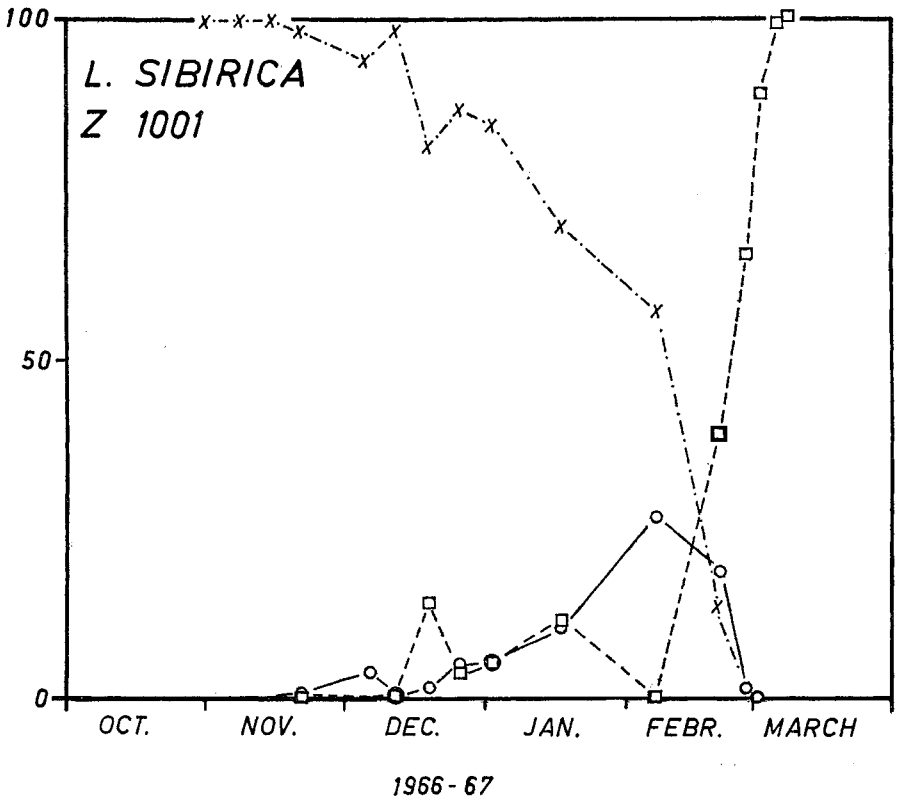

Fig. 16. The percentages of cells in diplotene (×), diakinesis—anaphase I (○), and tetrads—microspores (□) in clone Z 1001 of *Larix sibirica* on different occasions.

diplotene, as well as relatively advanced buds containing only microspores. Both will contribute to an increase of the standard error.

The large variation in the stage of development is partly due to differences between the grafts belonging to the same clone, which in turn might be attributed to differences in physiological activity of the individual grafts in a similar way as that discussed above for buds.

Although the standard errors might seem large, the most important factor for a proper pollen formation is with high probability the time for reaching diplotene as well as the time for initiation of further development from diplotene (cf. below). In these respects the four groups listed above were different.

Female meiosis was studied during spring 1967 in one *Larix decidua* tree growing in the garden of the Royal College of Forestry. The general applica-

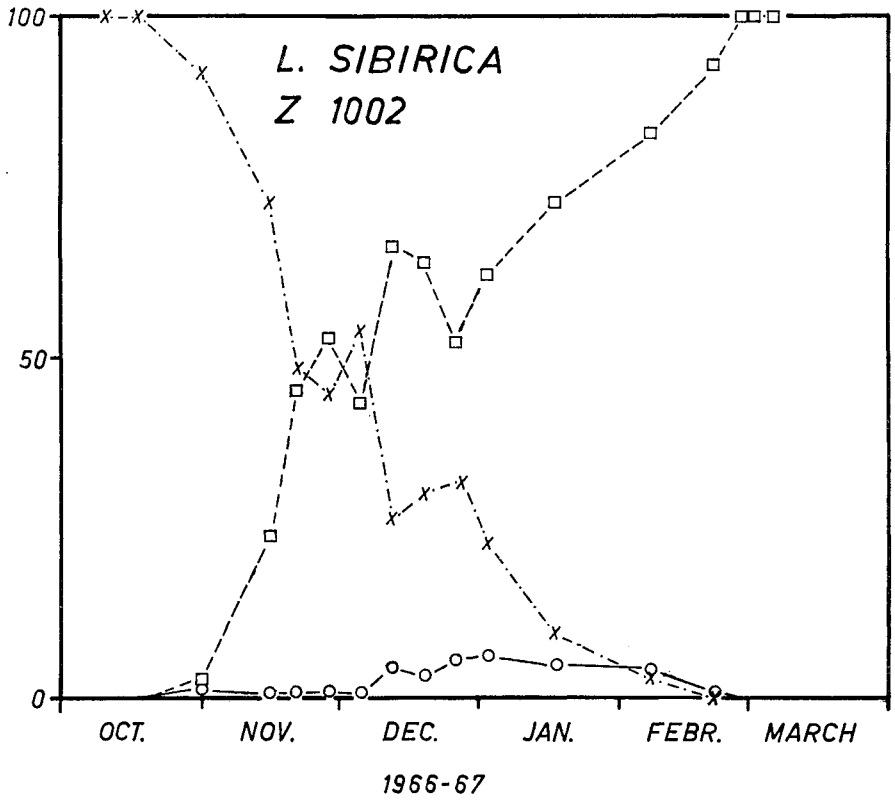

Fig. 17. The percentages of cells in diplotene (×), diakinesis—anaphase I (○) and tetrads—microspores (□) in clone Z 1002 of *Larix sibirica* on different occasions.

bility of these results might be debated, but they are interesting in the respect that the entire meiotic development seemed to take place during the latter part of April and early May 1967. There was a great variability with respect to stage of development within a cone, early prophase and tetrads occurring in the same cone. JUEL (1900) presented a detailed description of meiosis in the megaspore mother cells of *L. sibirica*. He studied the development in material which was forced to develop indoors. However, he stated that meiosis took place in April; this is in good agreement with the present observation. This means that the probability that frost damage will occur during female meiosis must be regarded as considerably lower than that it will do so during the male meiotic development. However, KIELLANDER (1966 B) reported that freezing of entire female strobili had occasionally been observed. In *L. decidua* growing in England SAXTON (1930) reported

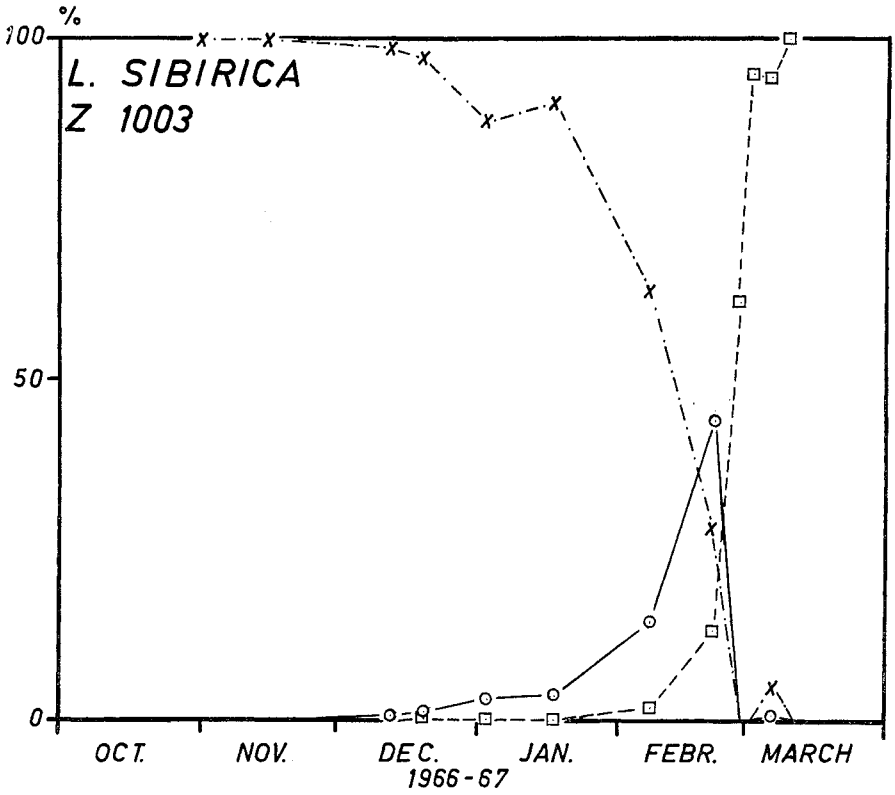

Fig. 18. The percentages of cells in diplotene (×), diakinesis—anaphase I (○), and tetrads—microspores (□) in clone Z 1003 of *Larix sibirica* on different occasions.

that megasporogenesis took place in end of February or end of March dependent upon the year.

The great variability of the stage of development within a female strobilus might constitute one reason for a low seed setting in *Larix*, supposing that some of the MMC do not complete meiosis in time to become receptive during the time of pollen dispersal. This constitutes a working hypothesis which needs careful experimental analysis. Support for the probability of this idea is obtained from the investigation of BARNER and CHRISTIANSEN (1960), where it was demonstrated that the receptiveness of the inflorescences was at the maximum during a ten-day period. This period might be longer, since the first part of the receptive period was not studied. It cannot be taken for granted that pollen dispersal in nature or in a seed orchard is distributed over such a long period as ten days (cf. SARVAS 1955). If not, only part of the potentially functionating female gametes would actually be able to take part in the fertilization. The rest would give rise to empty seeds.

Table 18. The percentages of cells of different stages in different clones on the fixing date when the standard errors were assumed to be close to their maxima.

	Fixing date	Pachy-tene	Diplo-tene	Meta-phase I	Ana-phase I	Telo-phase I	Inter-phase	Pro-phase II	Meta-phase II	Ana-phase II	Telo-phase II	Tetrads	Micro-spores
L. decidua													
SF-E 412	6 Mar. 67		34.5 ± 8.5	15.3 ± 4.8	0.77 ± 0.32	2.0 ± 0.57	23.8 ± 5.6	2.1 ± 0.89	3.0 ± 0.87	0.50 ± 0.25	1.9 ± 0.88	7.6 ± 2.0	8.2 ± 4.3
SF-E 655	21 Feb. 67		22.2 ± 6.5	15.9 ± 2.9	1.6 ± 0.37	2.7 ± 0.70	28.2 ± 4.4	4.1 ± 1.0	5.5 ± 1.3	0.92 ± 0.29	2.0 ± 0.71	11.6 ± 3.4	5.2 ± 2.9
SF-E 657	6 Mar. 67		17.5 ± 6.6	15.1 ± 3.0	2.1 ± 0.64	4.1 ± 1.0	36.4 ± 6.5	2.8 ± 1.1	7.0 ± 4.2	0.86 ± 0.58	1.3 ± 0.87	6.9 ± 3.4	5.9 ± 4.2
L. leptolepis													
L 1003	21 Feb. 67	14.3 ± 6.7	55.6 ± 6.1	13.3 ± 2.5	0.57 ± 0.20	0.36 ± 0.14	10.0 ± 1.8	0.46 ± 0.21	1.3 ± 0.41	0.21 ± 0.12	0.21 ± 0.12	2.7 ± 1.1	1.0 ± 0.74
L. sibirica													
Z 1001	21 Feb. 67		13.9 ± 7.7	16.9 ± 5.6	2.1 ± 0.92	2.8 ± 0.78	17.9 ± 4.8	0.75 ± 0.39	5.2 ± 2.2	0.12 ± 0.08	1.1 ± 0.44	21.8 ± 5.6	17.4 ± 8.6
Z 1002	5 Dec. 66		54.0 ± 10.6	0.70 ± 0.46	0.10 ± 0.07	0.25 ± 0.12	1.2 ± 0.54	0.25 ± 0.20	0.25 ± 0.12	0.40 ± 0.28	0.10 ± 0.10	21.2 ± 9.1	21.5 ± 8.8
Z 1003	21 Feb. 67		28.0 ± 7.3	41.4 ± 7.1	2.5 ± 0.76	3.6 ± 1.4	8.6 ± 3.5	0.13 ± 0.12	1.9 ± 0.95		0.50 ± 0.50	0.88 ± 0.44	12.5 ± 12.5

Irregularities during meiosis

During the course of the investigation it became evident that meiosis of the PMC was affected in several different ways. Obviously the appearance of the chromosomal irregularities is to some extent connected with the meiotic stage. Thus, the so-called "pulverization of meiosis" is confined to diplotene exclusively.

A classification of the irregularities is hard to carry out, owing to the frequent occurrence of transitions between different types. However, a classification has been performed in which the main purpose has been to facilitate the examination and the understanding of the factors responsible for the origin of the irregularity. Two main categories of irregularity have been distinguished:

1. Chromosomal irregularities.
2. Irregularities in cell division.

The first group comprises stickiness and fragmentation. Spindle abnormalities, polyspory, as well as univalents, were referred to the second group. Monads, dyads and triads might also originate from irregularities in cell division. Degeneration and micronuclei can arise as a consequence of both (1) and (2).

Stickiness. This term, coined by BEADLE (1932), means that the chromosomes stick together more or less. It soon appeared that the stickiness in the larch PMC varied in strength. Different types (A-, B-, and C-type) were therefore distinguished.

A few of the cells showing stickiness of A-type are presented in Plate 1 A—D. As seen from these micrographs a far-reaching aggregation of the chromatin has taken place. Furthermore, the cells show plasmolysis and the cytoplasm is frequently of abnormal appearance. It is probable that such cells are the expression of real frost damage, in the sense that the PMC had become frozen.

In the cells showing stickiness of B-type (Plate 1 E—F), no plasmolysis has taken place and the cytoplasm is more or less normal. As is the case for the A-type there is mostly no possibility of determining the meiotic stage in cells thus affected. However, the cells demonstrated in Plate 1 E—F were probably damaged during the pachytene stage, as may be seen from cells of transitional appearance between pachytene and the one shown in 1 E. Plate 1 F demonstrates the final appearance of such damage.

PMC affected by stickiness of the A- or B-type are arrested in development and will never complete the meiotic division, but start to degenerate.

Stickiness of C-type is illustrated in Plate 2 A—D. This group is characterized by less extreme forms of stickiness, comprising individual bivalents or the entire chromosome complement. The stage of development should be possible to identify in the C-type although the difficulties encountered are shown for instance, in Plate 2 D, where the appearance suggests metaphase I—anaphase I.

Bridges observed during anaphase I—telophase I and anaphase II—telophase II might be regarded as a special case of the C-type of stickiness. The origin of bridges might in some cases be due to the presence of paracentric inversions, but the number of bridges due to such structural changes must be regarded as considerably lower than the number of bridges caused by inaccurate separation of chromosomes due to stickiness.

It is possible that some of the PMC showing the C-type of stickiness will become more or less healed and may complete meiosis.

Stickiness is of wide occurrence among higher plants, and it might be genetical or environmental in origin. In his study of meiosis in the PMC of *Picea abies*, ANDERSSON (1947 A) reported three cases of stickiness. Their modificative origin was not ruled out completely by him, as the same type of stickiness was observed in PMC of normal trees exposed to the same conditions as the sticky spruces. No stickiness at all was detected in the normal trees when they were allowed to develop in the greenhouse.

Although CHIRA (1963, 1964, 1965 A and B, 1967, KANTOR and CHIRA, 1965) has frequently claimed that the reason for the low pollen fertility was damage induced during the meiosis, micrographs of sticky PMC are relatively rare in his papers. Therefore, it is hard to say whether stickiness was of any importance in the material studied by CHIRA. The irregularities designated as stickiness, fusion or pycnosis by CHRISTIANSEN (1960) were probably all related to the stickiness of C-type. No A- or B-type damage was shown by CHRISTIANSEN, which was probably due to the absence of such irregularities in his material. It should be mentioned that the PMC in his investigation were exposed to relatively moderate minimum temperatures, not lower than -2.3° C.

Degeneration. The causal connection between stickiness and degeneration is close as might be evident from the PMC of Plate 3 A—E. From Plate 3 A. C—E it may be seen that the chromosomes stain faintly and that the chromatin apparently starts to dissolve. Another type of degeneration is shown in Plate 3 B and F. In this case the chromatin is heavily stained. From Plate 5 D it is evident that the microspores originating from polyspory also will degenerate. No micrographs from conifers showing these irregularities

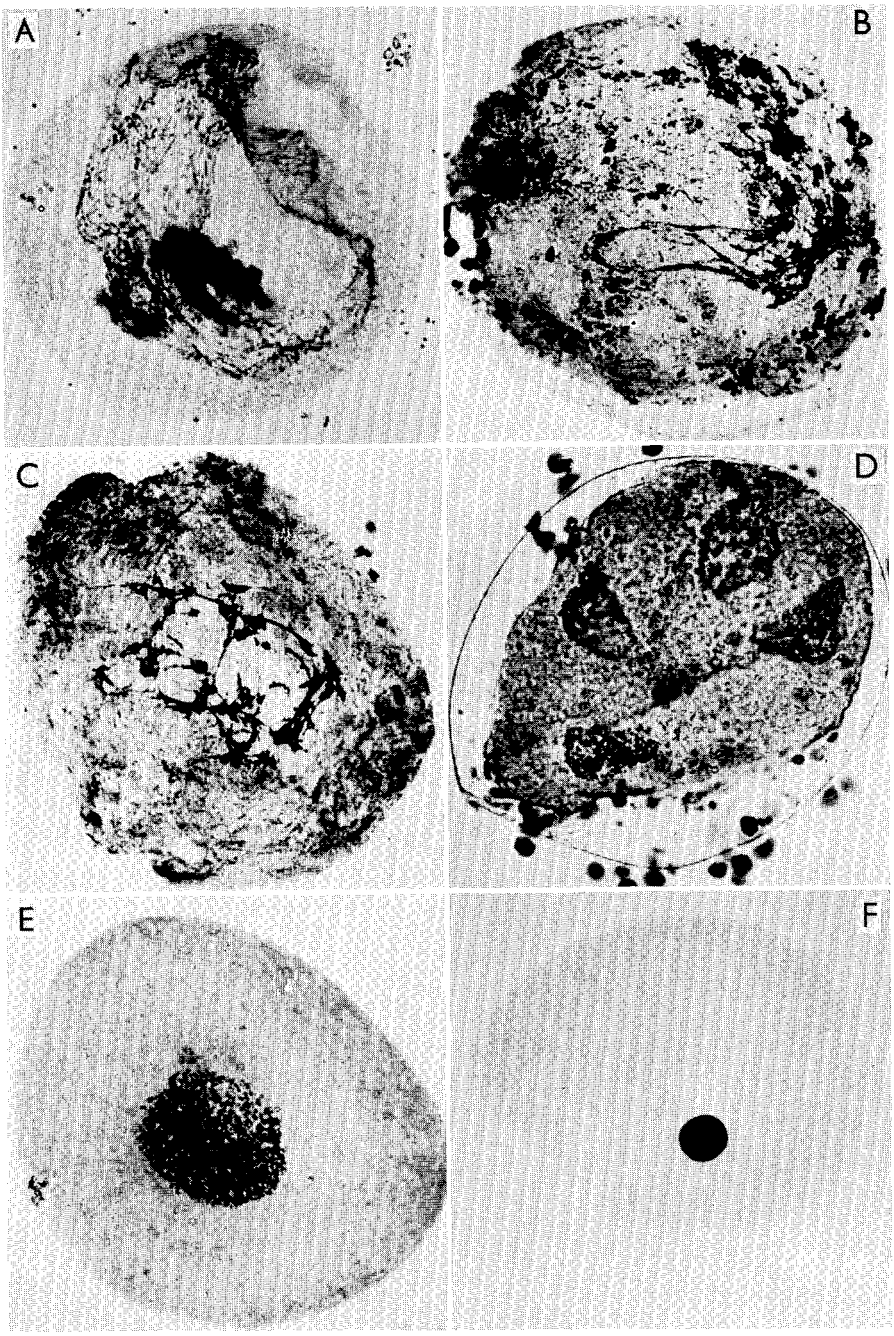

Plate 1. Stickiness of A-type (A—D) and B-type (E—F) (cf. text)

A.	Graft	Z 1002-10	L. sibirica	fixed on	25.1	1966
B.	"	Z 1003-19	L. sibirica	"	1.3	1966
C.	"	Z 1003-19	L. sibirica	"	1.3	1966
D.	"	SF-E 657-14	L. decidua	"	17.3	1966
E.	"	W 5006	L. sibirica	"	Nov.	1966
F.	"	L 1003-23	L. leptolepis	"	2.1	1967

magnification 1100—1500 x.

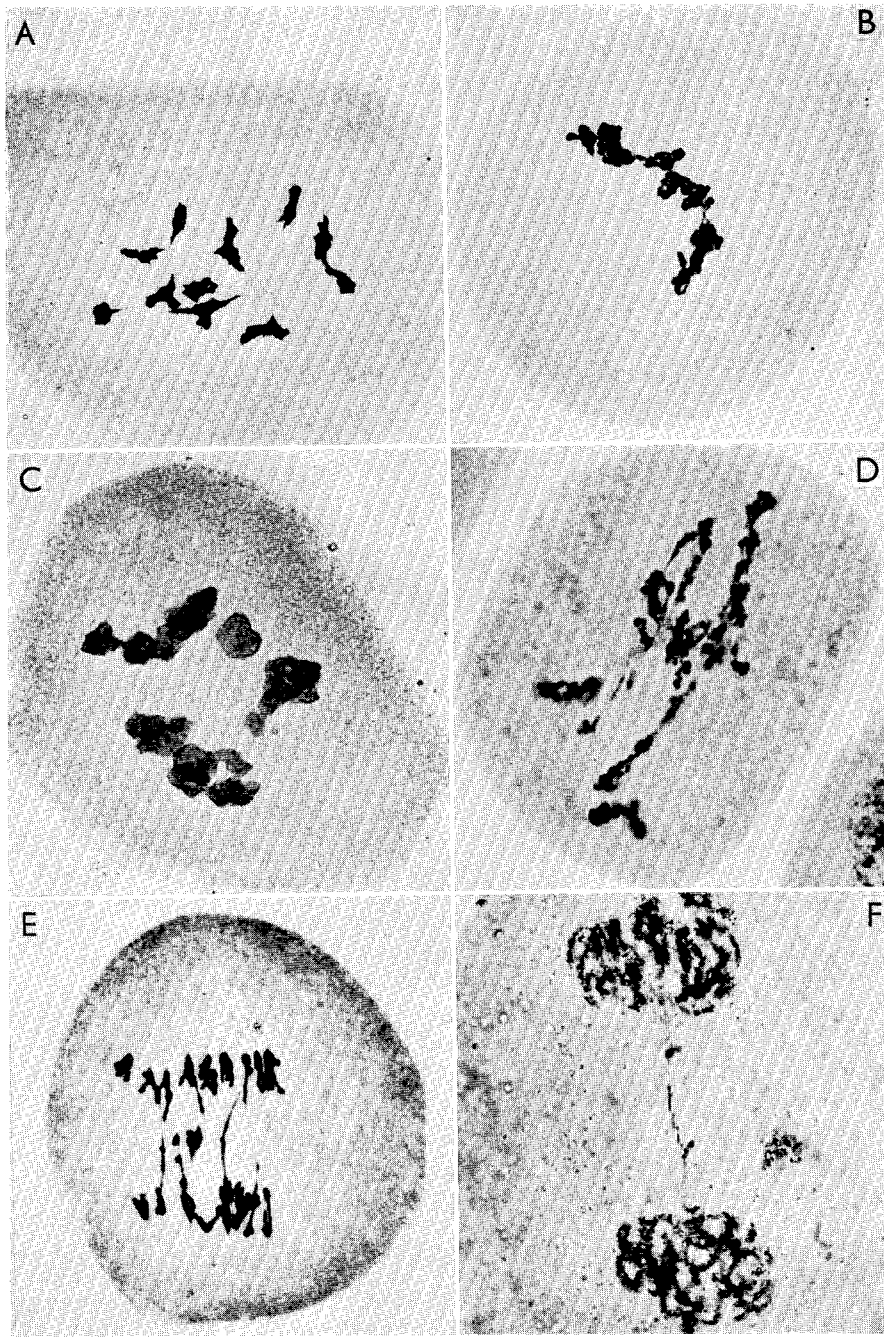

Plate 2. Stickiness of C-type (cf. text)

A.	Graft SF-E 412-7	L. decidua	fixed on	6.3		1967
B.	" Z 1003-20	L. sibirica	"	"	5.3	1966
C.	" L 1003-21	L. leptolepis	"	"	27.2	1967
D.	" L 1003-16	L. leptolepis	"	"	2.1	1967
E.	" Z 1002-14	L. sibirica	"	"	7.11	1966
F.	" L 2002	L. decidua	"	"	10.3	1967

magnification 950—1500 x.

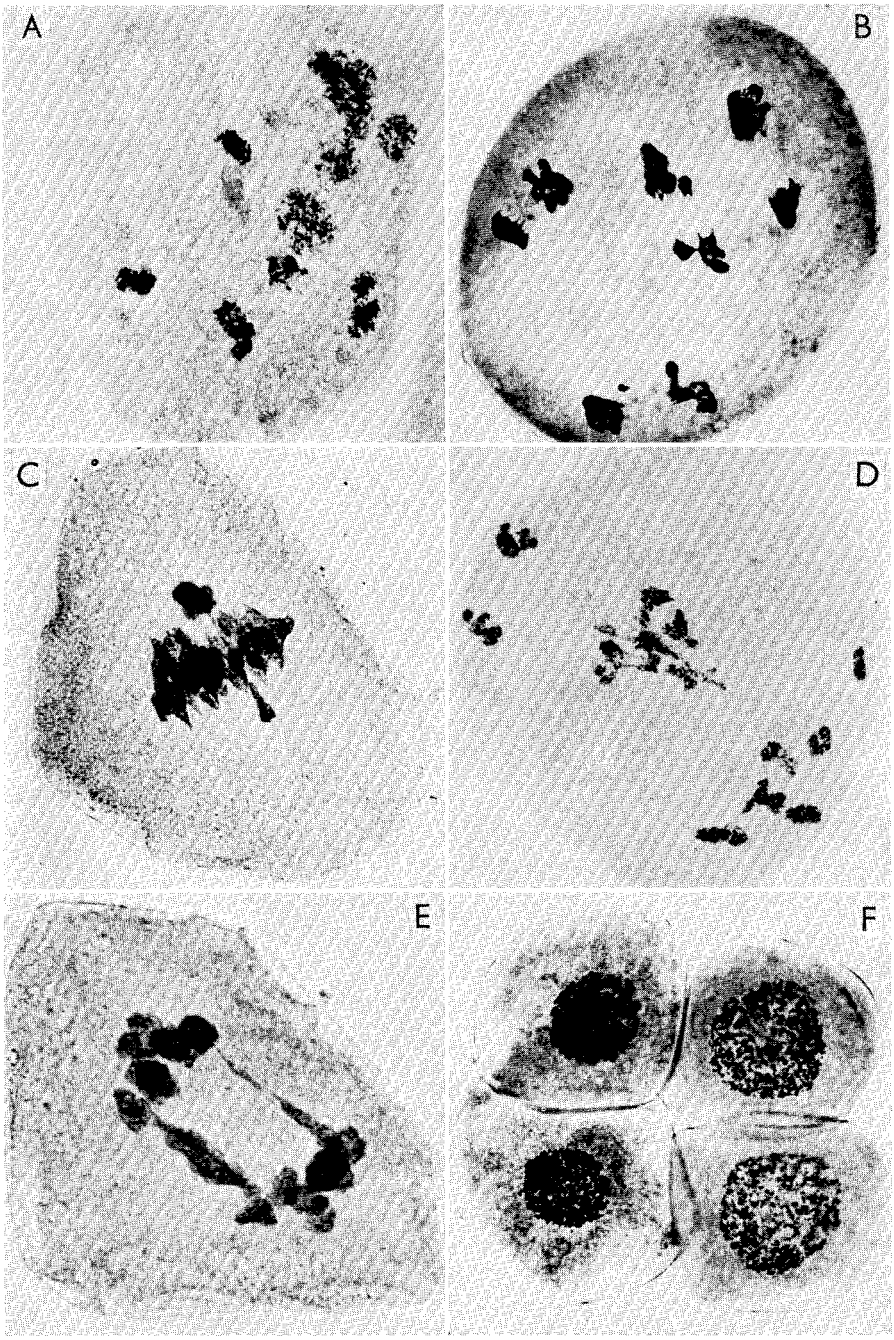

Plate 3. Degeneration during various meiotic stages (cf. text)

A. Graft L 1003-18 L. leptolepis fixed on 26.12 1966
B. " Z 1002-11 L. sibirica " " 21.11 1966
C. " L 1003-21 L. leptolepis " " 27.2 1967
D. " L 1003-16 L. leptolepis " " 2.1 1967
E. " L 1003-21 L. leptolepis " " 27.2 1967
F. " Z 1002-14 L. sibirica " " 7.11 1966

magnification 1100—1700 x.

have been published to my knowledge. As is obvious from the designation of this irregularity, it will cause pollen sterility if, on the whole, any pollen formation does take place.

Fragmentation. The "pulverized diplotene" (Plate 5 A) constitutes a special case of this irregularity which did not occur frequently in the present material. The classification of this aberration type is easy to carry out if chromosomal fragments arise as abundant as in Plate 5 B. This type of damage was also observed by CHRISTIANSEN (1960). The fragments observed in the present investigation are probably not related to the type of fragments observed during meiosis in triploids of *Allium schoenoprasum* by LEVAN (1935), where the fragments in some cases were connected with the spindle and thus could carry out a proper cell division. Fragmentation can give rise to the formation of micronuclei. It is probable that this type of irregularity is mostly connected with sterility.

Spindle abnormalities. All irregularities in which the appearance suggests an abnormal spindle formation or absence of spindle have been referred to this category. Thus this group is rather heterogeneous.

In Plate 4 A is shown a PMC containing about 48 chromosomes scattered all over the PMC. It is possible that this type of irregularity originates from a complete splitting of the bivalents during diplotene, although it cannot be ruled out that it was induced during anaphase II. At least there is a resemblance to anaphase II chromosomes.

Plate 4 B shows a PMC in which the chromosomes have a mitotic resembling appearance. This cell might be related to the one previously discussed. The only difference might be that the splitting in the centromere region took place at different times, being somewhat delayed in the cell in Plate 4 B. Both irregularities are relatively rare. They were most frequent in the PMC of *L. sibirica*, where the development from diplotene was initiated already during autumn 1966.

It might be possible that cells of this type give rise to diploid or even tetraploid pollen grains. Pollen of higher ploidy level from diploid trees has been reported by, amongst others AASS (1957) and CHRISTIANSEN (1960) and CHIRA (1965 B).

The cell in Plate 4 C contains univalents and there were no signs of the presence of a spindle in this cell. Plate 4 D shows an advanced stage of this abnormality. This cell contains more than ten micronuclei, and it is easy to understand that they have originated from individual, or few chromosomes which have become enclosed within a nuclear membrane.

In their report about meiosis in *Larix laricina* CHANDLER and MAVRODINEAU (1965) mentioned that the distribution of chromosomes during anaphase I was disturbed. However, the chromosomes (Fig. 1 D—E p. 69)

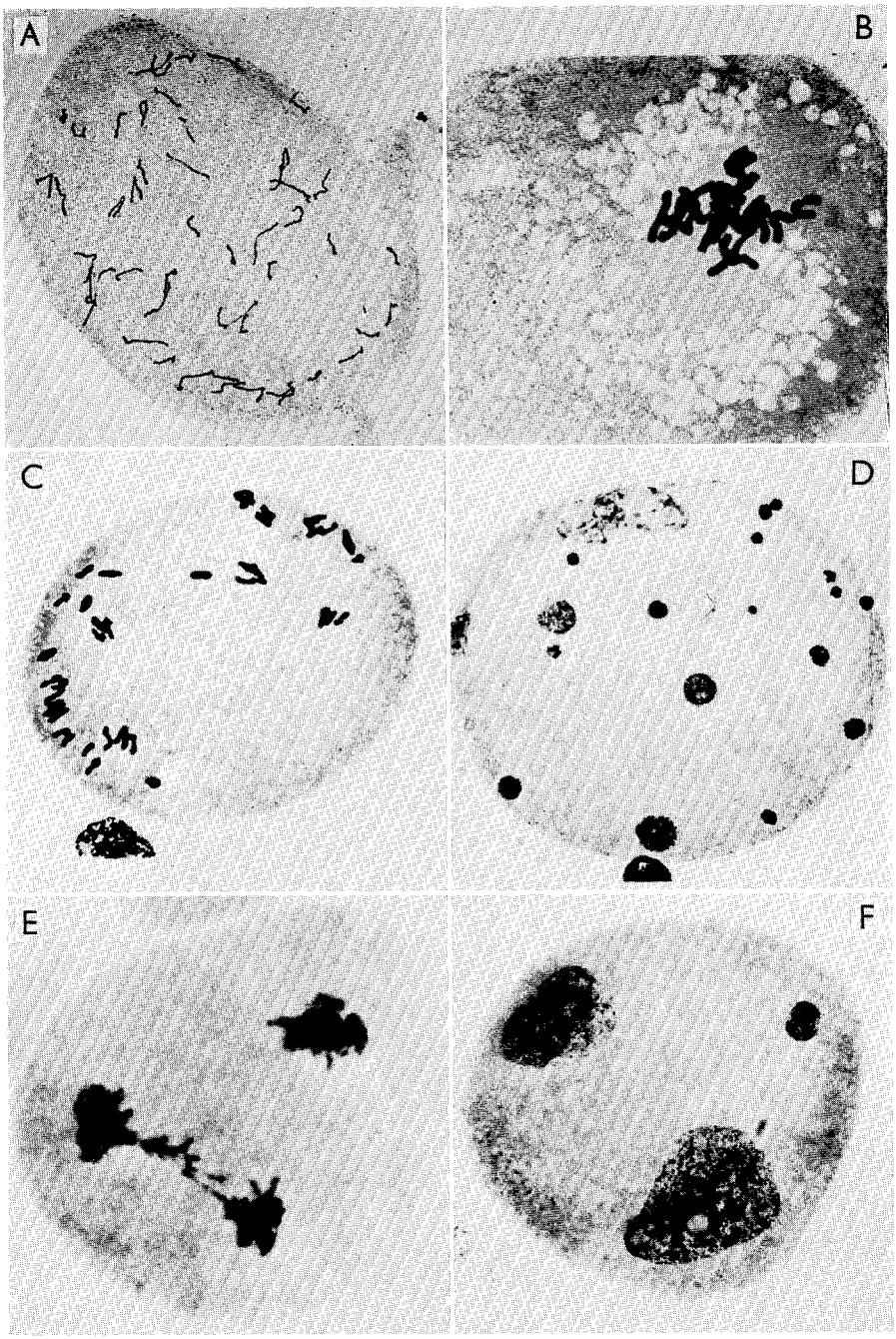

Plate 4. Various uncommon types of irregularity. For description see text.

A.	Graft Z 1002-11	L. sibirica	fixed on	21.11 1966
B.	" L 1003-17	L. leptolepis	" "	2.3 1967
C.	" Z 1002-13	L. sibirica	" "	21.11 1966
D.	" Z 1002-10	L. sibirica	" "	28.11 1966
E.	" Z 1002-10	L. sibirica	" "	14.11 1966
F.	" Z 1002-14	L. sibirica	" "	14.11 1966

magnification 750—1300 x.

shown by them resembled univalents more than anaphase I chromosomes. Since CHANDLER and MAVRODINEAU, at least partly studied the PMC from branches which were allowed to develop indoors, it might be possible that the univalents have arisen by precocious separation of bivalents. This seems to be a rather common phenomenon when the PMC are exposed to room temperature.

A detailed description of the behaviour of univalents in an asyndetic spruce was given by ANDERSSON (1947 A).

The PMC in Plate 4 E shows a cell probably containing a multipolar spindle, according to the terminology suggested for mitotic spindle abnormalities by ÖSTERGREN (1950). If the size of the chromosome groups varies, the end result might have the appearance like the one in Plate 4 F, which should be called apolar according to ÖSTERGREN (1950). However, the small nucleus of this cell might equally well have arisen from lagging chromosomes. This type of irregularity seems to have been frequent in the probably genetically conditioned asyndesis in spruce studied by ANDERSSON (1947 A and B) and in *Pinus edulis* examined by CHIRA (1967). In the last-mentioned investigation the irregularity was caused by low temperature (+ 2— + 4° C).

From the investigation by EHRENBERG (1946) it is known that the appearance of the spindle is dependent on the temperature. He measured the curvature of the outline of the spindle (defined as the inverted value of the radius) in root tip cells of *Salix fragilis* × *alba* and found that the curvature increased with decreasing temperature within the range of 3—29° C. Earlier EHRENBERG and ÖSTERGREN (1942) had reported the occurrence of multipolar spindles at 0° C in the cells of the root tip in *Salix*.

Polyspory. This type of irregularity has been described elsewhere (EKBERG *et al.* 1968). Polyspory *sensu stricto* should be confined to the eventually formed microspores. As all phases in the development until the formation of the polysporic microspores are probably induced in the same way, it is preferable to include all the stages in one group, as has been done in this investigation. The term polysporic unit will be used to designate all polysporic microspores originating from the same PMC.

At least in the uppermost division group of Plate 5 C it may be seen that the number of chromosomes in each daughter nuclei will be about six. This observation confirms the earlier statement that polysporic divisions occur without a preceding chromosome replication (cf. EKBERG *et al.* 1968). Frequently, apolar or multipolar spindles are seen during the polymitotic division.

The supernumerary separation of chromosomes might comprise all four microspores of a tetrad but sometimes only one is involved. Owing to the

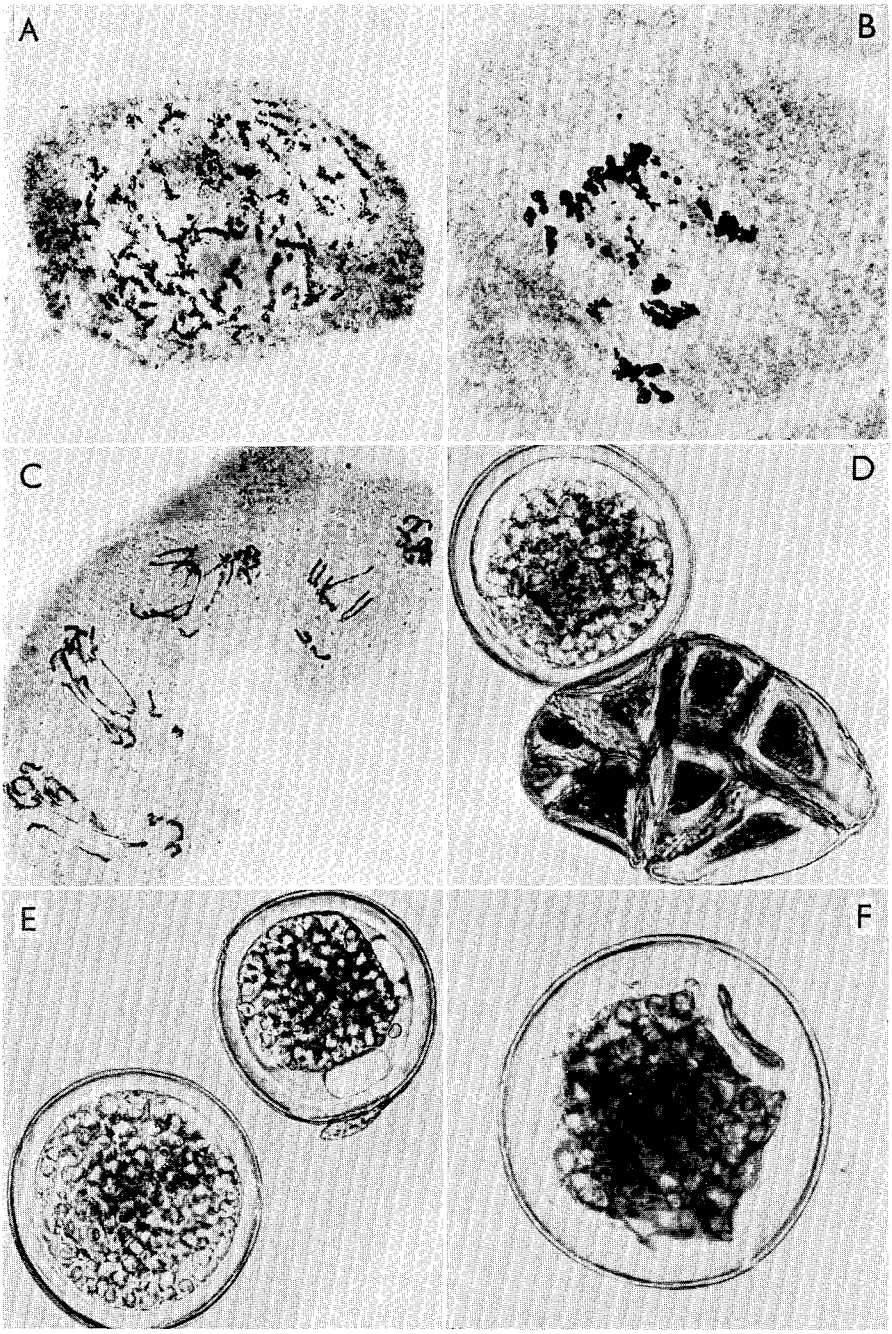

Plate 5. Fragmentation (A—B), polyspory (C—D), abnormal pollen (D—F), and one normal pollen grain (E, lower left corner).

A. Graft L 1003-25 L. leptolepis fixed on 8.11 1966
B. " L 1003-18 L. leptolepis " " 26.12 1966
C. " SF-E 412-7 L. decidua " " 6.3 1967
D. " Z 1001-1 L. sibirica " " 15.4 1967
E. " Z 1001-1 L. sibirica " " 15.4 1967
F. " Z 1001-1 L. sibirica " " 15.4 1967

magnification 900—1200 x.

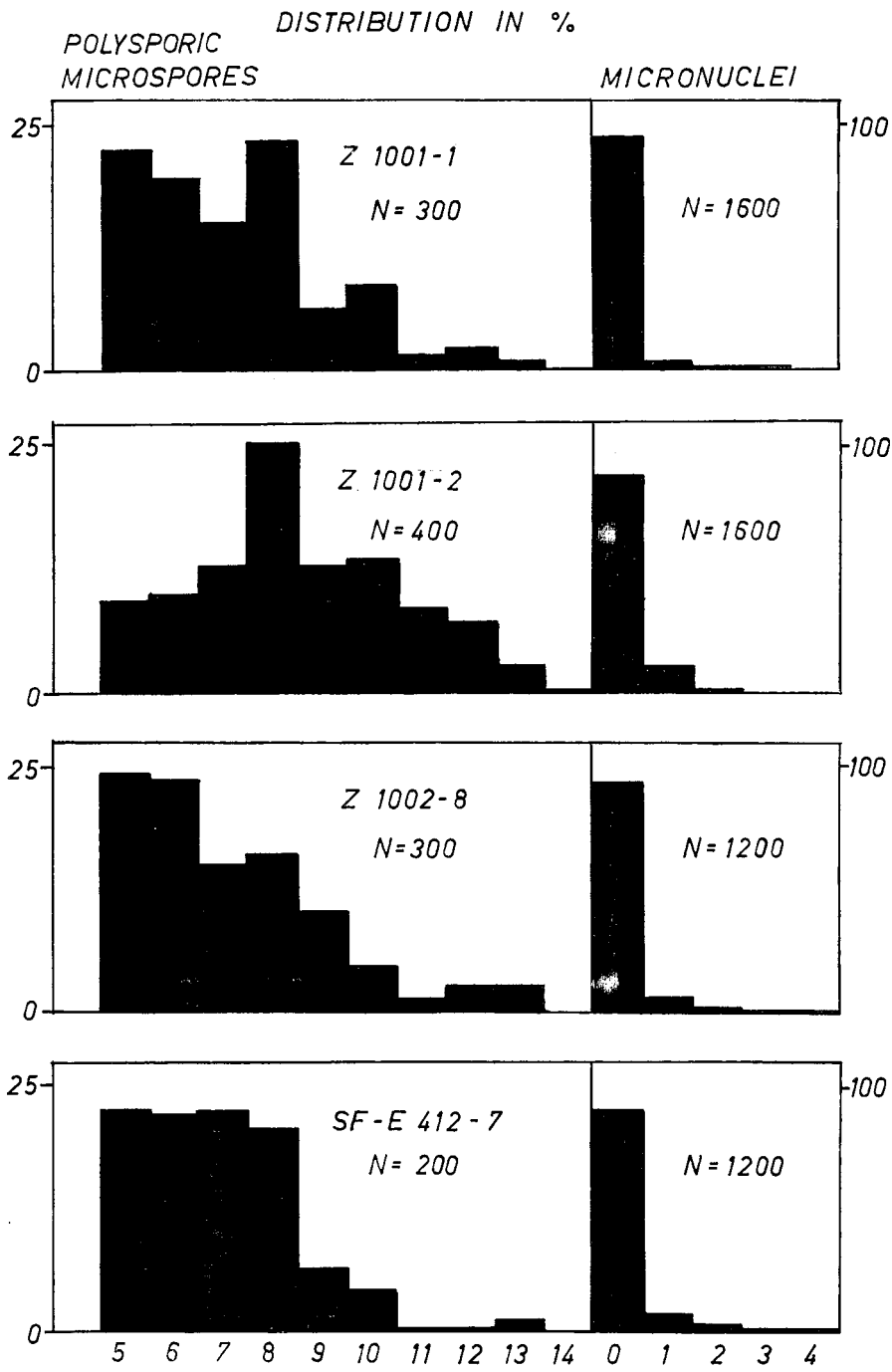

Fig. 19. The distribution of microspores in polysporic units as well as the number of micronuclei per microspore in the grafts Z 1001-1, Z 1001-2, and Z 1002-8 of *L. sibirica* and SF-E 412-7 of *L. decidua*.

simultaneous occurrence of multipolar or apolar spindles, the number of microspores formed might be considerably higher than the expected maximum of eight. To obtain some information about the distribution of different types of polysporic units a detailed classification was made in four grafts. From Fig. 19 it is seen that 5—8 microspores per polysporic unit is almost equally common in the material. It would be an interesting task to analyse the reasons for the supernumerary separation of chromosomes in one of the microspores of a tetrad but not in the other ones. It is also apparent that multipolar spindles have sometimes occurred frequently, e.g. in Z 1001-2, where polysporic units containing more than eight microspores are abundant.

As revealed in Fig. 19, micronuclei occurred in about ten per cent of the polysporic microspores. The micronuclei probably originated from lagging chromosomes or chromosomal fragments. The origin might also be dependent upon the occurrence of apolar or multipolar spindles.

Interestingly enough, polyspory was not observed at all during the season 1965—66. CHANDLER and MAVRODINEAU (1965) reported the same type of polyspory in *Larix laricina* as observed by us, although the origin of the irregularity in this case might be different. The frequent association of micronuclei with the polyspory was reported by them, too. In his study of the tetraploid *Larix decidua* CHRISTIANSEN (1950) noted several irregularities during meiosis. Amongst other things, they resulted in the formation of monads—hexads (1—6 microspores originating from one PMC).

Polyspory has been observed in other conifers as well (cf. ANDERSSON 1947 A, KHOSHOO 1957 A and B). In these cases the polyspory had probably arisen owing to genetically caused univalent formation, and is therefore not related to the polyspory observed in this investigation. The polyspory is not only confined to conifers. Thus it has been reported in such diverse genera as *Populus* (ERLANSON and HERMANN 1927), *Zea* (BEADLE 1931), *Allium* (LEVAN 1935), *Alopecurus* (JOHNSSON 1944), and *Salix* (SUDA 1963).

Monads, dyads, triads. The term monad means that only one microspore is formed from one PMC instead of the ordinary number of four. Dyads and triads are related to each other, in the first case two microspores containing the diploid chromosome number are formed from each PMC whereas three microspores are formed in the latter case, one being diploid. A monad might be formed from such a cell as the one in Plate 4 A. Compared to stickiness and polyspory, these types of irregularity were rather uncommon. Triads were reported by CHRISTIANSEN (1960) as one irregularity in diploid *L. decidua* following exposure to low temperatures. All these three types of irregularity were observed in the asyndetic pines studied by RUNQUIST (1968).

Factors influencing the origin of irregularities

The relations between stage of development and irregularities

Factors responsible for induction of irregularities can be of external or internal nature. The effect of low temperatures on the meiotic cells was discussed above and it was shown in several investigations (ANDERSSON 1947 A, 1954, 1966; CHIRA 1964, 1965 B, 1967; KANTOR and CHIRA 1965; EKBERG and ERIKSSON 1967; ERIKSSON et al. 1967; EKBERG et al 1968) that the PMC suffered from such temperatures. Also, high temperatures were in some cases responsible for irregularities (IWAKAWA and CHIBA 1952; CHIBA and WATANABE 1952). Besides the temperature, several other environmental conditions might be responsible for the origin of irregularities. However, only the temperature is easy to measure and almost no other climatic factor has been analysed in detail. However, ANDERSSON (1947 A) suggested that high wind velocities might strengthen the effect of low temperature and thus increase the rate of irregularities during the formation of microspores.

In some cases the irregularities are of genetic origin, as in the three asyndetic spruces and the "sticky" spruce studied by ANDERSSON (1947 A). Similarly the irregular meiosis observed by AASS (1957) and KHOSHOO (1957 A and B) in *Pinus silvestris* and *Cephalotaxus drupacea*, respectively, was probably also due to genetical reasons. Genetical reasons for the irregularities observed in the present investigation can be excluded, as this would require almost the same amount of irregularities in all buds within a clone which was not the case.

Previously, the stage of development and its importance for the induction of irregularities has not been considered to any great extent. This is perhaps not surprising, as this might be of greater importance in *Larix* than in other conifers owing to the considerably longer extension of meiosis in *Larix* than in other conifers. The long meiosis seems in *Larix* to be of fundamental importance for induction of frost damage at least indirectly. Therefore, a presentation of the relation between stage of development and irregularities will be given.

In Tables 19—23 the total percentages of irregularities at the different fixing dates are presented. The most common types of irregularity, stickiness and polyspory, are shown separately. As stickiness mostly occurred during the early part of meiosis and probably gave rise to degeneration during the

course of development, stickiness was replaced by degeneration in Tables 20, 21 B and 23 where the percentages of irregularity were presented on a microspore basis. The percentages of the most important stages, diplotene, diakinesis—anaphase I, as well as tetrads—microspores, are illustrated in the diagrams (Figs. 20—38). Diplotene and tetrads—microspores are of importance as they are probably rather insensitive to low temperatures (cf. below). Diakinesis—anaphase I are known to be sensitive to external agents like irradiation. Thus, almost all studies concerning radiosensitivity during meiosis have shown that these stages are the most sensitive for induction of chromosomal irregularities (cf. ERIKSSON and TAVRIN 1965).

Larix decidua. Owing to the suspect species classification of clone SF-E 655, the data from this clone will be omitted from the discussion. From Table 19 it is seen that stickiness is the dominating category of irregularity in *L. decidua*, at least when the estimation is made on a PMC basis. Besides stickiness, polyspory was relatively abundant in some cases. This abnormality could obviously not be detected until tetrad formation. Both stickiness and polyspory can result in degeneration, which was also relatively frequent, especially at the end of meiosis or following the completion of meiosis. This is well documented in Table 20 where it is seen that degeneration is the only irregularity on 28 March during the microspore stage. From Table 20 it may also be seen that polyspory is the other dominating category of irregularity of the young microspores.

In Figs. 20—27 the relations between meiotic stage and percentage of irregularities are demonstrated. One general and important trend is seen in all these diagrams, namely the simultaneous appearance of the irregularities and the stages diakinesis—anaphase I. This constitutes a confirmation of the earlier observation of the high resistance of diplotene to low temperatures down to -20 — -30° C (cf. EKBERG and ERIKSSON 1967). In this connection it should be mentioned that in *L. decidua* completely damaged buds (defined in EKBERG and ERIKSSON 1967) were detected before any passing of diplotene was observed. As they only appeared in the material fixed in March 1968, and not earlier, the induction of the damage cannot have occurred before the reaching of diplotene. Therefore, it seems that the induction may have taken place during the diplotene stage, in spite of its presumed high insensitivity to low temperatures. As diplotene is of long duration, containing as it does different phases (cf. EKBERG *et al.* 1968), it is possible that they vary with respect to frost sensitivity. During the end of diplotene, when the contraction of the chromosomes takes place, they may be as sensitive to low temperatures as they are during the subsequent highly sensitive stages. Therefore, the irregularities sometimes observed during diplotene are probably induced during the last part of this stage, when the

Table 19. Total number of pollen mother cells analysed as well as the number of normal PMC and aberrant PMC of various types on different fixing occasions in the grafts of L. decidua. The percentage of irregularities was calculated per PMC.

Clone and graft	Fixing date winter 66—67	Number of PMC investi-gated	Normal	Sticki-ness	Poly-spory	Others	% irr-egular
	2.1	300	300				0
	21.2	300	300				0
	27.2	300	296	4			1.3
	2.3	300	294	6			2.0
SF-E 412-1	6.3	400	330	70			17.5
	8.3	400	321	79			19.8
	10.3	300	291	9			3.0
	14.3	200	200				0
Sum		2500					
	2.1	300	300				0
	7.2	400	400				0
	21.2	300	298	2			0.7
SF-E 412-2	27.2	400	400				0
	2.3	300	272	26	2		9.3
	6.3	400	382	8	9	1	4.5
	8.3	300	284	2	10	4	5.3
Sum		2400					
	2.1	300	300				0
	21.2	100	100				0
	27.2	200	197	3			1.5
SF-E 412-3	2.3	200	198	2			1.0
	6.3	300	272	28			9.3
	10.3	300	291		7	2	3.0
Sum		1400					
	2.1	300	297	3			1.0
	17.1	300	299	1			0.3
	7.2	400	394	4	1	1	1.5
	21.2	300	264	15	4	17	12.0
SF-E 412-7	27.2	200	152	44	2	2	24.0
	6.3	400	349	31	19	1	12.8
	8.3	400	328	36	34	2	18.0
	10.3	200	190	7	2	1	5.0
	14.3	100	91		9		9.0
Sum		2600					
	2.1	300	298	2			0.7
	17.1	400	400				0
	7.2	400	344	56			14.0
SF-E 655-9	21.2	400	389	7	4		2.8
	27.2	300	300				0
	2.3	300	291	2	5	2	3.0
	6.3	400	392		1	7	2.0
Sum		2500					

Table 19 cont.

Clone and graft	Fixing date winter 66—67	Number of PMC investi-gated	Normal	Sticki-ness	Poly-spory	Others	% irreg-ular
	2.1	300	269	31			10.3
	17.1	200	178	16	6		11.0
	21.2	400	225	175			43.8
SF-E 655-20	27.2	300	190	110			36.7
	2.3	200	181		4	15	9.5
	6.3	400	382	1	8	9	4.5
Sum		1800					
	2.1	400	399	1			0.2
	27.2	300	299	1			0.3
SF-E 657-11	2.3	300	257	41		2	14.3
	6.3	400	360	37		3	10.0
	8.3	300	294	2		4	2.0
Sum		1700					
	2.1	300	300				0
	21.2	300	300				0
SF-E 657-13	27.2	300	276	22		2	8.0
	2.3	200	123	76		1	38.5
	6.3	400	379	19	1	1	5.2
	8.3	300	294			6	2.0
Sum		1800					
	2.1	300	300				0
	21.2	200	200				0
	27.2	400	396	4			1.0
SF-E 657-14	2.3	300	262	34		4	12.7
	6.3	300	216	84			28.0
	8.3	400	400				0
	10.3	300	300				0
Sum		2200					
	2.1	400	400				0
	21.2	300	299	1			0.3
	27.2	400	291	70		39	27.2
SF-E 657-16	2.3	400	364	36			9.0
	6.3	600	540	59		1	10.0
	8.3	300	298	2			0.7
	10.3	300	297		1	2	1.0
Sum		2700					

activity in the cell is at a high level or the irregularities arise during passing from diplotene to diakinesis.

The agreement between the maximum percentage of irregularity observed and the pollen sterility was in a few cases good (cf. Figs. 21—23).

Unless the material analysed is too limited, the percentage of irregularities

Table 20. Total number of microspores analysed as well as the number of normal microspores and aberrant microspores of various types on different fixing occasions in the grafts of L. decidua. The percentage of irregularities was calculated per microspore analysed.

Clone and graft	Fixing date winter 66—67	Number of microspores investigated	Normal	Degeneration	Polyspory	Others	% irregular
SF-E 412-1	8.3	1200	1200				0
	14.3	800	800				0
	28.3	800	796	4			0.5
Sum		2800					
SF-E 412-2	8.3	800	715		77	8	10.6
	10.3	1200	1049		148	3	12.6
	28.3	1200	1111	89			7.4
Sum		3200					
SF-E 412-3	10.3	1200	1132		66	2	5.7
	28.3	1200	1070	130			10.8
Sum		2400					
SF-E 412-7	8.3	372	323		37	12	13.2
	14.3	1170	996	2	84	88	14.9
	28.3	1200	989	211			17.6
Sum		2742					
SF-E 655-9	27.2	1200	1194		6		0.5
	2.3	1190	1139		42	9	4.3
	6.3	1600	1584		8	8	1.0
	28.3	1200	1111	89			7.4
Sum		5190					
SF-E 655-20	2.3	400	331	36	33		17.2
	6.3	1590	1541	5	40	4	3.1
	28.3	800	727	73			9.1
Sum		2790					
SF-E 657-11	8.3	1200	1194			6	0.5
	14.3	1200	1184	4	5	7	1.3
	28.3	1200	1192	8			0.7
Sum		3600					
SF-E 657-13	8.3	1200	1190			10	0.8
	28.3	1200	1179	21			1.8
Sum		2400					
SF-E 657-14	8.3	400	399			1	0.2
	10.3	1200	1200				0
	28.3	1200	1160	40			3.3
Sum		2800					
SF-E 657-16	10.3	1200	1190		8	2	0.8
	28.3	1600	1524	76			4.8
Sum		2800					

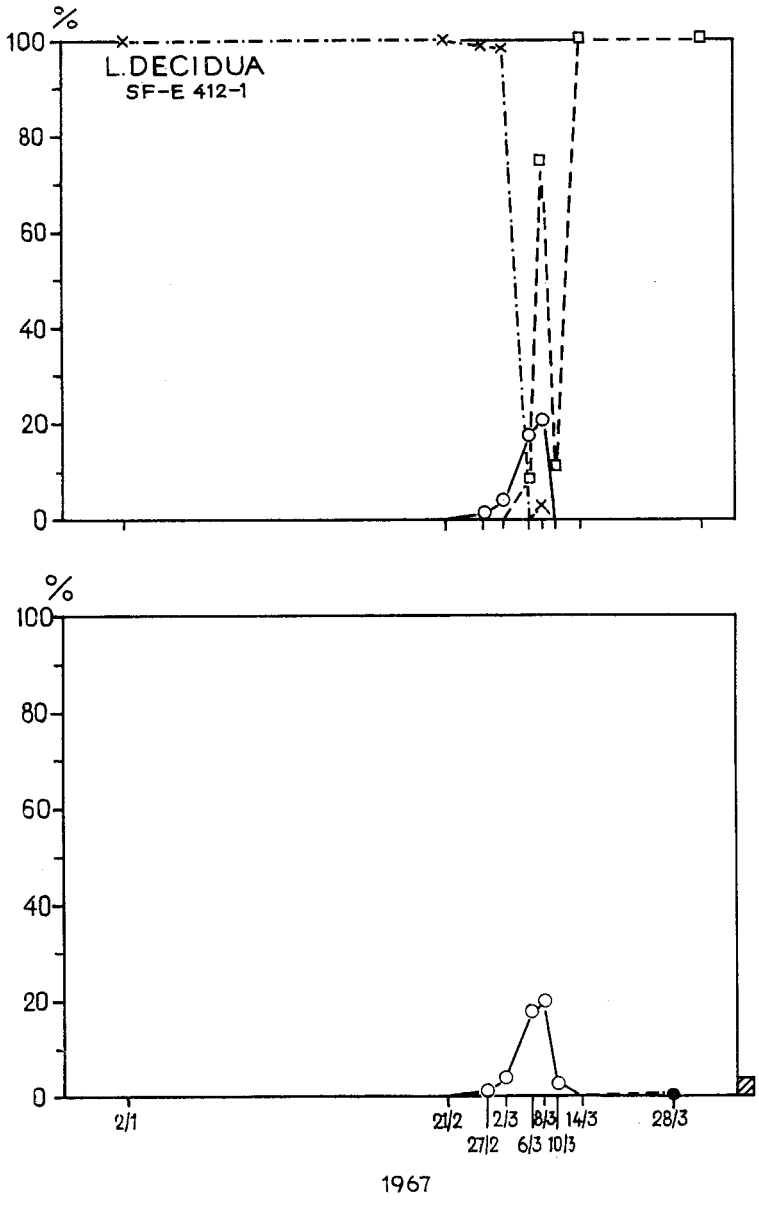

Fig. 20. Graft SF-E 412-1 of *L. decidua*. The percentages of cells in diplotene (×), diakinesis—anaphase I (○), and tetrads—microspores (□) are shown in the upper part of the diagram. Below, the percentages of irregularity in approximately the same cells are shown. The irregularities were estimated on a PMC basis (○) or on a microspore basis (●). The hatched column refers to the pollen sterility.

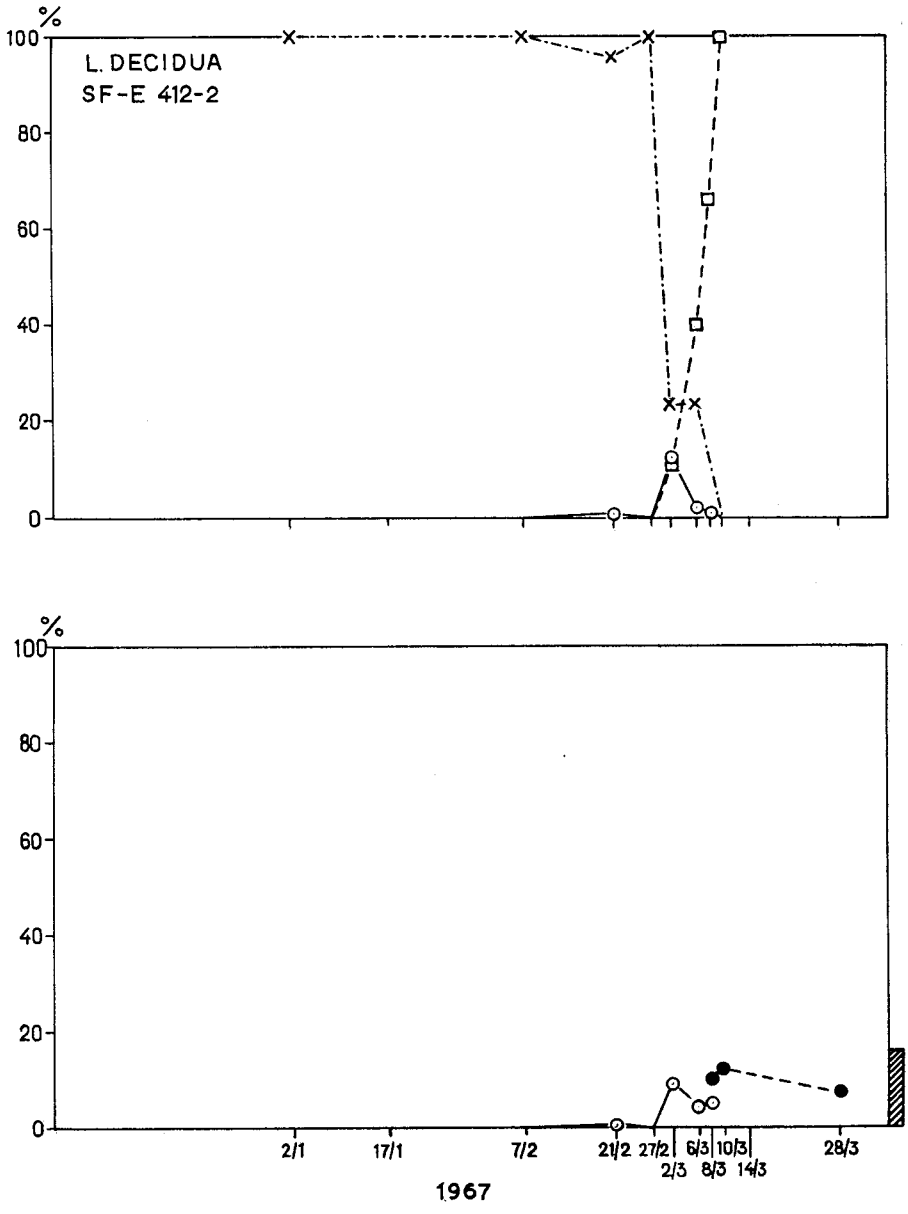

Fig. 21. Graft SF-E 412-2 of *L. decidua*. The percentages of cells in diplotene (×), diakinesis—anaphase I (○), and tetrads—microspores (□) are shown in the upper part of the diagram. Below, the percentages of irregularity in approximately the same cells are shown. The irregularities were estimated on a PMC basis (○) or on a microspore basis (●). The hatched column refers to the pollen sterility.

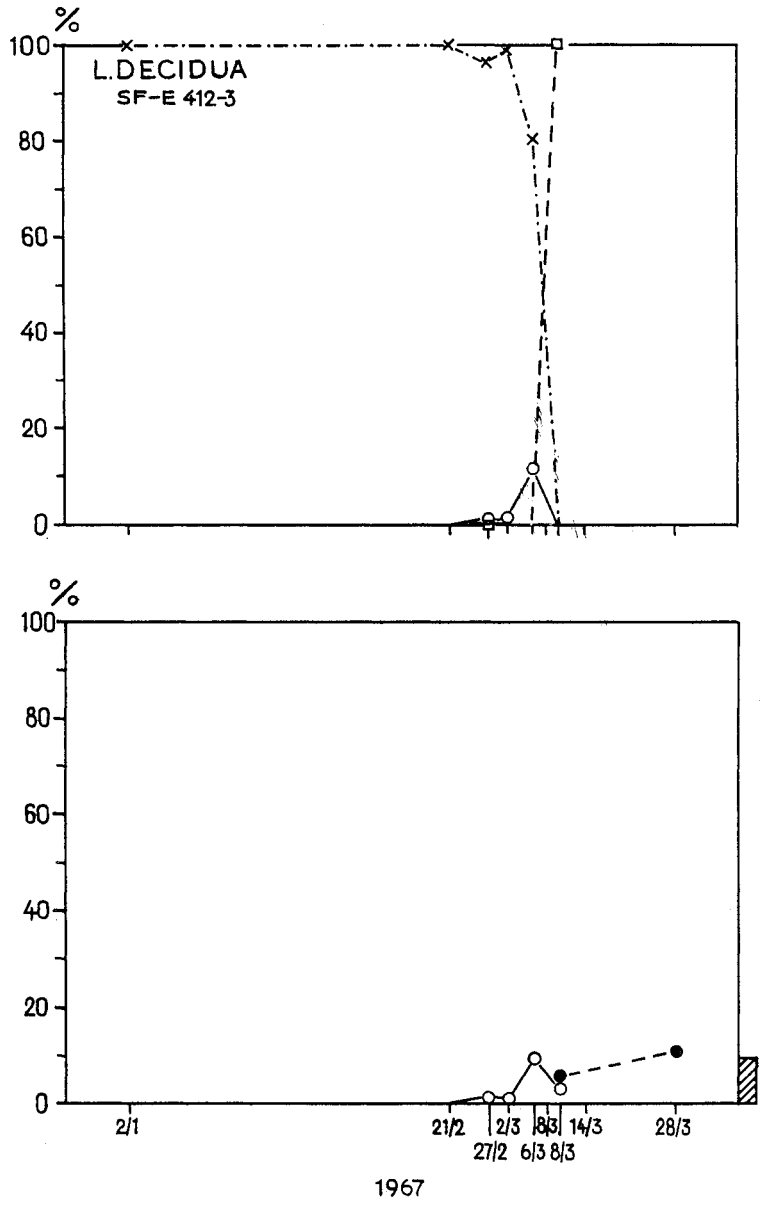

Fig. 22. Graft SF-E 412-3 of *L. decidua*. The percentages of cells in diplotene (×), diakinesis—anaphase I (○) and tetrads—microspores (□) are shown in the upper part of the diagram. Below, the percentages of irregularity in approximately the same cells are shown. The irregularities were estimated on a PMC basis (○) or on a microspore basis (●). The hatched column refers to the pollen sterility.

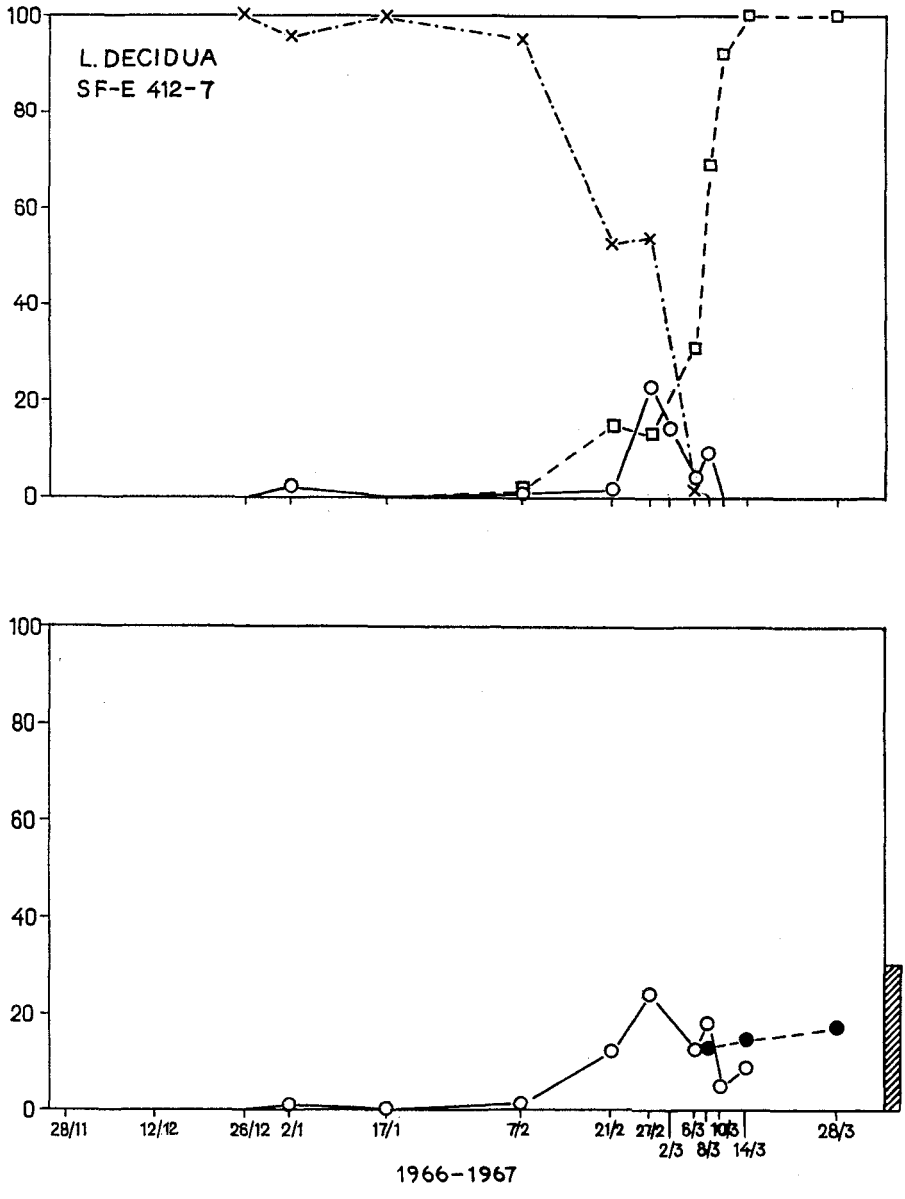

Fig. 23. Graft SF-E 412-7 of *L. decidua*. The percentages of cells in diplotene (×), diakinesis—anaphase I (○), and tetrads—microspores (□) are shown in the upper part of the diagram. Below, the percentages of irregularity in approximately the same cells are shown. The irregularities were estimated on a PMC basis (○) or on a microspore basis (●). The hatched column refers to the pollen sterility.

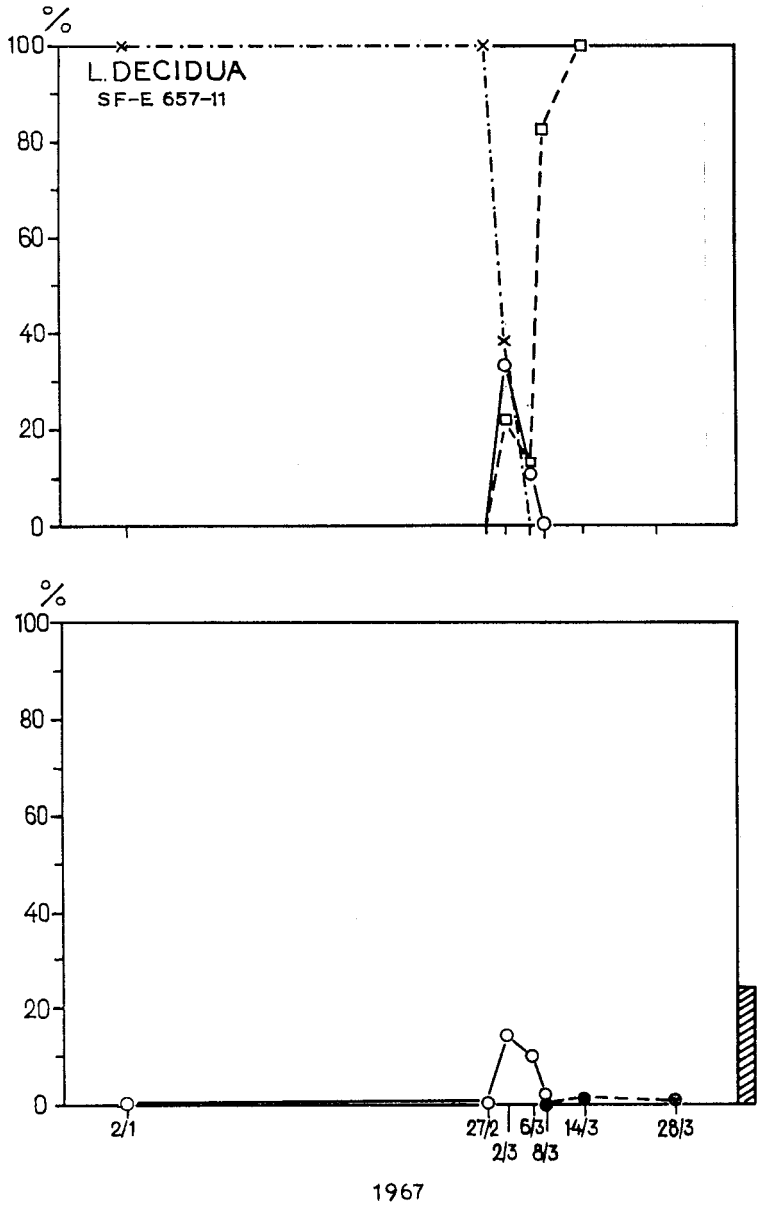

Fig. 24. Graft SF-E 657-11 of *L. decidua*. The percentages of cells in diplotene (×), diakinesis—anaphase I (○), and tetrads—microspores (□) are shown in the upper part of the diagram. Below, the percentages of irregularity in approximately the same cells are shown. The irregularities were estimated on a PMC basis (○) or on a microspore basis (●). The hatched column refers to the pollen sterility.

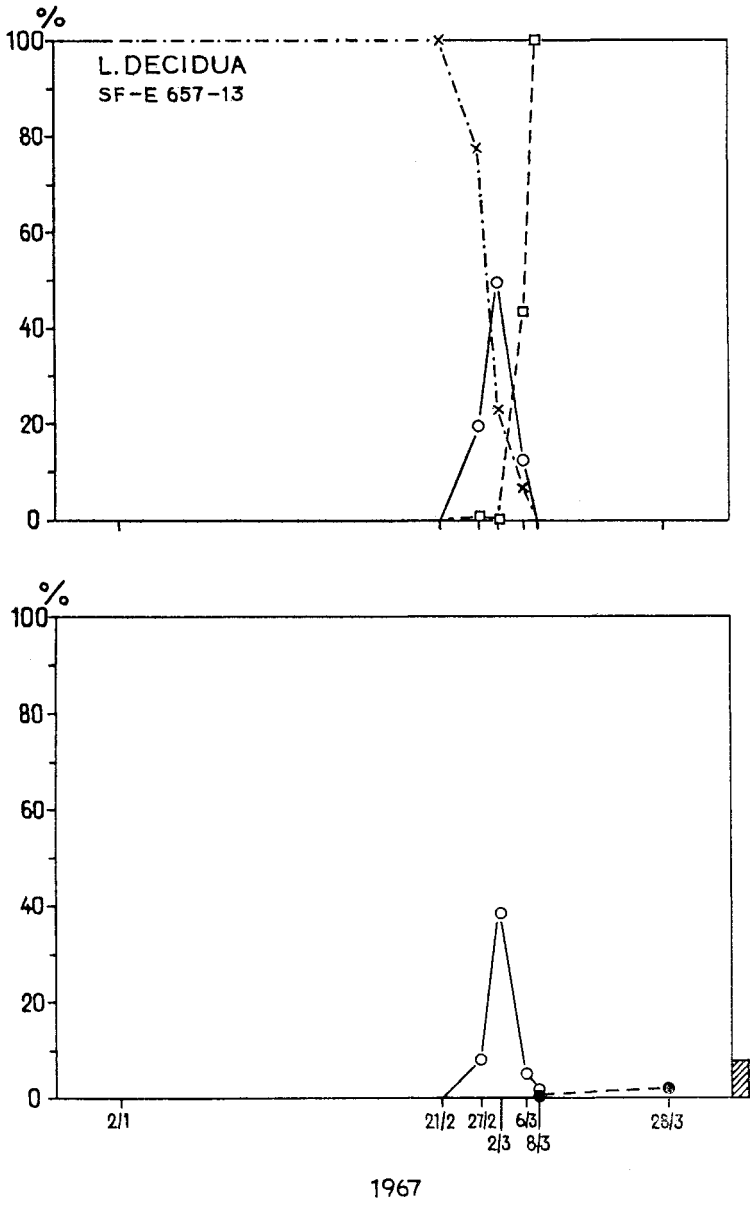

Fig. 25. Graft SF-E 657-13 of *L. decidua*. The percentages of cells in diplotene (×), diakinesis—anaphase I (○), and tetrads—microspores (□) are shown in the upper part of the diagram. Below, the percentages of irregularity in approximately the same cells are shown. The irregularities were estimated on a PMC basis (○) or on a microspore basis (●). The hatched column refers to the pollen sterility.

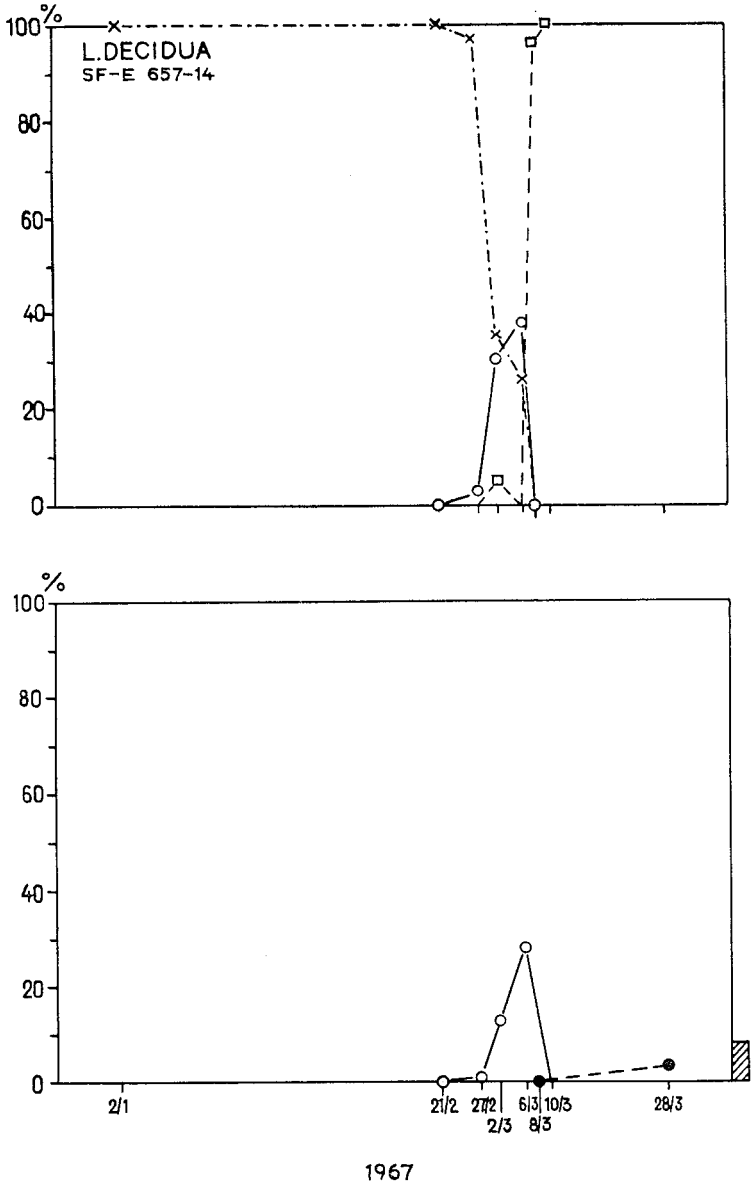

Fig. 26. Graft SF-E 657-14 of *L. decidua*. The percentages of cells in diplotene (×), diakinesis—anaphase I (○), and tetrads—microspores (□) are shown in the upper part of the diagram. Below, the percentages of irregularity in approximately the same cells are shown. The irregularities were estimated on a PMC basis (○) or on a microspore basis (●). The hatched column refers to the pollen sterility.

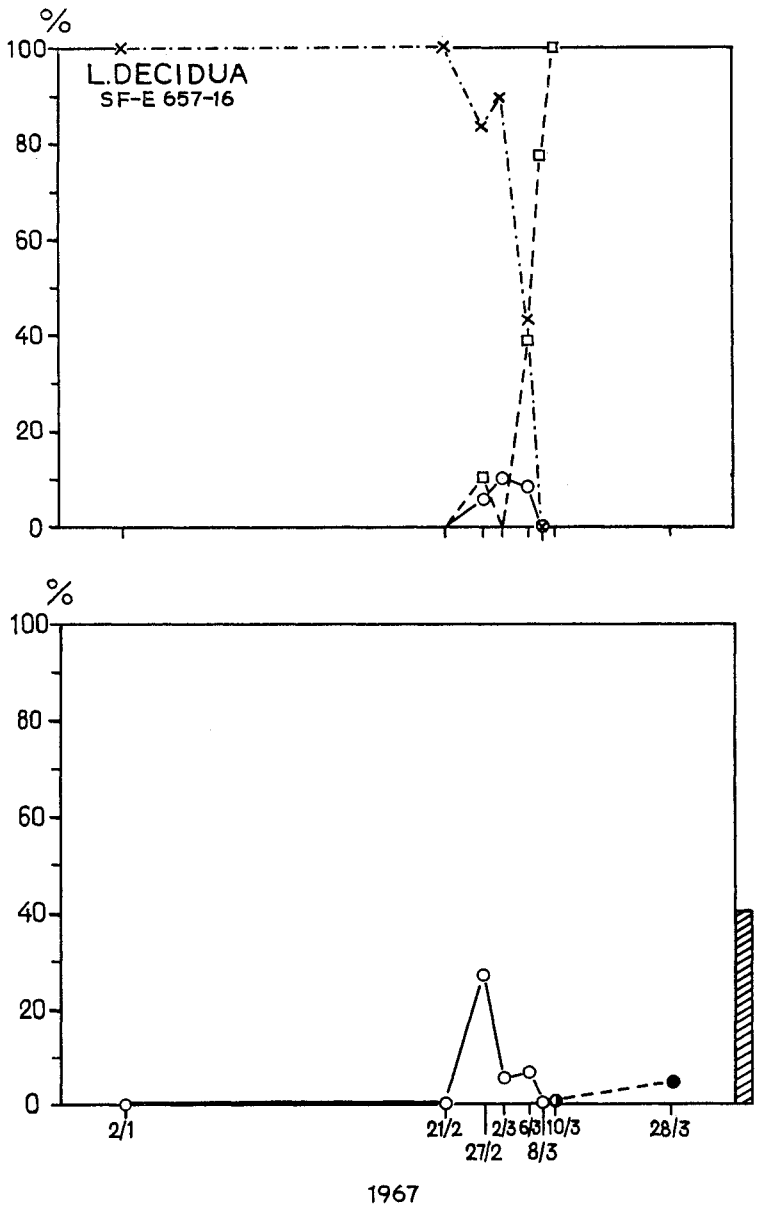

Fig. 27. Graft SF-E 657-16 of *L. decidua*. The percentages of cells in diplotene (×), diakinesis—anaphase I (○), and tetrads—microspores (□) are shown in the upper part of the diagram. Below, the percentages of irregularity in approximately the same cells are shown. The irregularities were estimated on a PMC basis (○) or on a microspore basis (●). The hatched column refers to the pollen sterility.

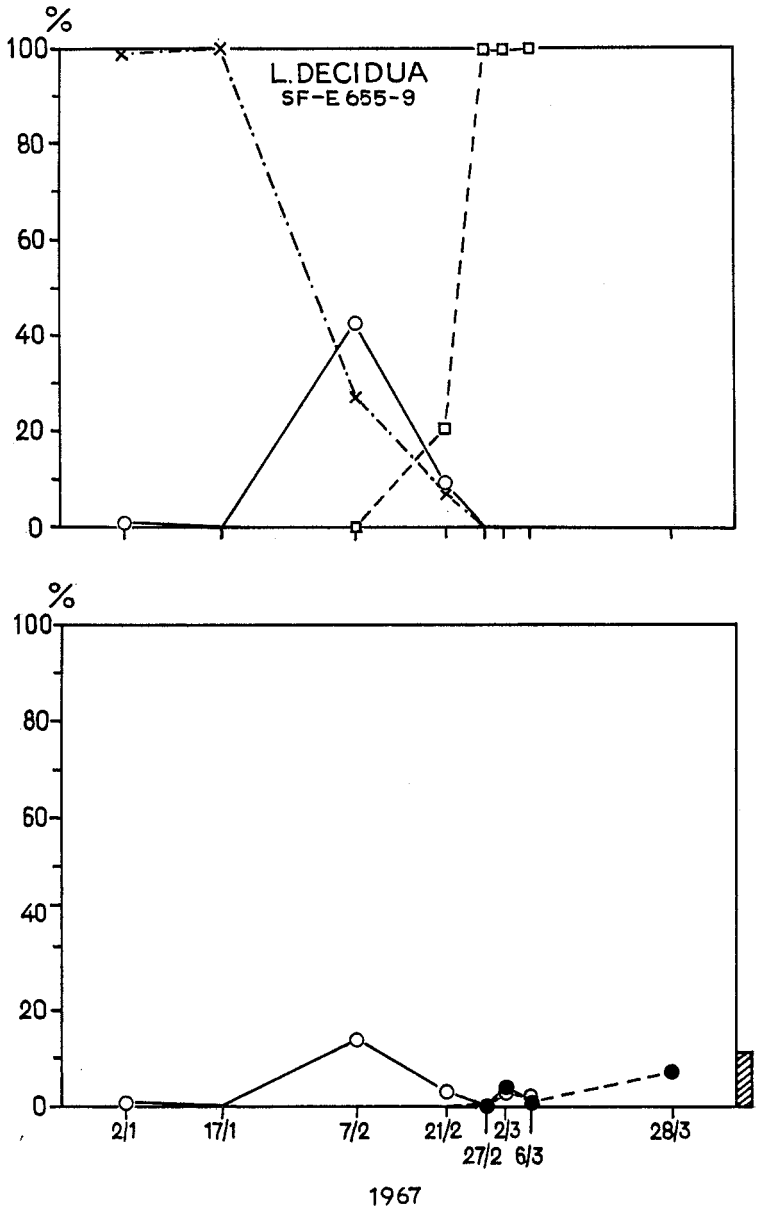

Fig. 28. Graft SF-E 655-9 of *L. decidua*. The percentages of cells in diplotene (×), diakinesis—anaphase I (○), and tetrads—microspores (□) are shown in the upper part of the diagram. Below, the percentages of irregularity in approximately the same cells are shown. The irregularities were estimated on a PMC basis (○) or on a microspore basis (●). The hatched column refers to the pollen sterility.

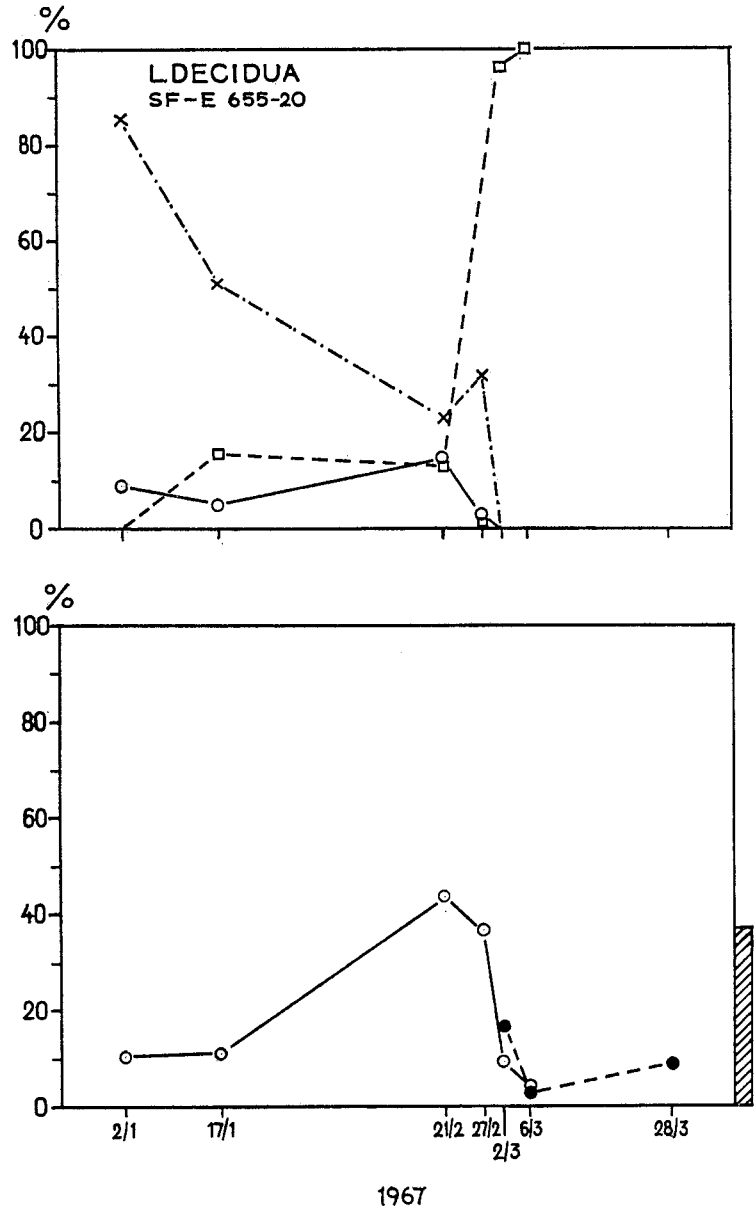

Fig. 29. Graft SF-E 655-20 of *L. decidua*. The percentages of cells in diplotene (×), diakinesis—anaphase I (○), and tetrads—microspores (□) are shown in the upper part of the diagram. Below, the percentages of irregularity in approximately the same cells are shown. The irregularities were estimated on a PMC basis (○) or on a microspore basis (●). The hatched column refers to the pollen sterility.

is not expected to decrease along with time. This will be discussed in connection with the temperature influence upon the induction of irregularities (see also Fig. 41).

In some cases (Figs. 20, and 25—27) a pronounced peak of the percentage of irregularities was observed. The decrease in the percentage of irregularities can be due to healing to a large extent. Support for this is obtained from those cases in which the pollen sterility was considerably lower than the percentage of irregularities observed during meiosis. This was the case for grafts SF-E 412-1, SF-E 657-13, and SF-E 657-14 (cf. Figs. 20, 25—26). As the mild stickiness of C-type was the dominating irregularity in these grafts, further support for the healing hypothesis is obtained. However, the damage might be so severe that no pollen formation at all took place, a situation resembling the one following irradiation during meiosis of *Vicia faba* (Ecochard perconal communication). Another explanation might be that it is impossible to detect irregularities in the cells fixed later than the appearance of the peak. Thus, some irregularities might be hidden e.g. during the microspore stage. This last explanation is not valid when the pollen sterility was found to be considerably lower than the percentage of irregularities, as was found in some cases (cf. above). A pronounced peak for the irregularities was also observed in SF-E 657-16 (Fig. 27). In contrast to the other grafts showing a peak, the pollen sterility of this graft was high, amounting to about 40 per cent. This high sterility is, however, somewhat suspect as the rapid passing of the sensitive cells during relatively favourable temperature conditions does not support the occurrence of a high pollen sterility.

Generally it can be stated that the percentages of irregularities in *L. decidua* were relatively low, in no case exceeding 50 per cent but mostly close to or less than 10 per cent. The rapid passing of the sensitive stages during relatively favourable temperature conditions is certainly responsible for the low sterility.

Larix leptolepis. The interpretation of the data from the grafts of *L. leptolepis* is somewhat complicated, partly owing to the occurrence of completely damaged buds in which the stage of development could not be determined. However, it is quite clear that the irregularities appear at the same time as the presumed sensitive stage (Figs. 30—31). Stickiness was the dominating irregularity during the first part of meiosis. The irregularities were of such a kind that any healing can be excluded as an explanation for the low percentages observed in the last few preparations. No definite conclusions can be drawn from this material.

Larix sibirica. From Table 22 it is seen that stickiness occurs to lesser extent in *L. sibirica* than in the other two species included in the investiga-

Table 21. Total number of cells analysed as well as the number of normal cells and aberrant cells on different fixing occasions in the grafts of L. leptolepis. The percentage of irregularities was calculated per PMC in A and per microspore in B respectively.

A.

Clone and graft	Fixing date winter 66—67	Number of PMC investigated	Normal	Stickiness	Polyspory	Others	% irregular
	2.1	200	192	8			4.0
	17.1	400	12	388			97.0
	7.2	400	105	295			73.8
L 1003-17	21.2	400	64	329		7	84.0
	27.2	200	0	200			100
	2.3	200	0	200			100
	6.3	400	247	114	15	24	38.2
Sum		2200					
	2.1	300	300				0
	17.1	300	187	113			37.7
	7.2	400	299	101			25.2
L 1003-21	21.2	200	88	112			56.0
	2.3	100	70	30			30.0
	10.3	100	97			3	3.0
Sum		1400					

B.

Clone and graft	Fixing date winter 66—67	Number of microspores investigated	Normal	Degeneration	Polyspory	Others	% irregular
L 1003-17	6.3	1173	1140		33		2.8
	28.3	800	655	144		1	18.1
Sum		1973					
L 1003-21	8.3	1600	1198	400		2	25.1
	10.3	400	399	1			0.25
Sum		2000					

tion. Instead, polyspory is the dominating category of irregularity, as is revealed in Tables 22—23.

The difference in the amount of irregularity between different grafts is most pronounced in *L. sibirica*. This is due to the difference in extent and time of initiation of further development from diplotene in this species. Thus, diplotene was passed late and only to a limited extent during November—January in Z 1001-1, Z 1001-2, 1 1001-7, and Z 1003-9 (cf. Figs. 32—34, 38). In these grafts the percentages of irregularity were considerably lower than in the

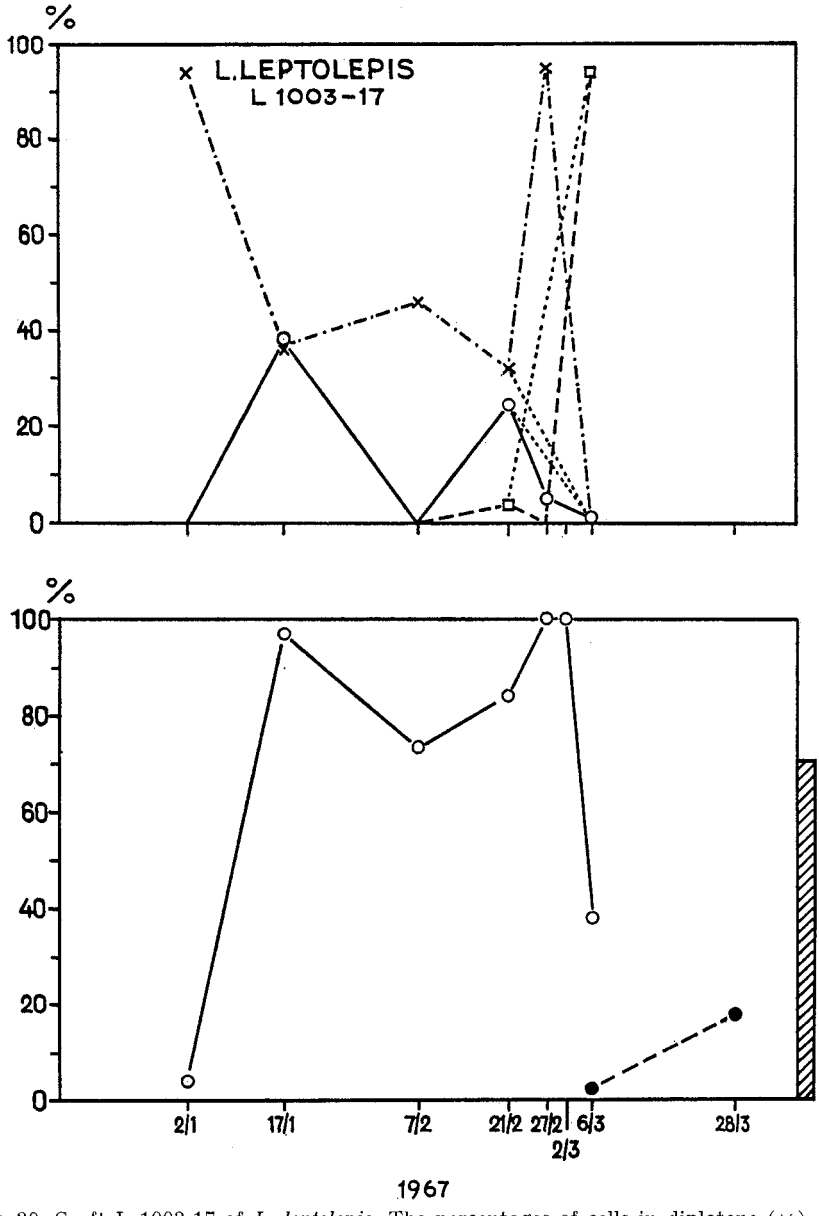

Fig. 30. Graft L 1003-17 of *L. leptolepis*. The percentages of cells in diplotene (×), diakinesis—anaphase I (○), and tetrads—microspores (□) are shown in the upper part of the diagram. Below, the percentages of irregularity in approximately the same cells are shown. The irregularities were estimated on a PMC basis (○) or on a microspore basis (●). Owing to the presence of two completely damaged buds on 27 February, the stage of development could not be determined accurately on that date. The dotted lines indicate the pattern of development when the data from 27 February are omitted. The hatched column refers to the pollen sterility.

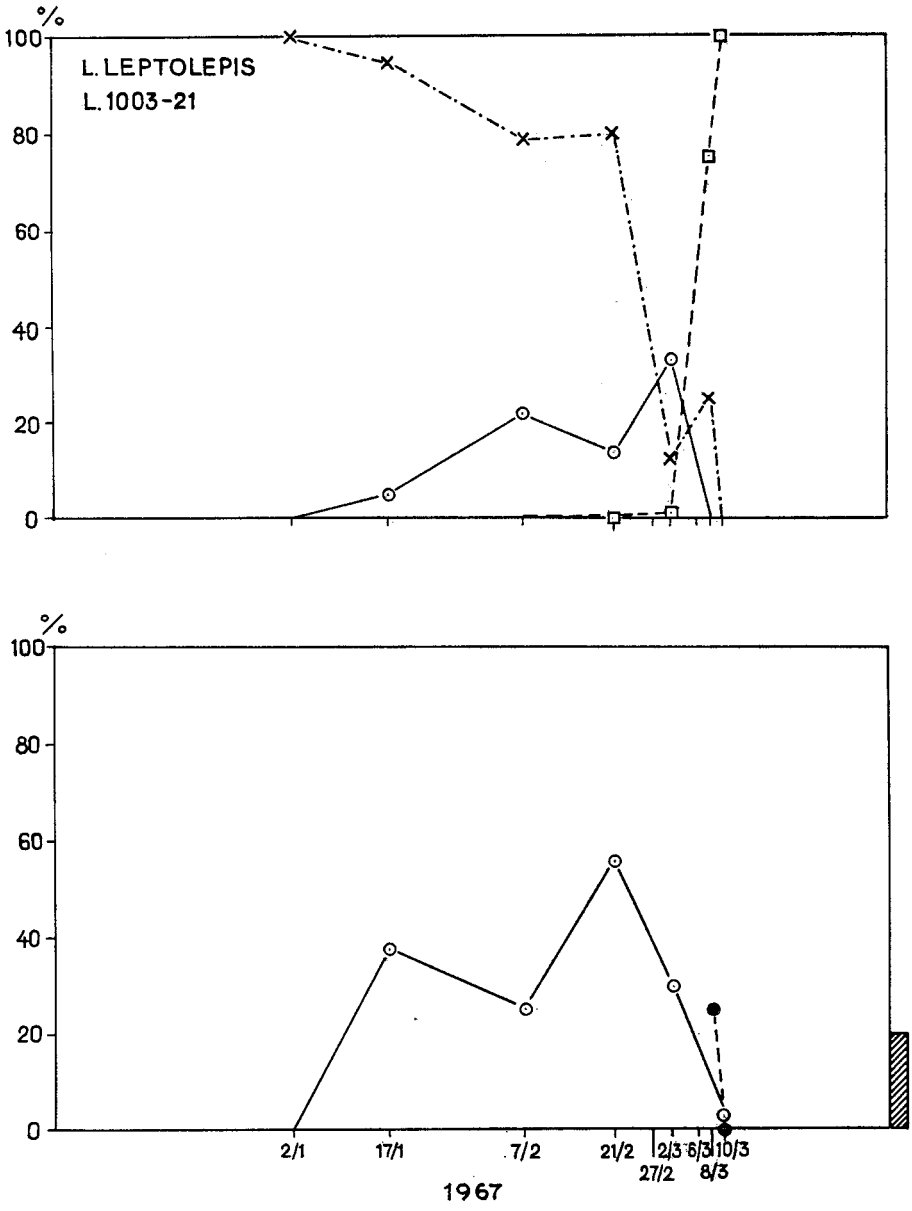

Fig. 31. Graft L 1003-21 of *L. leptolepis*. The percentages of cells in diplotene (×), diakinesis—anaphase I (○), and tetrads—microspores (□) are shown in the upper part of the diagram. Below, the percentages of irregularity in approximately the same cells are shown. The irregularities were estimated on a PMC basis (○) or on a microspore basis (●). The hatched column refers to the pollen sterility.

Table 22. Total number of pollen mother cells analysed as well as the number of normal PMC and aberrant PMC of various types on different fixing occasions in the grafts of L. sibirica. The percentage of irregularities was calculated per PMC.

Clone and graft	Fixing date winter 66—67	Number of PMC investigated	Normal	Stickiness	Polyspory	Others	% irregular
	26.12	200	200				0
	2.1	300	300				0
	17.1	400	400				0
Z 1001-1	7.2	400	398	1		1	0.5
	21.2	397	384		10	3	3.3
	27.2	600	580	8	8	4	3.3
	2.3	100	98	1		1	2.0
Sum		2397					
	26.12	300	300				0
	2.1	300	292	6	2		2.7
Z 1001-2	17.1	400	395	5			1.2
	7.2	300	290	9		1	3.3
	21.2	200	171	4	17	8	14.5
	27.2	400	332	7	42	19	17.0
Sum		1900					
	2.1	200	200				0
	17.1	300	300				0
Z 1001-7	7.2	300	297	3			1.0
	21.2	100	100				0
	27.2	300	281	15		4	6.3
	6.3	200	181	3	7	9	9.5
Sum		1400					
	28.11	200	200				0
	12.12	300	278	22			7.3
	26.12	200	160	26	12	2	20.0
	2.1	400	337	26	33	4	15.8
Z 1002-8	17.1	385	332	11	40	2	13.8
	7.2	341	233	6	88	14	31.7
	21.2	155	115	1	38	1	25.8
	27.2	236	150		70	16	36.4
	2.3	340	174		61	105	48.8
	6.3	303	180		105	18	40.6
Sum		2860					
	21.11	400	368	14	12	6	8.0
	28.11	100	90		9	1	10.0
Z 1002-11	12.12	200	117	8	30	45	41.5
	2.1	100	24	4	45	27	76.0
Sum		800					

Table 22 cont.

Clone and graft	Fixing date winter 66—67	Number of PMC investi-gated	Normal	Sticki-ness	Poly-spory	Others	% irreg-ular
	5.12	300	300				0
	19.12	400	371	29			7.2
	26.12	200	200				0
	2.1	400	393	7			1.8
Z 1002-17	17.1	400	369	30		1	7.8
	7.2	400	346	46	4	4	13.5
	21.2	100	74	2	22	2	26.0
	2.3	200	186		12	2	7.0
	6.3	100	92		8		8.0
Sum		2500					
	12.12	300	299	1			0.3
	19.12	300	297	3			1.0
	2.1	299	281	16	1	1	6.0
	17.1	400	385	11		4	3.8
Z 1003-9	7.2	400	343	57			14.2
	27.2	289	270	4	11	4	6.6
	2.3	298	275		23		7.7
	6.3	400	358		41	1	10.5
Sum		2686					

grafts in which the diplotene stage was passed early, such as Z 1002-8 and Z 1002-11 (cf. Figs. 35—36). The last graft was extreme in the respect that the level of damage throughout the investigation was high, about 60 per cent. In agreement with the other two species the irregularities appeared at the same time as the stages diakinesis—anaphase I. However, the percentage of irregularities was frequently low during this phase of development (cf. Figs. 32—34, and 37—38). This suggests that the sensitivity of these meiotic stages in *L. sibirica* is lower than in *L. decidua*.

As with *L. decidua*, the agreement between the maximum percentage of irregularities during meiosis and pollen sterilities was in most cases relatively good (cf. Figs. 32, 33, 36, and 37). The situation in grafts Z 1001-7 (Fig. 34), Z 1002-8 (Fig. 35) and Z 1003-9 (Fig. 38) needs special comment. As may be seen from Figs. 34 and 38, the level of irregularities was considerably lower than the observed pollen sterilities. Owing to the inaccuracy of the estimation of the pollen sterility it might be suggested that the pollen sterility in these two cases was actually lower than the values reported in Table 30. Such an explanation is probably not true for Z 1002-8. This is partly based on the observation of the complete break-down of development in this graft on 28 March. Therefore, it is rather probable that the irregularities were to some extent hidden during the microspore stage and that they became visible at a late stage of development.

Table 23. Total number of microspores analysed as well as the number of normal microspores and aberrant microspores of various types on different fixing occasions in the grafts of L. sibirica. The percentage of irregularities was calculated per microspore analysed.

Clone and graft	Fixing date winter 66—67	Number of micro-spores in-vestigated	Normal	Degen-eration	Poly-spory	Others	% irreg-ular
	21.2	382	321		61		16.0
	2.3	800	753		47		5.9
	6.3	2693	2109	6	525	53	21.7
Z 1001-1	8.3	1527	1137		390		25.5
	10.3	1200	909		291		24.2
	28.3	3200	2978	222			6.9
Sum		9802					
	21.2	792	649	4	139		18.0
	27.2	1936	1477	5	441	13	23.7
	2.3	709	480	1	228		32.3
Z 1001-2	6.3	2538	1819	2	717		28.3
	8.3	1737	1119	2	613	3	35.6
	10.3	1369	702		667		48.7
	28.3	1200	860	340			28.3
Sum		10281					
	27.2	390	365		25		6.4
Z 1001-7	6.3	394	357		37		9.4
	28.3	1200	1118	82			6.8
Sum		1984					
	26.12	392	314		78		19.9
	2.1	349	191		158		45.3
	17.1	1474	909	1	563	1	38.3
	7.2	1065	667	10	388		37.4
Z 1002-8	21.2	1407	926	12	469		34.2
	27.2	1449	1006	43	400		30.6
	2.3	1506	1063	222	221		29.4
	6.3	1481	1029	53	399		30.5
	28.3	800		800			100
Sum		9923					
	21.11	793	701	56	36		11.6
	28.11	1187	460	706	21		61.2
	12.12	1568	676	780	112		56.9
Z 1002-11	2.1	1600	478	537	583	2	70.1
	7.2	1600	488	486	616	10	69.5
	21.2	1600	572	716	294	18	64.2
	8.3	1600	111	982	500	7	93.1
	28.3	1600		1600			100
Sum		11548					

Table 23 cont.

Clone and graft	Fixing date winter 66—67	Number of micro-spores investigated	Normal	Degen-eration	Poly-spory	Others	% irreg-ular
	2.3	778	724	1	53		6.9
Z 1002-17	6.3	1452	1070	4	378		26.3
	8.3	2000	1649	9	277	65	17.6
	28.3	1200	985	215			17.9
Sum		5430					
	27.2	1173	1068	1	102	2	9.0
Z 1003-9	2.3	1188	1102		86		7.2
	6.3	1572	1417	1	154		9.9
	28.3	1200	913	287			23.9
Sum		5133					

Throughout the diagrams (Fig. 20—38) the observation of the simultaneous appearance of irregularities and the stages diakinesis—anaphase I is conspicuous. This could be due to their high sensitivity to low temperatures or to the fact that irregularities could only be detected during these stages but not in earlier or later ones. Although it is believed that irregularities might be hidden in some cases during the microspore stage, severe irregularities will in all probability be detected during this stage. The explanation for the absence of irregularities during diplotene 1966—67 is probably due to the great insensitivity of the resting part of this stage as pointed out above. If diplotene were a sensitive stage, a delayed appearance of the irregularities must be assumed. This would mean that the same amount of irregularity should occur within one clone as the cells of the different grafts were exposed to approximately the same low temperatures. As the amount of irregularity varied considerably such an explanation must be ruled out. Thus, the only tenable explanation for the simultaneous appearance of irregularities and the stages diakinesis—anaphase I is their high sensitivity against low temperatures accompanied with an immediate manifestation of the irregularities. This is not true for degeneration. However, this type of irregularity is a secondary one originating from other irregularities. It will not be stated that diakinesis—anaphase I are the only sensitive stages. It is probable that all stages during the active division in meiosis are of high sensitivity. Stated in another way, insensitive stages should be the resting phases of diplotene and the interphase.

There are several similarities between low temperatures and radiation when the induction of irregularities is concerned. Thus, the same types of irregularity have been observed following exposure to these two agents.

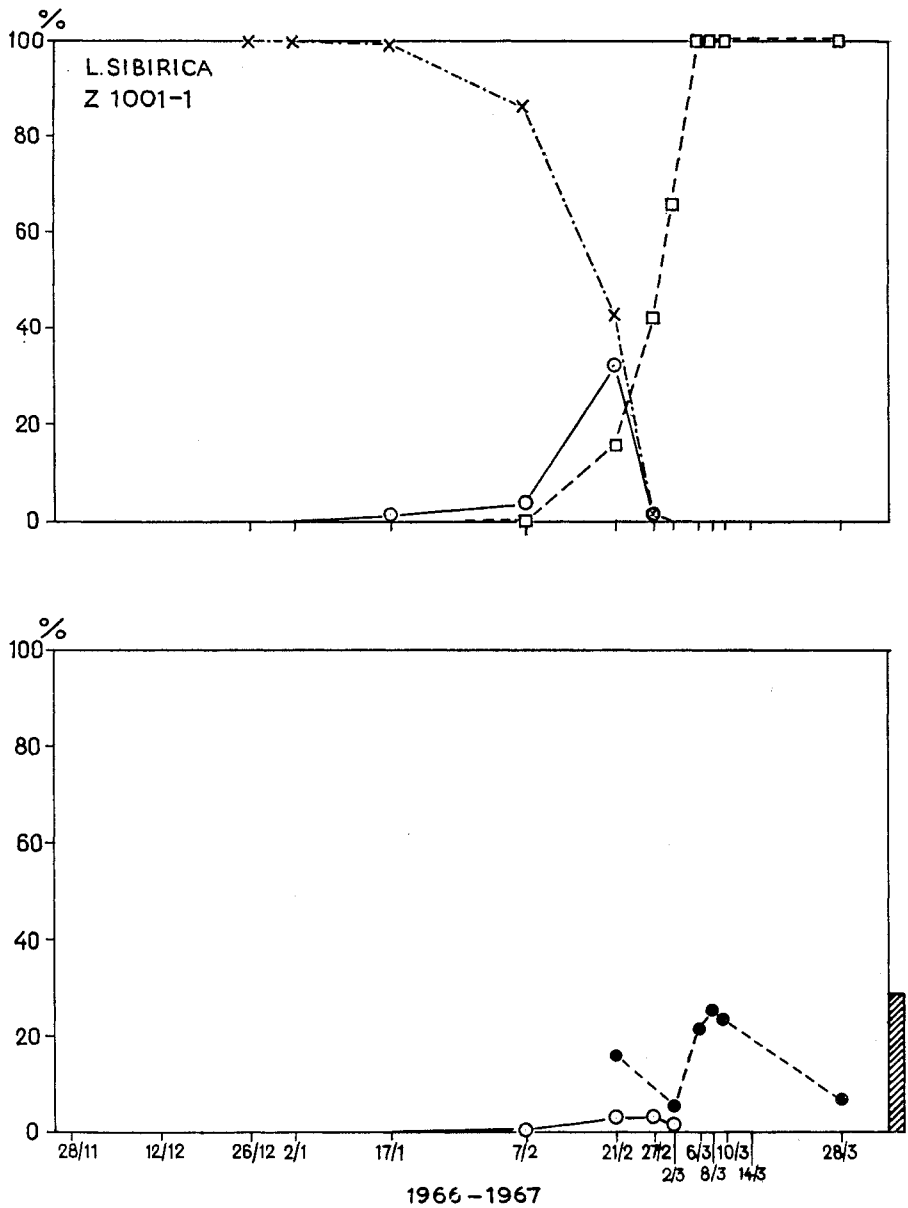

Fig. 32. Graft Z 1001-1 of *L. sibirica*. The percentages of cells in diplotene (×), diakinesis —anaphase I (○), and tetrads—microspores (□) are shown in the upper part of the diagram. Below, the percentages of irregularity in approximately the same cells are shown. The irregularities were estimated on a PMC basis (○) or on a microspore basis (●). The hatched column refers to the pollen sterility.

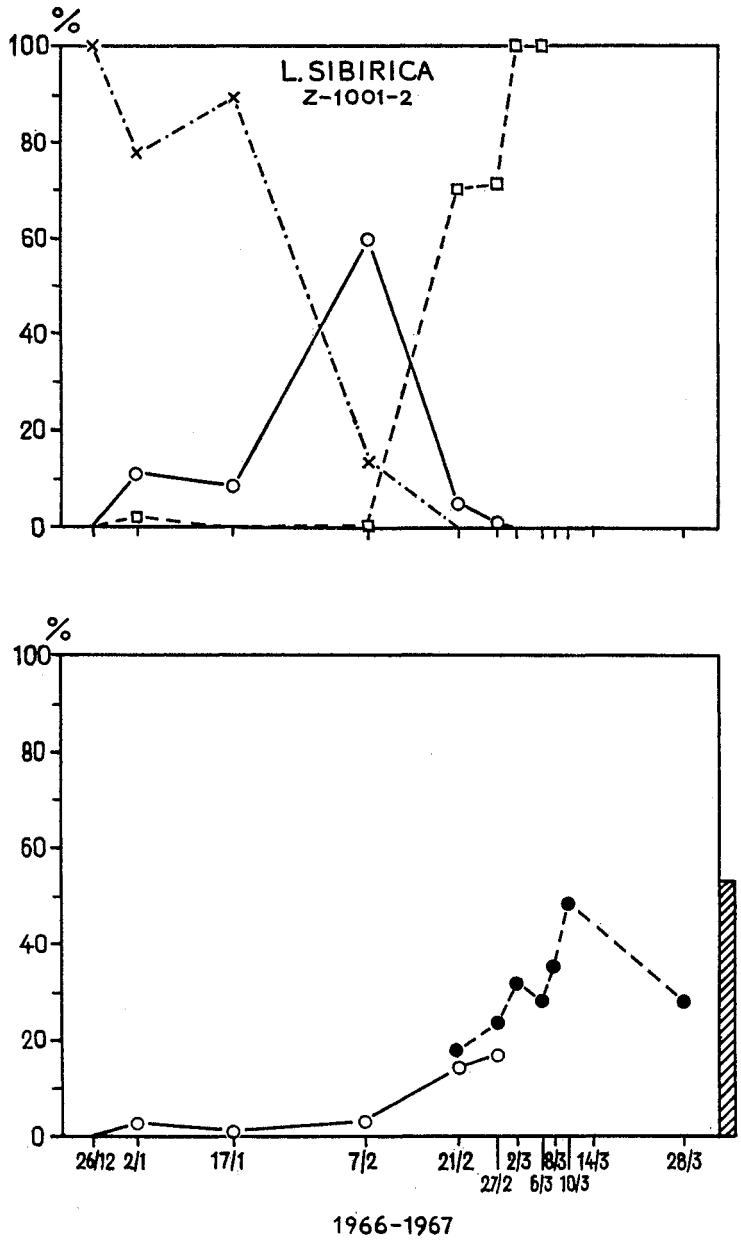

Fig. 33. Graft Z 1001-2 of *L. sibirica*. The percentages of cells in diplotene (×), diakinesis —anaphase I (○), and tetrads—microspores (□) are shown in the upper part of the diagram. Below, the percentages of irregularity in approximately the same cells are shown. The irregularities were estimated on a PMC basis (○) or on a microspore basis (●). The hatched column refers to the pollen sterility.

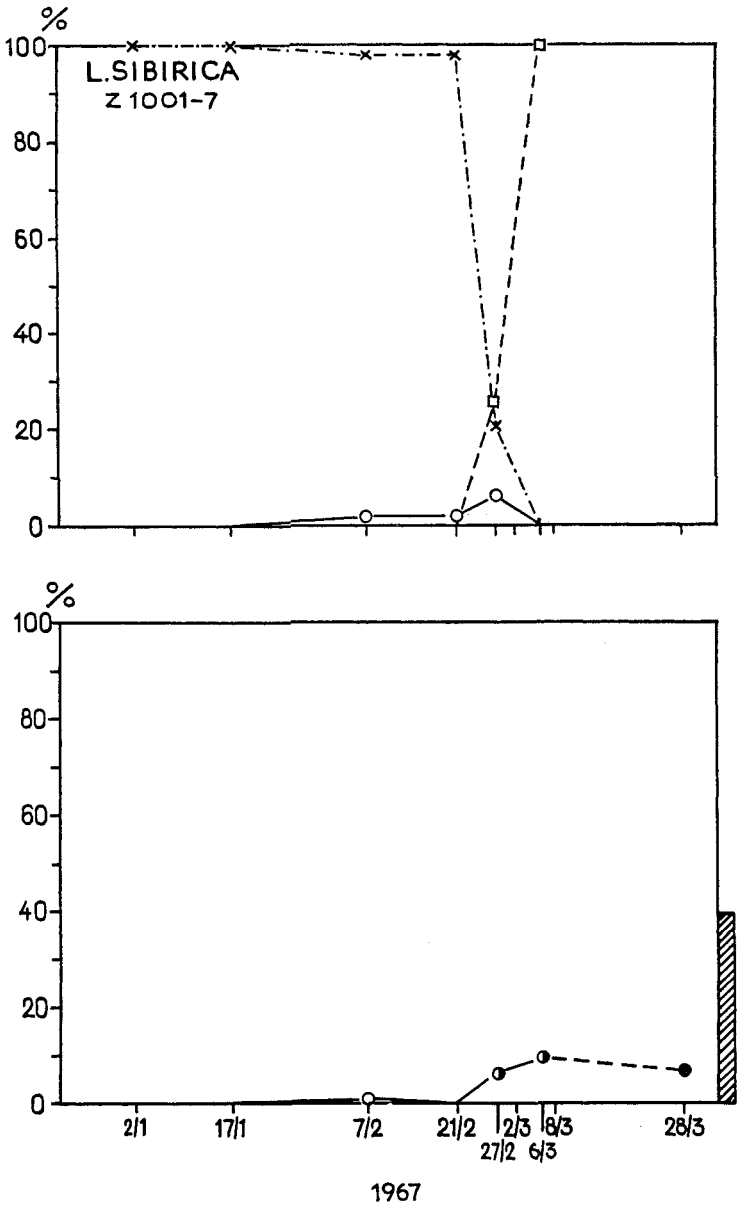

Fig. 34. Graft Z 1001-7 of *L. sibirica*. The percentages of cells in diplotene (×), diakinesis —anaphase I (○), and tetrads—microspores (□) are shown in the upper part of the diagram. Below, the percentages of irregularity in approximately the same cells are shown. The irregularities were estimated on a PMC basis (○) or on a microspore basis (●). The hatched column refers to the pollen sterility.

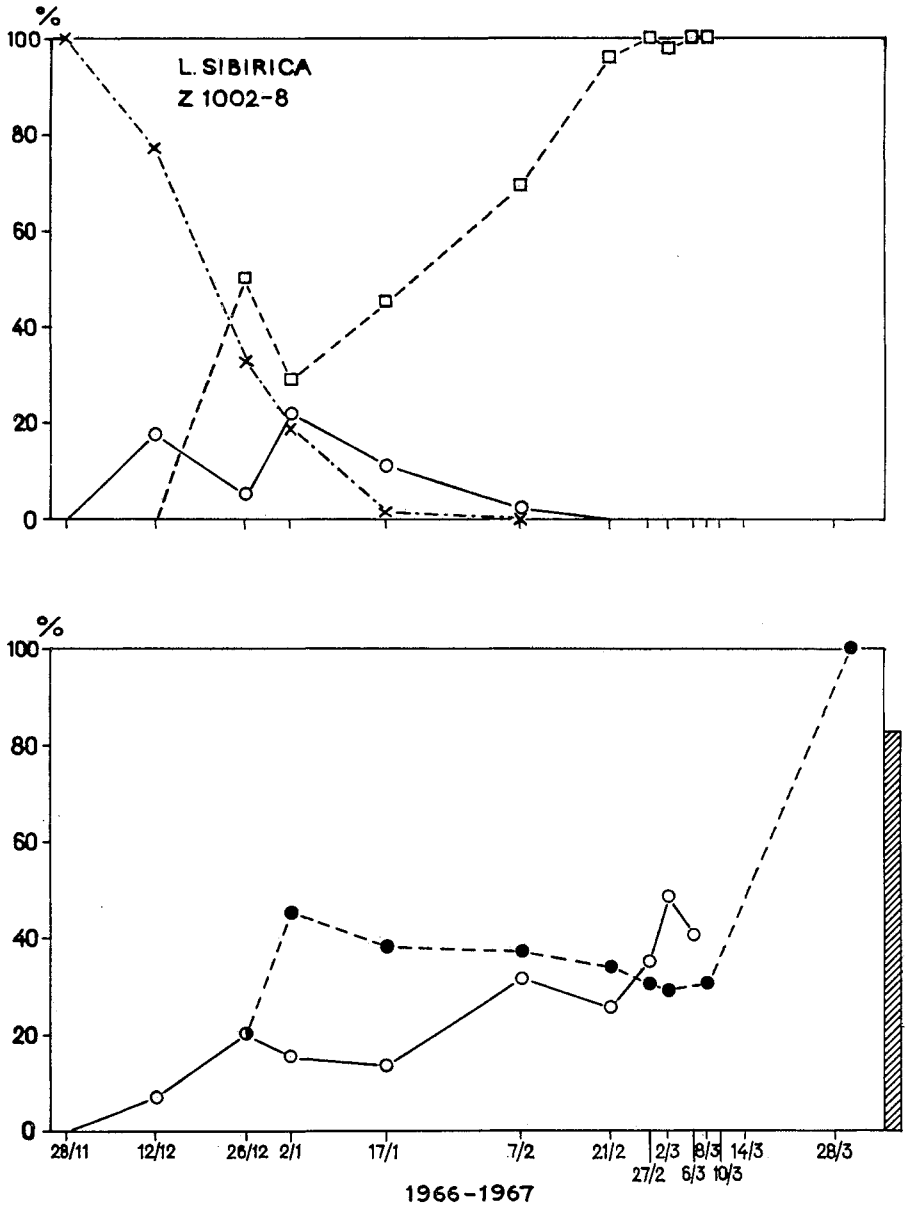

Fig. 35. Graft Z 1002-8 of *L. sibirica*. The percentages of cells in diplotene (×), diakinesis —anaphase I (○), and tetrads—microspores (□) are shown in the upper part of the diagram. Below, the percentages of irregularity in approximately the same cells are shown. The irregularities were estimated on a PMC basis (○) or on a microspore basis (●). The hatched column refers to the pollen sterility.

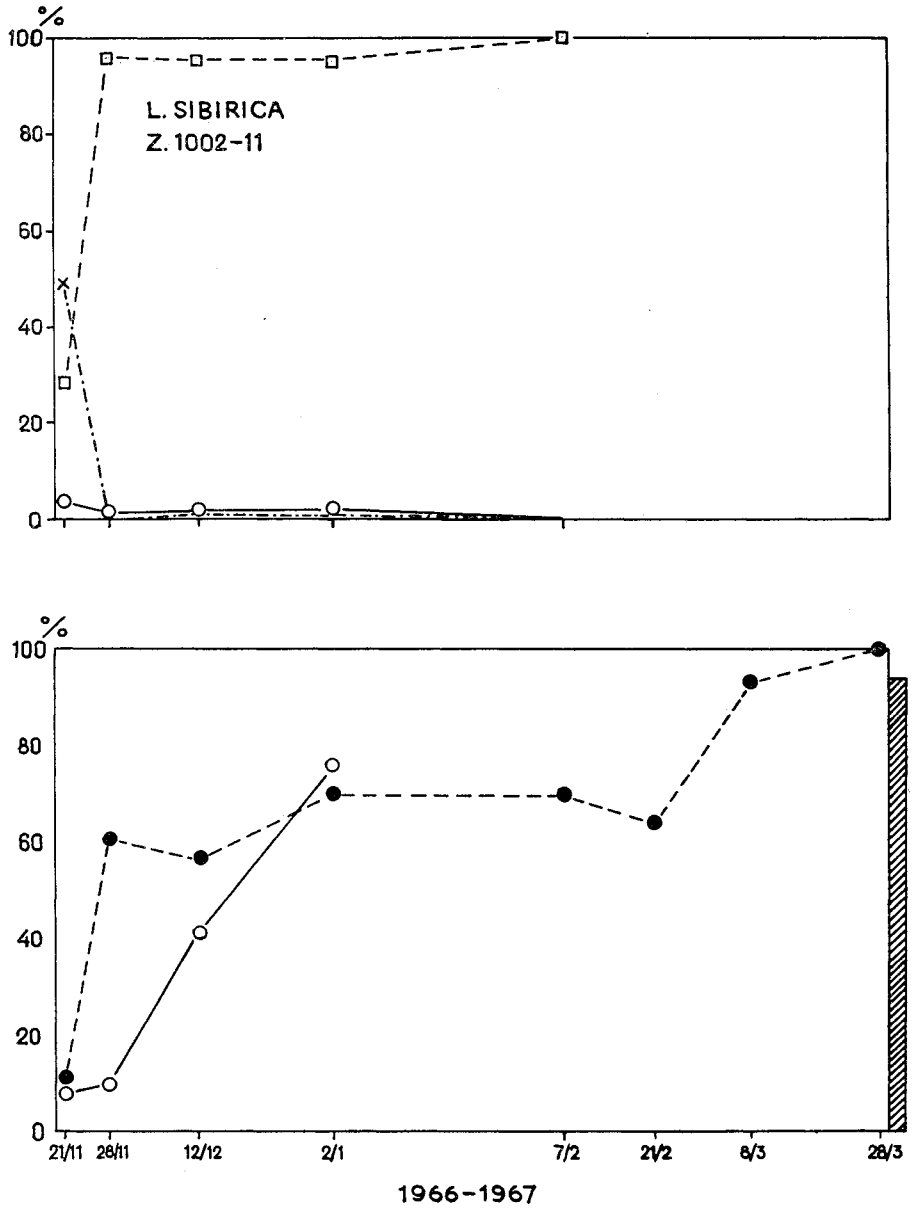

Fig. 36. Graft Z 1002-11 of *L. sibirica*. The percentages of cells in diplotene (×), diakinesis —anaphase I (○), and tetrads—microspores (□) are shown in the upper part of the diagram. Below, the percentages of irregularity in approximately the same cells are shown. The irregularities were estimated on a PMC basis (○) or on a microspore basis (●). The hatched column refers to the pollen sterility.

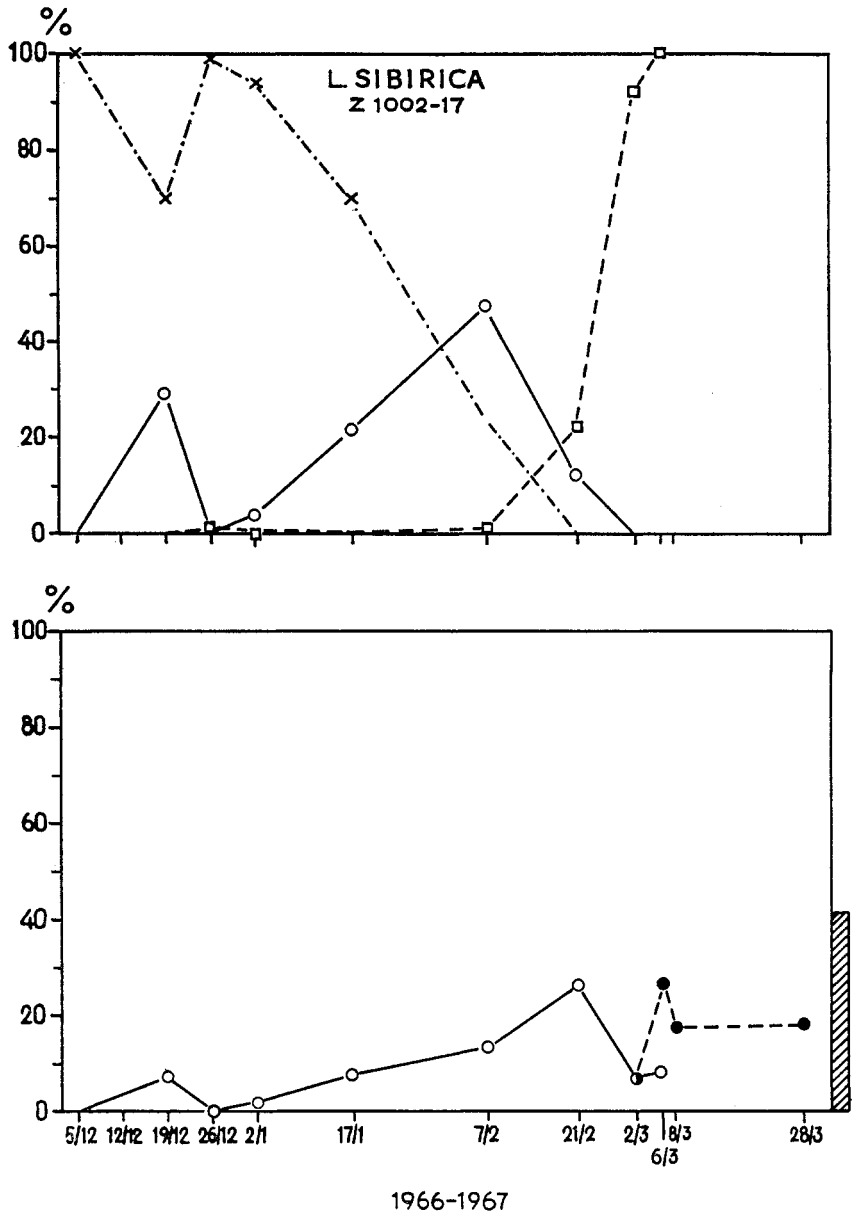

Fig. 37. Graft Z 1002-17 of *L. sibirica*. The percentages of cells in diplotene (×), diakinesis—anaphase I (○), and tetrads—microspores (□) are shown in the upper part of the diagram. Below, the percentages of irregularity in approximately the same cells are shown. The irregularities were estimated on a PMC basis (○) or on a microspore basis (●). The hatched column refers to the pollen sterility.

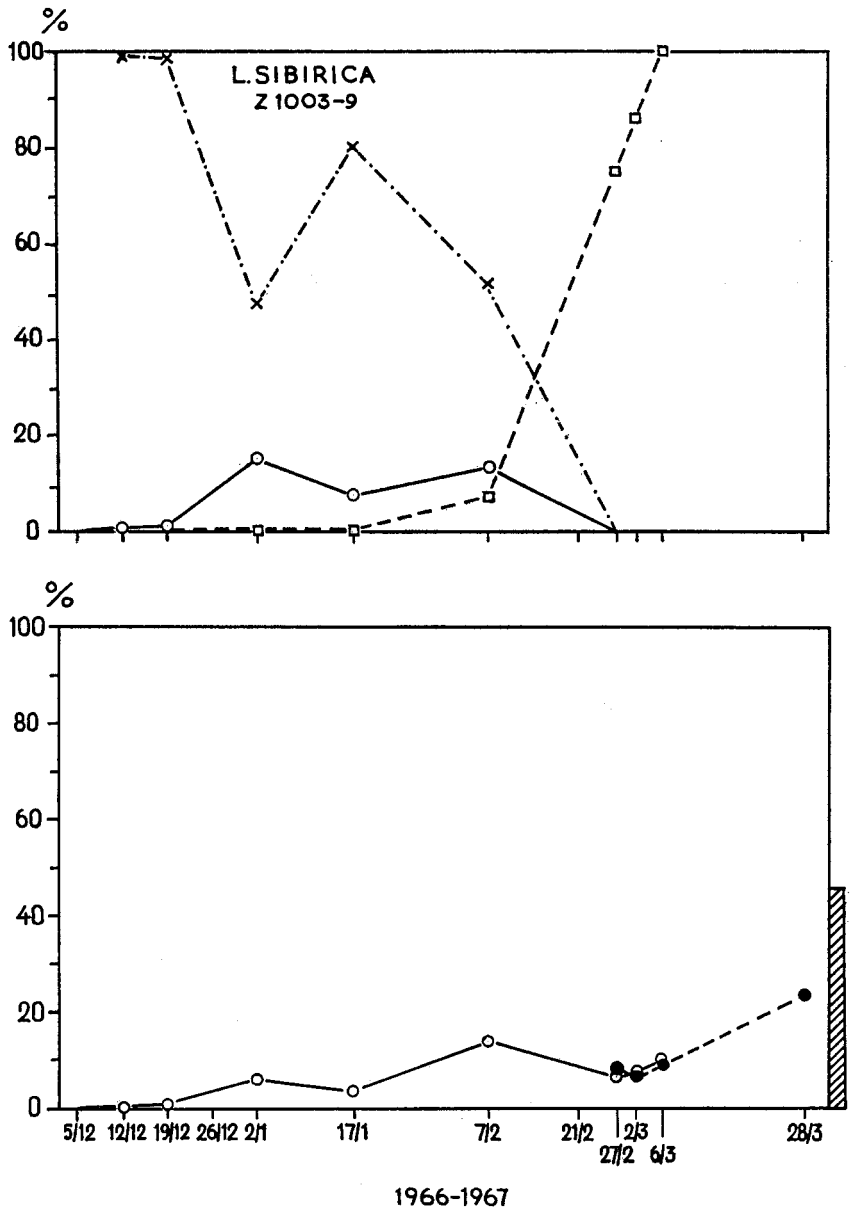

Fig. 38. Graft Z 1003-9 of *L. sibirica*. The percentages of cells in diplotene (×), diakinesis—anaphase I (○), and tetrads—microspores (□) are shown in the upper part of the diagram. Below, the percentages of irregularity in approximately the same cells are shown. The irregularities were estimated on a PMC basis (○) or on a microspore basis (●). The hatched column refers to the pollen sterility.

Besides degeneration, low temperatures have provoked an immediate manifestation of the irregularities, whereas radiation causes both immediate and delayed effects. This difference in the mode of action of the two external agents is probably due to the fact that energy is dissipated in discrete units following radiation whereas it is not after exposure to low temperatures. The low temperatures probably act completely or not at all.

Relation between irregularities, stage of development and temperature

Above it was shown that irregularities did not appear until the sensitive stages were present (cf. Figs. 20—38). Obviously the cells will not be damaged unless the sensitive cells are exposed to unfavourable conditions such as low temperatures.

To illustrate the possibilities of quantitatively predicting the relation between sensitive cells and low temperatures on the one hand, and irregularities on the other hand, the diagram in Fig. 39 was constructed. In this diagram the percentage of sensitive cells is shown on the time axis, as well as three hypothetical minimum temperature curves (I, II and III). If irregularities are assumed to be induced exclusively below 0° C, only curve II will be effective in explaining the induction of irregularities, as only in this case are sensitive cells and low temperatures simultaneously present. Thus the occurrence of low temperatures before or after the period when the sensitive cells are present is of no significance.

The simplest possible means of predicting the amount of irregularities induced would be to use the product of the area between the temperature curve II and the time axis and the area between the curve for sensitive cells and the time axis. However, the product thus obtained does not consider the change in percentage of sensitive cells and low temperatures along the time axis. The best approach would be to have a continuous registration of percentage of sensitive cells and low temperatures and use the integrated product for prediction of irregularities (such a multiplication means that a linear increase of the effect of low temperatures is assumed). This is not feasible but could be done for certain time intervals. This has been done, giving curve IV as the result. This curve describes along the time axis, in relative units, the probability for induction of irregularities by low temperatures. Stated in another way, the area limited by curve IV and the time axis is an estimate of the amount of irregularities. The larger is this area, the higher is the probability of obtaining irregularities caused by low temperature. For simplicity, areas calculated in this way will in the following be referred to as "prediction areas".

In Fig. 40 the actual situation for graft SF-E 412-1 (*L. decidua*) is de-

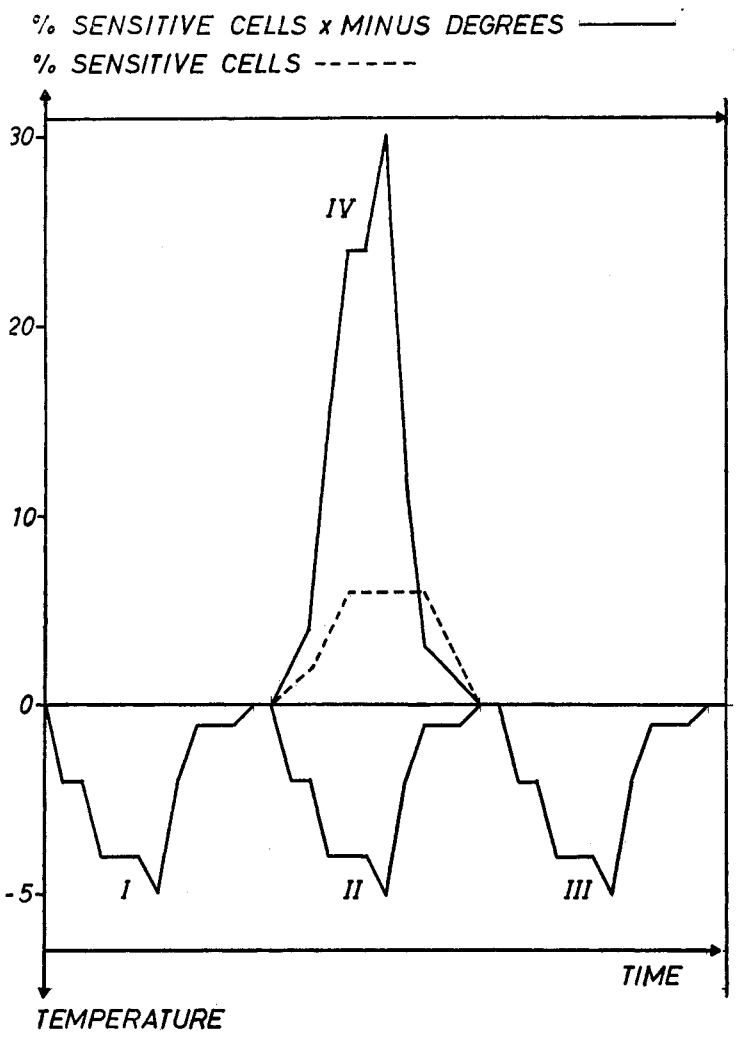

Fig. 39. The figure illustrates the approaches made for a prediction of the amount of irregularities expected based on the simultaneous occurrence of sensitive cells and low temperatures. For explanation see text.

monstrated. The area which ought to represent the amount of irregularity might at first glance seem to be large. However, compared to the situation in *L. sibirica* where sensitive cells might be present for several months, the area is small. In agreement with this the amount of irregularity was small in this graft of European larch.

It is not expected *a priori* that irregularities are induced as soon as 0° C is passed. It might be expected that the limit for the induction of irregularities

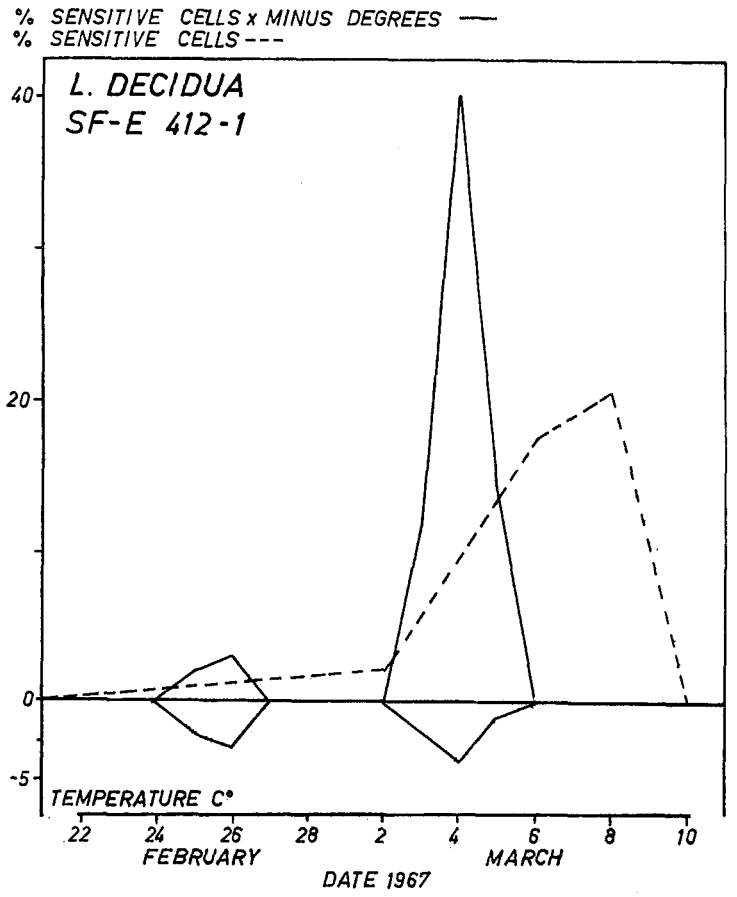

Fig. 40. The percentage of sensitive cells (diakinesis—anaphase I) and the prediction area (for definition see text). The minimum temperature curve below 0° C in presence of sensitive cells is shown.

is situated at −4° C, as is the case in *Picea abies* reported by ANDERSSON (1954) or even at temperatures above 0° C, the situation in *Pinus edulis* (cf. CHIRA 1967). Therefore, to test the relationship between sensitive cells, low temperatures and irregularities, several curves like curve IV must be constructed, based on arbitrary limits such as 0°, −2°, −4° C, etc. Different temperature responses of various irregularities will still further complicate the relationships.

Throughout the discussion above it was assumed that the effect of the low temperatures increased linearly. This assumption might be wrong. It can equally well be assumed that there is a threshold effect at a certain level e.g. all temperatures below 0° C might provoke an effect independent of how

low the temperature is. In the case of a threshold effect, the curve showing the percentage of sensitive cells in the presence of the damage-inducing temperatures will describe the probability for induction of low-temperature irregularities. If a threshold effect is assumed at 0° C in the constructed example in Fig. 39, the area limited by the curve for sensitive cells and the time axis would constitute the prediction area. Still more complicated assumptions could be suggested. Thus there might be an increase of the temperature effect within a certain temperature region and a threshold effect within another region. An exponential increase of the temperature might also be assumed. In the case of an effect at or above 0° C the temperature must be counted from the level at which the temperature effect is assumed to start, before multiplication such as that outlined above can be carried out.

The next question concerns the possibility of testing the hypotheses of relations between sensitive cells, low temperatures and irregularities. To make this point clear the diagram in Fig. 41 was constructed. In the upper part of this diagram a curve for percentage of sensitive cells and a minimum temperature curve are shown. For this hypothetical case a linear increase in the effect of the low temperatures, starting at 0° C, was assumed. Based on this assumption the amount of irregularity, in arbitrary units on different days, is shown in the lower part of this diagram. From the diagram it may be seen that there is a steady increase of the irregularities as long as sensitive cells and temperatures below 0° C are present. When the sensitive stages have been passed the irregularities will be at a constant level. This level constitutes the cumulative percentage of irregularities induced on various occasions during the sensitive phase. The percentage of irregularities at the constant level should correspond to a "prediction area" if the assumptions concerning the temperature effects are true; therefore, it seems clear that the relationships between low temperatures, sensitive cells and irregularities should be tested by plotting percentages of irregularity at the constant level and "prediction areas".

However, complications might occur. Thus, healing of the irregularities was frequently observed, especially in *L. decidua* (cf. above and Figs. 20, 25, 26). This means that no constant level was observed. Constancy is difficult to reach also in those cases where no healing took place, owing to the limited number of buds available for a test on each fixing date.

Therefore, another test of the relations between sensitive cells, low temperatures and observed irregularities must be carried out. The best approach seems to be to use the aberration area (i.e. the area limited by the curve for irregularities and time axis) as this area ought to reflect the expected amount of irregularity based on the simultaneous presence of sensitive cells

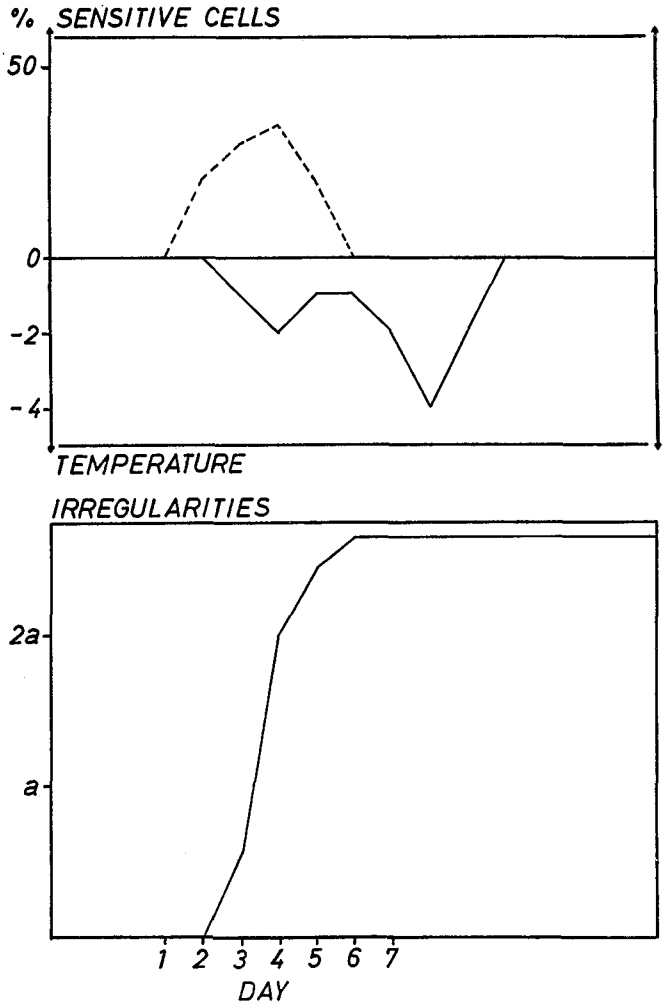

Fig. 41. The figure illustrates that the percentage of irregularities is expected to increase as long as sensitive cells and low temperatures simultaneously are present. It is further shown that the percentage of irregularities is not expected to decrease unless healing occurs.

and low temperatures. In the material several different aberration areas could be calculated. One could be obtained with the aid of irregularities calculated on a PMC basis (1), a second could be obtained by making use of irregularities calculated on a microspore basis (2). Another area which might be described as the maximal area (3) of irregularities could be constructed by using the percentages showing the highest values on the individual fixing dates.

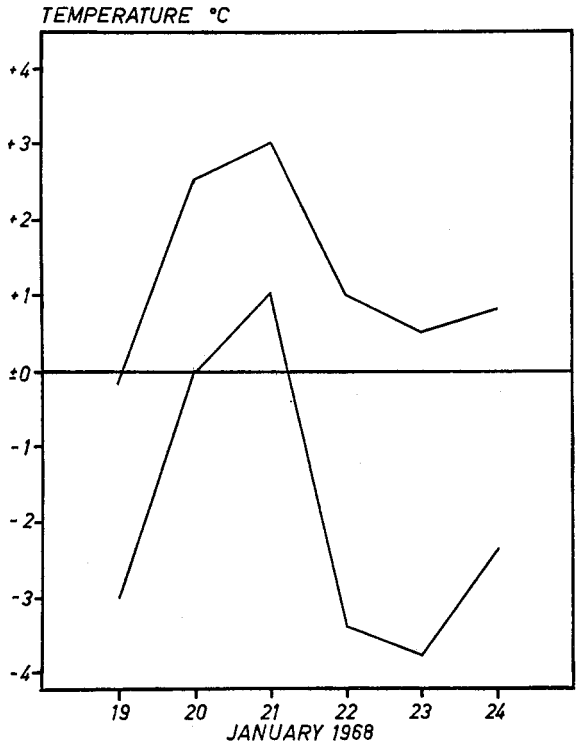

Fig. 42. The maximum and minimum temperatures responsible for the initiation of further development from diplotene and for the induction of irregularities in the PMC of graft W 5006, *L. sibirica* growing at Ernvik.

In the preceding chapter it was stated that the sensitivity of the resting phases to temperatures below 0° C was low. Therefore, all aberration areas including the time following the disappearance of the sensitive cells must not be used for a test of the relationships between sensitive cells, low temperatures on the one hand and irregularities on the other hand. This means that (2) and (3) can immediately be ruled out, since they mainly covered the period when only insensitive cells were present. The first possibility (1) might contain part of the constant period (i.e. from day 6 on in Fig. 41) or alternatively the period when only healing of irregularities took place. However, for simplicity (1) will be used, since the contribution of insensitive cells (tetrads) in this area is considerably lower than in (2) and (3).

Before the relationship between stage of development, and low temperature on the one hand and irregularities on the other hand are discussed, one further example, especially illuminating, concerning the temperatures responsible for the induction of irregularities, will be presented.

Table 24. The numbers and percentages of normal and damaged post diplotene PMC observed in graft W 5006, L. sibirica fixed on January 23rd, 1968 at Ernvik.

	Normal	Stickiness				Poly-spory	Total
		A-type	B-type	C-type	Bridges		
Number	78	1	5	130	2	5	221
%	35	0.4	2.3	59	0.9	2.3	

Above, the initiation of the further development from diplotene in graft W 5006 at Ernvik was discussed. In Fig. 42 the minimum and maximum temperatures immediately before the fixing carried out on 23 January are shown. Since the initiation of further development was shown to be due to mild weather occurring at that time, all the irregularities observed in the PMC fixed 23 Jan. 1968 must have been induced by the low temperatures observed on 22 and 23 January. Thus, this material is suitable for use in evaluating the effect of low temperatures on PMC. In the eight buds in which diplotene had been passed, an examination of the post diplotene stages was made. The data observed are listed in Table 24. From this table it may be seen that only 35 per cent of the PMC which had passed diplotene did not show any irregularities, whereas the rest were more or less damaged. However, the dominating category of irregularity was stickiness of C-type, amounting to almost 60 per cent. From these observations it can be stated with confidence that temperatures of -3 — -4° C cause a considerable amount of irregularity. However, it is not known what importance such temperatures have for pollen sterility, since stickiness of C-type might be healed. Healing can be ruled out for the other categories of irregularity which amounted to about 5 per cent in the post diplotene PMC. Therefore, the actual temperatures provoked irregularities leading to pollen sterility in at least 5 per cent of the PMC. However, it is probable that this percentage is considerably higher in this particular case as the PMC showing stickiness of C-type constituted transitions to the A- or B-types.

This may not be generally applicable. Thus, the temperature response might vary within *L. sibirica* as well as between the species. However, the variability is probably not great. Irregularities were also observed at temperatures above -4° C in *L. decidua* (cf. below and CHRISTIANSEN 1960). These two observations suggest that irregularities in *Larix* are induced at somewhat higher temperatures than in Norway spruce, where -4° C was regarded as a threshold temperature by ANDERSSON (1954).

The relationships tested numerically are shown in Tables 25—27. Besides the ones shown in these tables, others were tested ocularly and the data

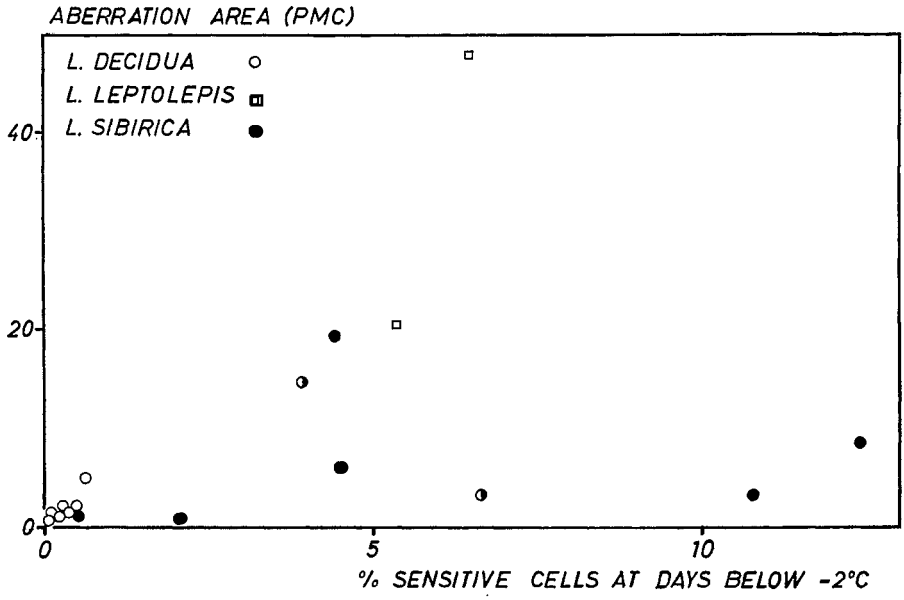

Fig. 43. One of the first approaches for a test of the relation between low temperatures and sensitive cells on one hand and irregularities on the other. In this case a threshold effect at — 2° C was assumed to be acting. ◑ = clone SF-E 655.

were in some cases scattered in such a way that no numerical calculation was needed to exclude significance for a test of the regression. One of the first approaches which was of this type is illustrated in Fig. 43. This figure might be used as a starting point for the analysis of the relationships. It may be observed that the European larch grafts are located within a small range in the lower left corner of this diagram. Furthermore, most of the Siberian larches have a small aberration area. This suggests different mechanisms or modes of induction of irregularities in *L. decidua* and L. *sibirica*. Therefore, separate tests of the relationships were performed. Another complication, especially expressed in *L. sibirica*, was the occurrence of two main types of irregularity, *viz.* polyspory and stickiness (cf. Table 22). In *L. decidua* stickiness was the dominating category (cf. Table 19). There is every reason to believe that stickiness can be induced during any of the stages diakinesis —T I and P II—T II. On the other hand it is difficult to understand how low temperatures during the first meiotic division should be able to provoke induction of polyspory. Rather, it is probable that low temperatures at the end of meiosis are responsible for this irregularity. Therefore, separate relationships were tested for this irregularity in *L. sibirica*. Owing to the absence or low percentages of this irregularity in *L. decidua* no such test was carried out for this species.

Table 25. Regressions in L. decidua of aberration areas on sensitive cells × low temperatures as well as regressions of aberrations on areas of sensitive cells.

No.	Relationship studied	Variance ratio (F) for testing regression
1	Aberration area (PMC) —% cells (diak—A I) × minus degrees below 0° C	24.9**
2	" " " —% cells (diak—A I) at minus degrees below –2° C	18.6**
3	" " " —% cells (diak—A I) × minus degrees below –2° C	91.2***
4	" " " —% cells (diak—T I and P II—T II) × minus degrees below 0° C	57.2***
5	" " " —% cells (diak—T I and P II—T II) at minus degrees below –2° C	61.4***
6	" " " —% cells (diak—T I and P II—T II) × minus degrees below –2° C	41.3***
7	" " " —area of diakinesis—A I	3.91
8	" " " —area of diakinesis—T I and P II—T II	17.3**
9	" " " –polyspory—% cells (diak—A I) × minus degrees below 0° C	26.3**
10	" " " – " —% cells (diak—A I) at minus degrees below –2° C	27.0**
11	" " " – " —% cells (diak—A I) × minus degrees below –2° C	34.3***
12	" " " – " —% cells (diak—T I and P II—T II) × minus degrees below 0° C	23.9**
13	" " " – " —% cells (diak—T I and P II—T II) at minus degrees below –2° C	26.9**
14	" " " – " —% cells (diak—T I and P II—T II) × minus degrees below –2° C	16.8**
15	" " " – " —area of diakinesis—A I	6.8*
16	" " " – " —area of diakinesis—TI + P II—T II	16.8**
17	Aberration area (PMC) –stickiness of C-type—% cells (diak—A I) × minus degrees below –2° C	43.8***
18	" " " – " " " —% cells (diak—T I and P II—T II) × minus degrees below –2° C	69.7***
19	Aberration area (PMC) -(stickiness of C-type + polyspory)—% cells (diak—A I) × minus degrees below –2° C	24.0**
20	" " " – " " " + " —% cells (diak—T I and P II—T II) × minus degrees below –2° C	22.1**

Larix decidua. From Table 25 it may be seen that the variance ratios for testing regression are frequently of high significance. Interestingly enough, the variance ratios for the relationships 1—6 were in most cases significant at the 0.1 per cent level, whereas the corresponding ones (9—14) where polyspory was excluded from the calculation, were only significant at the 1 per cent level. This was not expected, as polyspory was believed to be induced only during the end of meiosis, as discussed above.

From Table 25 it may also be seen that the best agreements within the two groups (1—6 and 9—14, respectively) were obtained for Nos 3 and 11 which were both of the same type except for the absence of polyspory in the aberration area in No. 11. Therefore, it might be suggested that this way of estimating the amount of irregularity is a good approach. It cannot be claimed that the integrated product of degrees below $-2°$ C and the percentage of cells of the stages diakinesis—A I is the only way of predicting irregularities, but it constitutes one of the best approaches. No numerical comparison between the individual regressions was performed.

Although stickiness was the dominating category it could be divided into at least three different components, each one of which might show a specific temperature response, thus complicating the interpretation of the relationships.

Another limiting factor is the absence of daily fixations, which was a result of the paucity of male buds available. This factor was not as serious in *L. decidua* as in *L. sibirica*, where sensitive cells appeared for several months.

Relationships between aberration areas and areas of sensitive cells were also tested, see Table 25 (7—8 and 15—16). The variance ratios in those cases are, on the average, low compared with the other ones, which stresses the importance of the simultaneous presence of sensitive cells and low temperatures for induction of irregularities.

Larix sibirica. As the sensitive phase of the development was not well covered in graft No. Z 1002-11, the data from this graft were excluded from all tests.

Two main approaches were followed in this species for testing the relations between sensitive cells, low temperatures and irregularities. One concerned all irregularities except polyspory, whereas the second concerned polyspory.

Owing to the early passing of diplotene in graft Z 1002-8, many of the polysporic microspores had probably given rise to degeneration. Therefore, it was justifiable to subtract not only cells showing polyspory but also the degenerating cells from the aberration area in this particular graft to avoid further complications. As may be seen from Table 26 the regression was

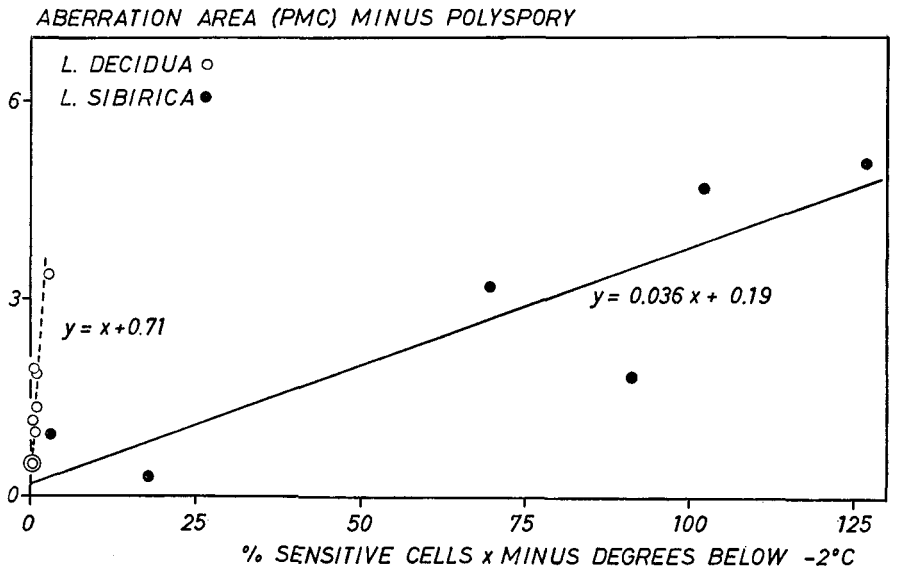

Fig. 44. The relationships between the aberration area (for definition see text) minus polyspory and percentage of sensitive cells multiplied by minus degrees below — 2° C in *L. decidua* (○) and *L. sibirica* (●). The equations of the 2 regression lines are given. The coefficients of x constitute a measure of the sensitivity of the PMC for induction of all types of irregularity besides polyspory. The linear regressions demonstrated refer to No. 11 in Table 25 and No. 5 in Table 26.

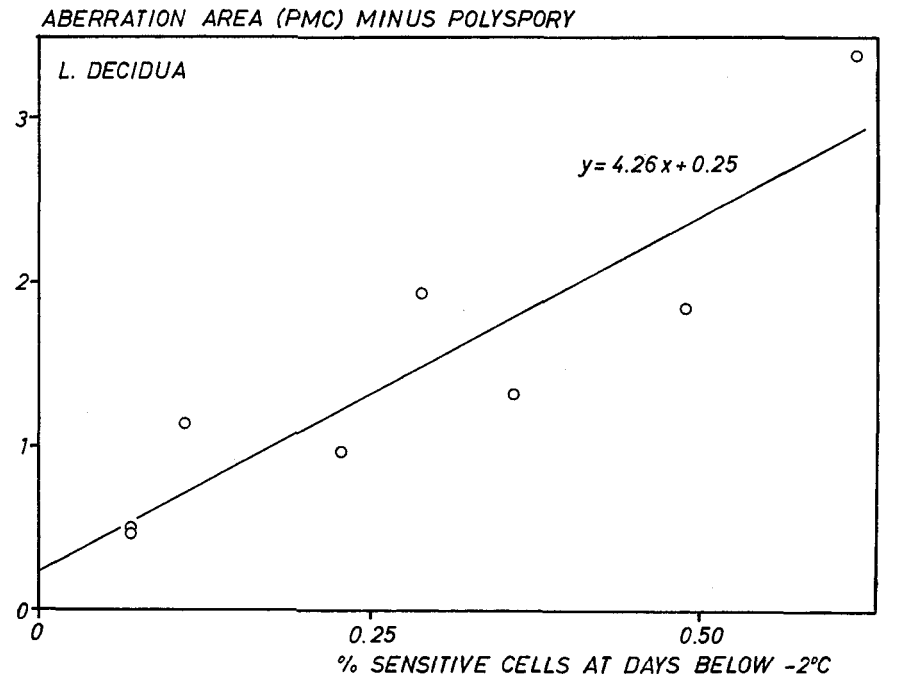

Fig. 45. The relationship between the aberration area (for definition see text) minus polyspory and percentage of sensitive cells at days with a temperature below — 2° C. The equation of the regression line is given. The linear regression demonstrated refers to No. 10 in Table 25.

Table 26. Regressions of aberration area minus polyspory in L. sibirica on sensitive cells × low temperatures.

No.	Relationship studied	Variance ratio (F) for testing regression
1	Aberration area (PMC) minus polyspory —% cells (diak.—T I and P II—T II) × minus degrees below –2° C	7.79*
2	" " " " " and degeneration in Z 1002-8—% cells (diak.—A I) × minus degrees below –4° C	2.27
3	" " " " " " " " Z 1002-8—% cells (diak.—A I) × minus degrees below –2° C	2.17
4	" " " " " " " " Z 1002-8—% cells (diak.—T I and P II—T II) × minus degrees below –4° C	14.6*
5	" " " " " " " " Z 1002-8—% cells (diak.—T I and P II —T II) × minus degrees below –2° C	13.9*

Table 27. Regressions of polyspory area (calculated in different ways) in Larix sibirica on the percentage of PMC in the stages anaphase II—telophase II × various low temperatures.

No.	Relationship studied	Variance ratio (F) for testing regression
1	Polyspory area —% cells A II—T II × minus degrees below –2° C	25.2**
2	" " —% cells A II—T II × minus degrees below –4° C	31.0**
3	Polyspory area to the fixing date where A II—T II became 0 %—% cells A II—T II × minus degrees below –4° C	52.2**
4	Ditto —% cells A II—T II × minus degrees below –2° C	39.2**

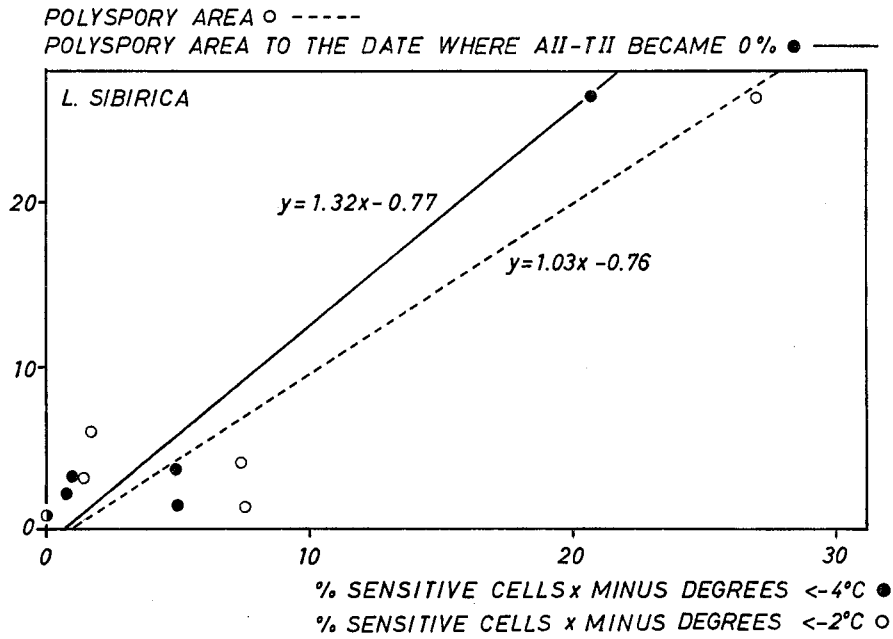

Fig. 46. The figure illustrates the relationships between polyspory areas calculated in 2 different ways and percentage of sensitive cells multiplied by minus degrees below — 2° C or by — 4° C. The linear regressions demonstrated refer to relationships 1 (○) and 3 (●) in Table 27.

almost significant in three cases and not significant in two cases. It is worth mentioning that significance was only obtained where the relationships (1, 4—5) included the stages diakinesis—T I + P II — T II but not when the stages diakinesis—A I were the only stages (2—3).

Both anaphase II and telophase II were included in the tests of the reasons for induction of polyspory, although it was assumed that this irregularity was induced during the very end of meiosis. However, it is difficult to set a correct boundary between the two stages. Therefore, they were taken together. The polyspory area could be estimated also after the completion of meiosis. At that time all induction of polyspory had ceased. Such an area can only in its first part (when A II—T II were present) along the axis of time be related to a continuous process. When A II—T II are passed the area will increase linearly in the same way as discussed above for the aberration areas calculated on a microspore basis. To eliminate this complication, the polyspory area was calculated to the fixing date on which A II—T II became 0 per cent. Although significance was obtained independent of the estimate of the polyspory area (cf. Table 27), the appearance of the regression lines in Fig. 46 suggests that the significance may be due to the presence

of a single point far away from the other. A short delay in the manifestation of polyspory must also be assumed, which still more complicates the interpretation.

From the presentation above it may be concluded that the relationships are weaker in *L. sibirica* than in *L. decidua*. The reason for this is probably the complexity of the irregularities observed in the former species as well as the broader temperature range acting during the sensitive phases in *L. sibirica*. Furthermore, the sensitive phase of meiosis could not be followed in detail owing to its long extension in time.

It is not believed that the exact temperature response of the PMC can be revealed in outdoor experiments of the present design, where the temperature varied considerably during the period of high sensitivity. The response can only be obtained if the temperatures can be controlled during the experiment.

The most important point about the relationships obtained is that they have shown that the approaches made for understanding the complicated relationships between sensitive cells and low temperatures on the one hand and irregularities on the other hand were made satisfactory. Furthermore, they have given valuable suggestions for the design of phytotron experiments which constitute the only possibility of finally solving the problems concerning the temperature response of different meiotic stages for induction of certain irregularities. Therefore, the experience from this investigation should be used to avoid wasting money in the expensive phytotron experiments.

It is believed that relations similar to those between low temperatures, sensitive cells and irregularities could be used in studies in which a heterogeneous cell population is exposed to an external agent. This is met within tests of radiosensitivity during meiosis and in tumor irradiation as well as in other cases.

Pollen sterility

First, it must once more be pointed out that there is no possibility in *Larix* precisely to determine the pollen sterility. Pollen grains of shrunken or shrivelled appearance such as those in Plate 5 F and Fig. 4 B—C in the paper by ERIKSSON *et al.* (1966) can obviously not be functional. On the other hand, some of the pollen grains of a fertile appearance might be sterile, as in the situation suggested for barley by ERIKSSON (1965).

The data from the estimates of the pollen sterilities have been listed in Tables 28—30 and Figs. 47—49. From Table 28 it may be seen that the pollen sterility frequently did not exceed 10 per cent in the two European larch clones SF-E 412 and SF-E 657. The low sterility in *L. decidua* was expected from the relatively favourable conditions at the time of development from diplotene to microspores (cf. Tables 11 and 13 as well as Figs. 3, 12 and 14). The average sterility of the dubious clone SF-E 655 was a little, although not significantly, higher than that in the two pure European larch clones. Somewhat higher sterility was expected, as the development from diplotene

Table 28. Pollen sterility of L. decidua growing in Stockholm 1967.

Clone	Graft	% sterility and standard error
SF-E 412	1	3.8 ± 0.7
SF-E 412	2	16.1 ± 4.2
SF-E 412	3	9.4 ± 3.2
SF-E 412	4	2.9 ± 0.6
SF-E 412	5	19.9 ± 10.0
SF-E 412	7	30.1 ± 4.7
SF-E 655[1]	8	24.0 ± 8.7
SF-E 655[1]	9	11.5 ± 2.2
SF-E 655[1]	10	27.3 ± 3.8
SF-E 655[1]	15	12.7 ± 1.1
SF-E 655[1]	17	20.8 ± 5.7
SF-E 655[1]	18	11.3 ± 1.8
SF-E 655[1]	20	37.0 ± 7.4
SF-E 657	11	24.1 ± 7.3
SF-E 657	12	7.8 ± 2.1
SF-E 657	13	7.7 ± 1.1
SF-E 657	14	8.0 ± 1.2
SF-E 657	16	40.4 ± 11.2
SF-E 657	19	4.4 ± 0.5

[1] species classification dubious.

Table 29. Pollen sterility of L. leptolepis growing in Stockholm 1967.

Clone	Graft	% sterility and standard error
L 1003	4	49.1 ± 14.5
L 1003	8	26.4 ± 6.0
L 1003	10	52.2 ± 7.0
L 1003	12	48.1 ± 3.8
L 1003	14	59.0 ± 9.7
L 1003	15	33.3 ± 4.8
L 1003	17	70.4 ± 5.4
L 1003	18	70.1 ± 8.1
L 1003	19	27.8 ± 3.5
L 1003	21	20.0 ± 1.6
L 1003	23	98.0 ± 0.8
L 1003	24	97.9 ± 7.1

was initiated earlier in this clone than in the two other clones (cf. Figs. 12—14).

The pollen sterility in *L. leptolepis* was at a considerably higher level, ranging from 20 per cent to almost complete sterility in grafts L 1003-23 and L1003-24 (cf. Table 29 and Fig. 48). Relatively low sterilities (20—30 per cent) were observed in four cases. The low sterilities could be due to favourable conditions during the development from diplotene to microspores or to a complete elimination of the most severely damaged buds, in the same

Table 30. Pollen sterility of L. sibirica growing in Stockholm 1967.

Clone	Graft	% sterility and standard error
Z 1001	1	29.2 ± 4.4
Z 1001	2	53.1 ± 6.5
Z 1001	3	63.8 ± 6.8
Z 1001	4	54.6 ± 4.3
Z 1001	5	66.9 ± 3.1
Z 1001	6	46.6 ± 6.6
Z 1001	7	39.2 ± 7.3
Z 1002	8	82.6 ± 4.1
Z 1002	10	98.0[1]
Z 1002	11	94.3 ± 1.8
Z 1002	12	48.2 ± 5.4
Z 1002	13	86.9 ± 3.4
Z 1002	17	41.0 ± 2.7
Z 1003	9	45.6 ± 2.9
Z 1003	19	62.6 ± 4.3
Z 1003	20	55.7 ± 3.5

[1] Only 2 buds developed any pollen. In both cases a sterility of 98 % was observed.

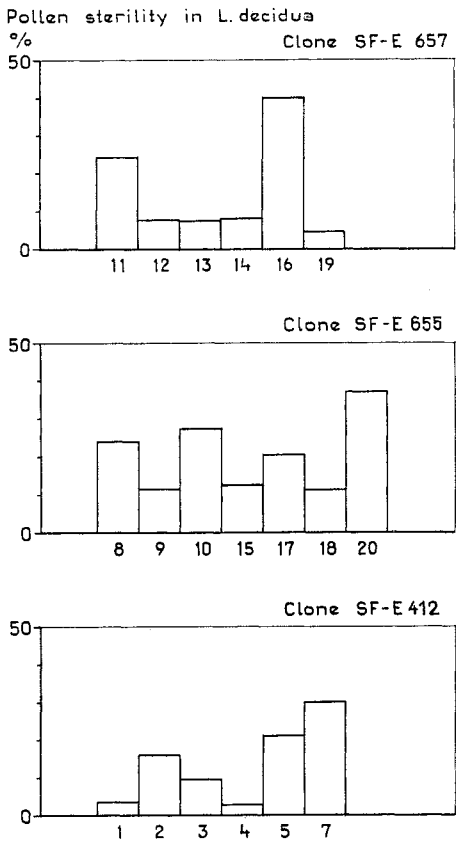

Fig. 47. The percentages of sterile pollen grains of the individual grafts of *L. decidua* growing in Stockholm 1966—67.

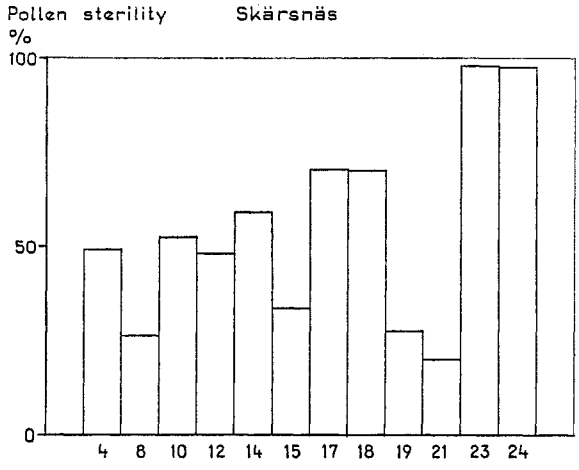

Fig. 48. The percentages of sterile pollen grains of the individual grafts of *L. leptolepis* growing in Stockholm 1966—67.

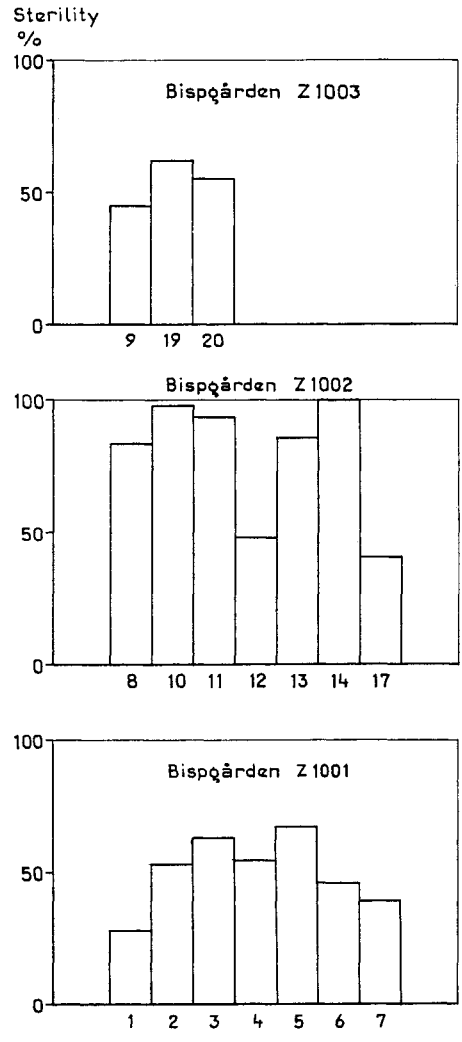

Fig. 49. The percentages of sterile pollen grains of the individual grafts of *L. sibirica* growing in Stockholm 1966—67. Only one bud developed pollen in Z 1002-14.

way as was observed for all buds of *L. leptolepis* during the spring of 1966. The first explanation can at least partly be ruled out, as the initiation took place in January, thus allowing the exposure of the sensitive PMC to low temperatures on several occasions. The second explanation is rather probable, as completely damaged buds were frequently observed during meiosis. During the course of spring such buds become more and more dried and no pollen formation will take place in them. Owing to this drying-out and lack of pollen formation they will mostly not be included in the fixing at pollen maturity. Therefore, the values for pollen sterility in the present investiga-

tion constitute an estimate of the sterility of the pollen population which is available for dispersal from the stamens. It might be debated whether the pollen sterility estimated in this way or that estimated on primarily formed PMC is of greater importance for seed setting (cf. below).

From Table 30 and Fig. 49 it is evident that the pollen sterility in the Siberian larch grafts in most cases was at a high level, rarely below 50 per cent.

The high sterility in clone Z 1002 is conspicuous. This high sterility was also expected from the early initiation of the development from diplotene.

Pollen measurements

Additional information concerning pollen quality might be obtained from the measurement of the pollen grains. The grafts selected for the measurement studies were those which were included in the analysis of irregularities during meiosis. The average values presented in Table 31 do not reveal any

Table 31. The average size of microspores and pollen grains in scale units as measured from the fixings carried out on 28 March and 15 April 1967. 100 μ is equal to 49.5 scale units. Mostly 300 cells emanating from 3 different buds were measured.

Species	Clone	Graft	Fixing date	
			28.3	15.4
L. decidua	SF-E 412	1	28.5 ± 0.1[1]	39.0 ± 0.1
" "	SF-E 412	2	30.7 ± 0.2	38.7 ± 0.2
" "	SF-E 412	3	31.4 ± 0.1	40.6 ± 0.2
" "	SF-E 412	7	30.5 ± 0.2	36.5 ± 0.2
" "	SF-E 655	9	36.7 ± 0.2[2]	42.2 ± 0.2
" "	SF-E 655	20	32.0 ± 0.3[1]	41.5 ± 0.2
" "	SF-E 657	11	32.1 ± 0.1[2]	42.7 ± 0.2
" "	SF-E 657	13	32.9 ± 0.2	40.8 ± 0.3
" "	SF-E 657	14	30.4 ± 0.1	39.2 ± 0.1
" "	SF-E 657	16	31.2 ± 0.1[3]	36.0 ± 0.1
L. leptolepis	L 1003	17	31.3 ± 0.3[1]	41.4 ± 0.3
" "	L 1003	21		40.3 ± 0.2
L. sibirica	Z 1001	1	33.0 ± 0.2[4]	41.0 ± 0.3
" "	Z 1001	2	33.3 ± 0.3	40.2 ± 0.5
" "	Z 1001	7	33.0 ± 0.2	40.0 ± 0.4
" "	Z 1002	8	30.2 ± 0.3	37.0 ± 0.5
" "	Z 1002	11		34.5 ± 1.1[5]
" "	Z 1002	17	31.2 ± 0.2	40.2 ± 0.3
" "	Z 1003	9	33.8 ± 1.4	45.0 ± 0.4

[1] 200 cells measured
[2] 320 " "
[3] 600 " "
[4] 310 " "
[5] 100 " "

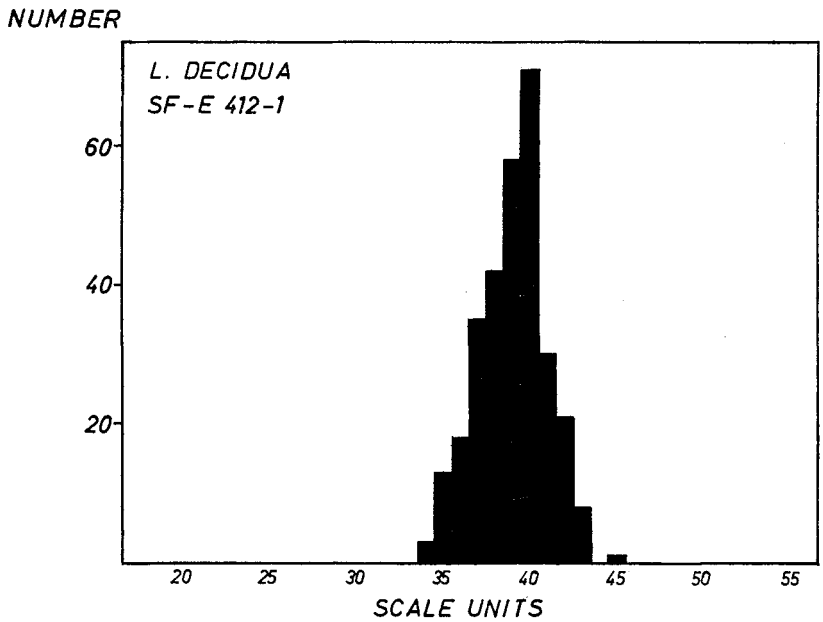

Fig. 50. The distribution of pollen grains in graft SF-E 412-1, *L. decidua*, according to size. 49.5 scale units = 100 μ.

conspicuous differences. The standard errors are mostly small. Of greater interest is the investigation of the distribution of different sizes of pollen grain. A few cases have been illustrated in Figs. 50, 52—53 and 55—56.

Graft SF-E 412-1 showed a low pollen sterility, amounting to 3.8 per cent. The distribution of pollen grains according to their size revealed a relatively narrow range from 34—45 scale units. Distributions of this type were also observed in grafts SF-E 655-9, SF-E 657-14, and SF-E 657-16 (*L. decidua*). As is indicated in Fig. 51, there was good agreement with a normal distribution in this graft. Graft SF-E 412-7 (*L. decidua*) showed another type of distribution (Fig. 52). In this case extremely small pollen grains occurred as well as a few relatively large ones. The estimated pollen sterility amounted in this case to 30.1 per cent. During meiosis the percentage of polyspory was observed to amount to somewhat less than ten per cent. Therefore, it would be expected that the same percentage of small pollen grains would be observed at pollen maturity. The number of small pollen grains which originated from polyspory was, however, lower, as is revealed in the diagram. It must be pointed out that the pollen in the polysporic units of the type illustrated in Plate 5 D could never be measured, whereas they were included in the estimate of the pollen sterility. Thus, when the pollen was measured, only the diameters of individual pollen grains

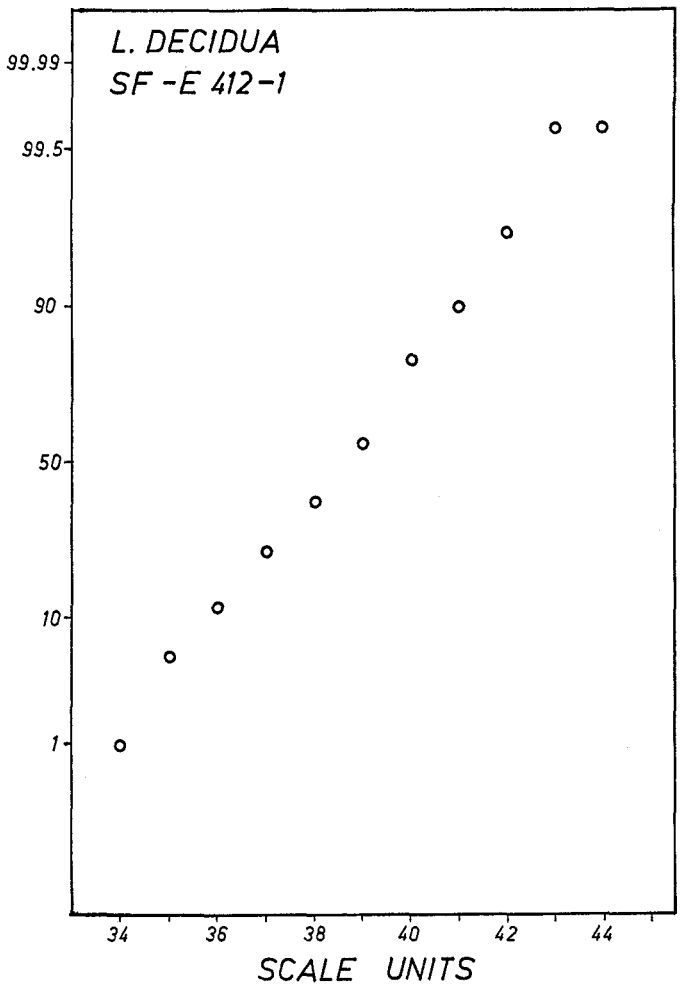

Fig. 51. Test of normal distribution concerning size of the pollen grains in graft SF-E 412-1, *L. decidua*.

could be determined. There is a more or less continuous variation in pollen-grain size, from the smallest to the largest. This means that such small pollen grains, if they have a fertile appearance, might be classified as good pollen although they probably have only half the number of chromosomes and are not capable of giving rise to viable offspring. However, these pollen grains are almost exclusively of the type shown in Fig. 4 C in the paper by Eriksson *et al.* (1966). Therefore, they will not seriously interfere with the estimation of sterility.

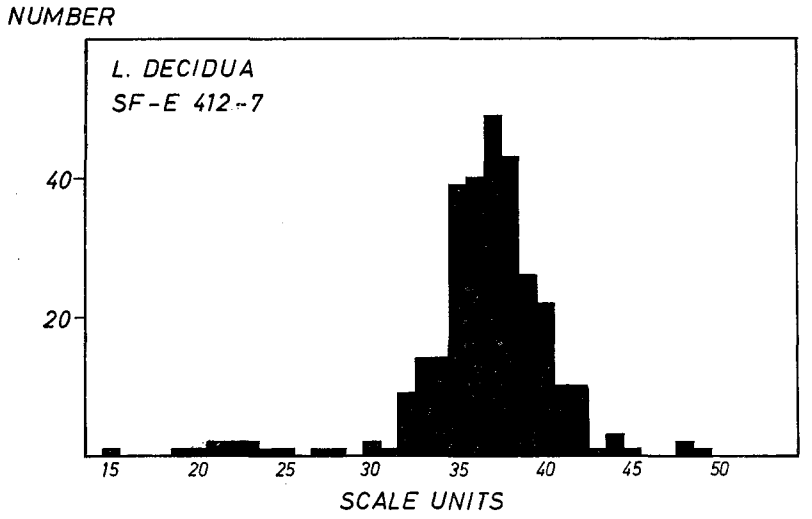

Fig. 52. The distribution of pollen grains in graft SF-E 412-7, *L. decidua*, according to size. 49.5 scale units = 100 μ.

As polyspory occurred in low percentages in the grafts of *L. decidua*, the bimodal type of distribution shown in SF-E 412-7 was not accentuated in this species. Similar distributions were observed in grafts SF-E 412, SF-E 412-3, SF-E 655-20, SF-E 657-11, and SF-E 657-14. On the other hand a clear bimodal distribution occurred frequently in the grafts of *L. sibirica*. The situation in Z 1001-2 is demonstrated in Fig. 53. As may be seen from this diagram the bimodal type of distribution is pronounced in this graft. A high percentage of small pollen grains was also expected from the high percentage of polyspory observed. In Fig. 54 the pollen sizes were tested with respect to the occurrence of normal distributions. According to the diagram it is probable that three separate distributions were present. The average pollen size of the individual normal distributions can be estimated by plotting the individual distributions in separate diagrams. The values obtained in this way amounted to 21, 32, and 45 scale units respectively. It should be noted that the pattern is more complicated than could be given by three pure normal distributions. According to expectation the pollen originating from polysporic units would have half the volume of the ordinary pollen grains. However, this is probably only true during the initial phase of polyspory, as it might be expected that the polysporic microspores would not increase in size, at least not to the same extent as the ordinary pollen. Another complication is the frequent occurrence of multipolar or apolar spindles in connection with polyspory (cf. above) which cause the formation

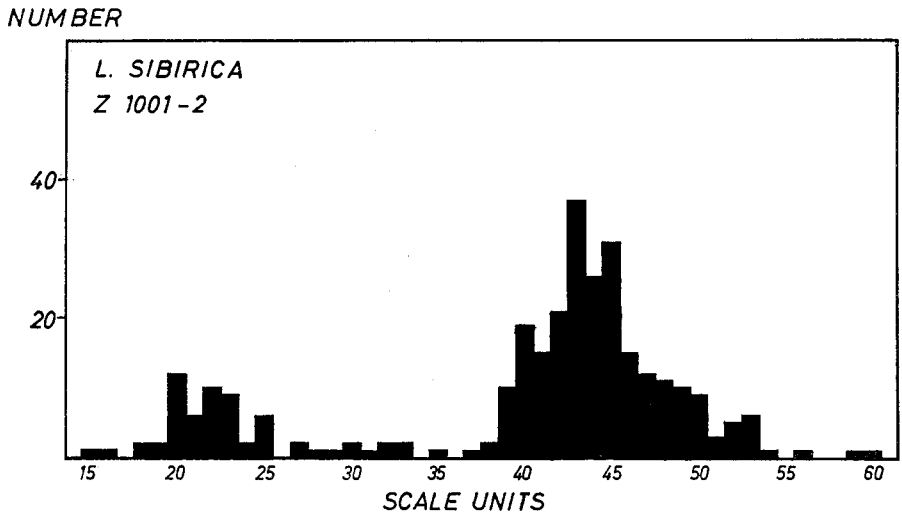

Fig. 53. The distribution of pollen grains in graft Z 1001-2, *L. sibirica*, according to size. 49.5 scale units = 100 μ.

of still smaller microspores. Therefore, it is impossible to conclude anything definite concerning the pollen involved in the three distributions. However, it might be suggested that the smallest category is constituted by the polysporic pollen, the intermediate group originated from PMC arrested in development during meiosis whereas the third category would comprise normal, fertile pollen grains as well as sterile pollen in which the cause of sterility acted less drastically than in the two previous groups. Distributions of the type demonstrated in Fig. 53 were also revealed in grafts Z 1001-1, Z 1002-17, and Z 1003-9.

The bimodal type of distribution in graft L 1003-17, *L. leptolepis*, (Fig. 55) probably originates from two normal distributions. The first distribution (maximum at 32—34) contains probably the end products of PMC arrested in their development during meiosis, whereas the other should contain the normal pollen grains (maximum at 46) as well as relatively slightly damaged pollen grains.

The interpretation of the broad distribution observed in graft Z 1002-8 (*L. sibirica* Fig. 56) is difficult. It should be recalled that the percentage of polyspory was high, amounting to 48 per cent at its maximum. This means that a relatively large number of small pollen grains was expected. Polyspory alone could not be responsible for the sterility in this graft, as the percentage of sterile pollen grains amounted to 82.6 per cent. If this percentage was not excessive, it is probable that PMC to a considerable extent were arrested in

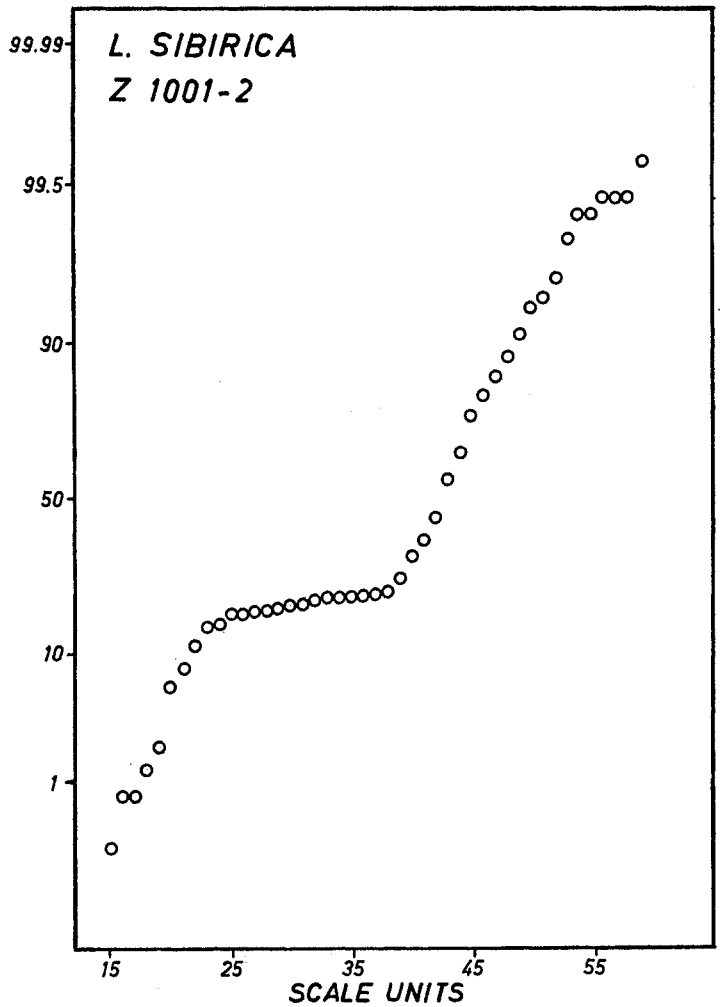

Fig. 54. Test of normal distribution concerning size of the pollen grains in graft Z 1001-2, *L. sibirica*.

development during meiosis. These PMC should constitute the intermediate group between the extremely small pollen grains and the ordinary pollen grains. Besides these categories a fourth group should be composed of the giant pollen grains (cf. CHRISTIANSEN 1960; ERIKSSON *et al.* 1966) which probably arose by formation of restitution nuclei at some time during meiosis. The pattern in Fig. 56 is explained if the boundaries between these four categories overlap.

It is hardly to be supposed that measurement of the pollen grains will be

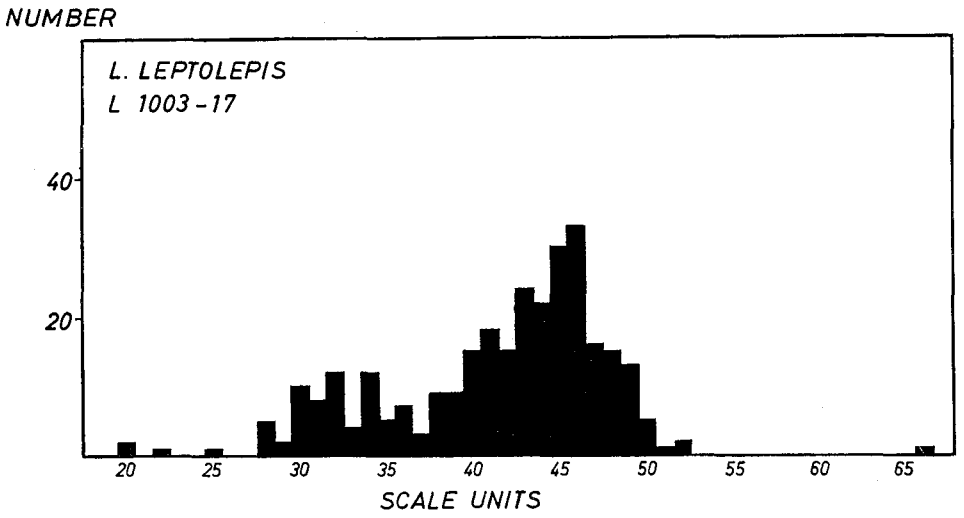

Fig. 55. The distribution of pollen grains in graft L 1003-17, *L. leptolepis*, according to size. 49.5 scale units = 100 μ.

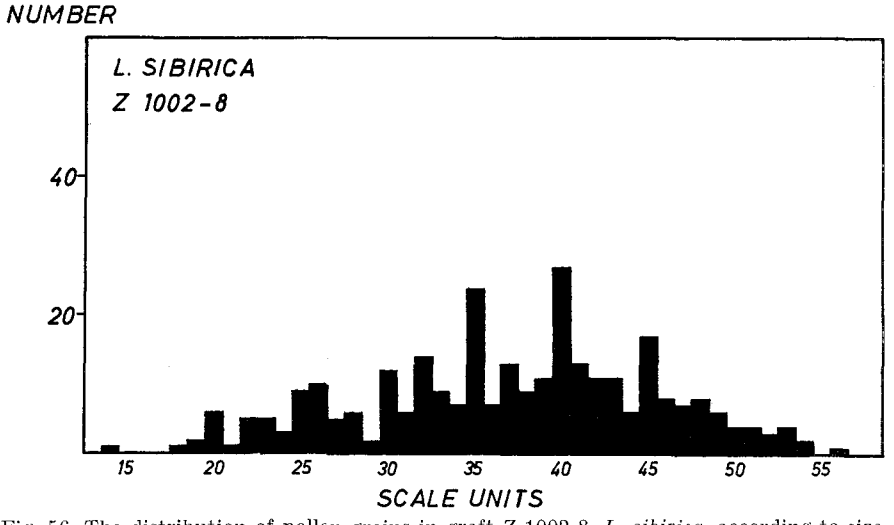

Fig. 56. The distribution of pollen grains in graft Z 1002-8, *L. sibirica*, according to size. 49.5 scale units = 100 μ.

of any practical importance for seed orchard work. Its laborious nature constitutes a great limitation on this method, especially as considerably greater samples of pollen could be tested for fertility by conventional staining methods in the same time. However, for an understanding of all sequences leading to the formation of sterile pollen grains it is justifiable or even necessary.

Relation between irregularities during meiosis and pollen sterility

Above, it was frequently pointed out that there was good agreement between the maximum percentage of irregularities during meiosis and the observed pollen sterility. It was also stated that the percentage of irregularities would not decrease unless healing or other complications occurred. If the irregularities observed cause pollen sterility, a direct relationship between the percentages of irregularity during meiosis and pollen sterility is to be expected. This relationship might be disturbed by genetic factors which are expressed subsequent to meiosis or to induction of damage, e.g. during the pollen mitoses. According to unpublished data by ERIKSSON, the average radiosensitivity during the entire meiosis in barley was about 20 times higher than that during the first pollen mitosis. If individual meiotic stages were analysed the difference in radiosensitivity increased a further 20 or 100 times. If there is a parallel between the influence of radiation and low temperature on the male germ line, the induction of low temperature damage during the pollen mitoses could be neglected. As the same types of abnormality have been registered both following irradiation and exposure to low temperatures, there is every reason to believe that at least some parallel exists between these two external physical agents.

Table 32. Regressions of pollen sterility on maximum percentage of irregularities during meiosis.

No.	Species	Relationship studied	Variance ratio (F) for testing regression
1	*L. decidua*[1]	Pollen sterility—maximum percentage of irregularities during meiosis minus stickiness of C-type	5.95
2	*L. sibirica*	Pollen sterility—maximum percentage of irregularities during meiosis minus stickiness of C-type	13.9*
3	*L. decidua* + *L. sibirica*	Pollen sterility—maximum percentage of irregularities during meiosis minus stickiness of C-type	52.6***
4	*L. decidua* + *L. leptolepis* + *L. sibirica*	Pollen sterility—maximum percentage of irregularities during meiosis minus stickiness of C-type	39.9***
5	*L. decidua* + *L. leptolepis* + *L. sibirica*	Pollen sterility—maximum percentage of irregularities during meiosis	15.7***

[1] Clone SF-E 655 not included owing to its dubious classification.

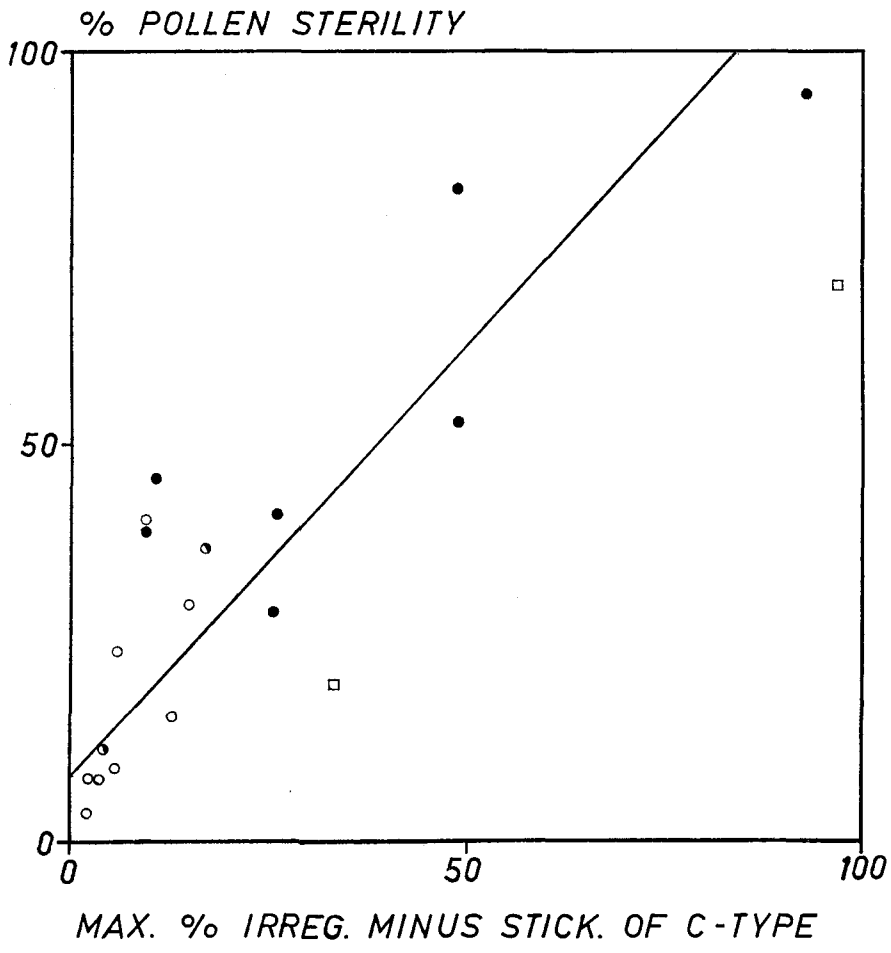

Fig. 57. The relationship between observed pollen sterility during spring 1967 and the maximum percentage of irregularity during meiosis minus stickiness of C-type. The regression line shown refers to number 4 in Table 32. ○ = *L. decidua* ● = *L. sibirica* □ = *L. leptolepis* ◑ = *clone* SF-E 655 of *L. decidua* (cf. material and methods).

In Table 32 the numerically tested relationships between the maximum percentage of irregularities during meiosis (the data from the fixation on 28 March 1967 were in all cases excluded as meiosis had been passed at that time) and pollen sterility are presented. Several different approaches were tried. The data observed and two of the calculated regression lines are shown in Figs. 57—58. As stickiness of C-type was regarded as relatively slight damage, it was assumed that cells affected by this abnormality could be healed in some cases (cf. above and Figs. 20, 25—26). Therefore, it might

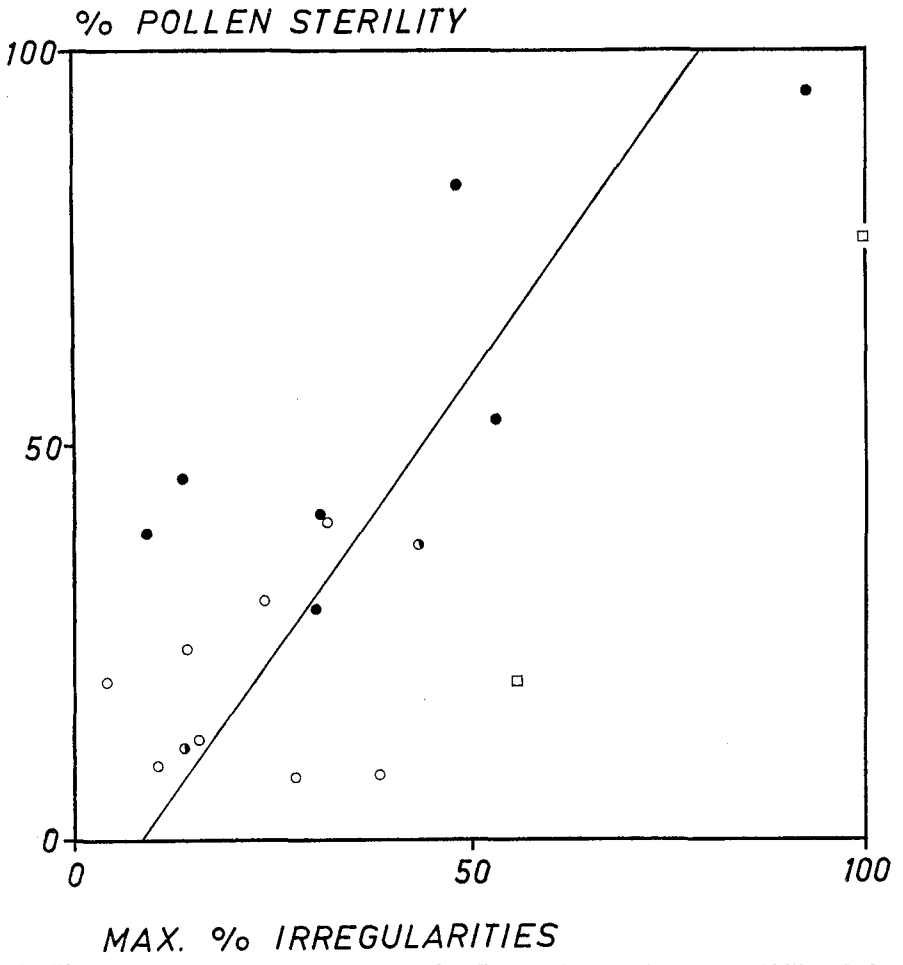

Fig. 58. The relationship between observed pollen sterility during spring 1967 and the maximum percentage of irregularity during meiosis. The regression line shown refers to number 5 in Table 32. ○ = *L. decidua* ● = *L. sibirica* □ = *L. leptolepis* ◑ = clone SF-E 655 of *L. decidua* (cf. material and methods).

be justifiable to exclude this type of irregularity from the tests as was done in relationships Nos 1—4 in Table 32. However, as may be seen from the table there was a high significance for the agreement between sterility and irregularities, independent of the presence of stickiness of C-type, when the data from all species were combined. No significance or only a weak agreement was observed when the data from *L. decidua* and L. *sibirica* were tested individually. The disagreement in *L. decidua* is probably caused by healing of some stickiness of C-type but not others. The true relationship

between sterility-provoking irregularities during meiosis and pollen sterility is probably intermediate to the two extreme ways of estimating the relationships, either including stickiness of C-type or totally excluding it.

The ideal condition would be an equation $y = x$, where y stands for pollen sterility and x for meiotic irregularities. This means that the percentage of irregularities during meiosis should be equal to the percentage of pollen sterility.

The intersection of the regression line on the y-axis at a considerable distance from 0, as was the case for *L. sibirica*, suggests that non-environmental conditions are responsible for the sterility, which should be approximately equal to the numerical value of the intersection point on the y-axis, unless the sterility is exaggerated (see also page 78). This interpretation resembles the one given by SAX (1960) for the pollen sterility in pine hybrids, where the irregularities during meiosis were at a lower level than the observed pollen sterility.

A coefficient for $x < 1$ suggests that the irregularities are partly healed or that buds containing irregularities during meiosis are excluded from the analysis of pollen sterility as discussed above.

Concluding remarks

In the Introduction it was pointed out that the type of meiotic development known for PMC of *Larix* is unique among the conifers. This has been confirmed in the present investigation. However, there is no uniformity of the meiosis in the different species, at least not under Swedish conditions.

The extremely long duration of meiosis in the PMC of *Larix* was believed to be a disadvantage to proper pollen development. This was based mainly on the idea that frost damage might be induced any time during meiosis (cf. EKBERG and ERIKSSON 1967). This is probably not true, since the PMC during most of the time are resting and insensitive to low temperatures. Rather, the data obtained in the present investigation indicate that the phases of active division during meiosis are the most sensitive to low temperatures. According to the data presented in this paper, as well as in earlier papers (EKBERG and ERIKSSON 1967, EKBERG *et al.* 1968), the prerequisites for proper pollen formation could be summarized as follows:

A. Reaching of the diplotene stage before the frost appears during the autumn.

B. Stability of the resting diplotene stage against short temperature fluctuations around 0° C (-5 — $+5$° C).

C. Rapid reaching of the tetrad stage when the development from diplotene was initiated.

To be able to give any forecast for the pollen quality it is necessary to discover whether these three requirements are fulfilled by the different species. The following characteristics might be arrived at from the detailed study 1966—67 as well as from later data which partly are unpublished:

1. Diplotene is reached early in *L. sibirica*. The initiation of further development from diplotene takes place early, even during autumn in *L. sibirica*.

2. Diplotene is reached relatively early in *L. decidua*. The initiation of the further development from diplotene takes place late in *L. decidua*.

3. Diplotene is reached relatively late in *L. leptolepis*. The initiation of the further development takes place somewhat earlier in *L. leptolepis* than in *L. decidua*.

Although some overlapping between the species occurs (cf. Table 6) the trend for reaching of diplotene in the sequence *L. sibirica*—*L. decidua*—*L. leptolepis* seems to be clear. This means that the probabilities for irregularities caused by autumn frosts will increase in this order for the three larch

species. Owing to the late reaching of diplotene in *L. leptolepis*, a considerable contribution to the amount of damage will originate from autumn frosts. At certain localities, e.g. Sundmo, all species will probably suffer from autumn frosts (cf. Table 6 and Fig. 11).

If requirement B is not fulfilled, the sensitive stages diakinesis—telophase I, and prophase II—telophase II will with a high probability be exposed to low temperatures, with a disastrous effect on pollen formation, as was the case in the Siberian larch clones Z 1001, Z 1002 and Z 1003.

As only one clone of *L. leptolepis* was continuously studied during 1966—67, the early passing of diplotene cannot be regarded as a characteristic of this species, especially as diplotene only was passed to small extent in the other clones investigated at Ekebo and Brunsberg in the middle of January 1967 (cf. EKBERG *et al.* 1968). The observations from 1967—68 lend further support for the impression that clone L 1003 was outstandingly early concerning further development from diplotene.

Knowledge is relatively limited concerning the time needed for completion of meiosis when further development from diplotene has been initiated. The data from 1966—67 give the impression that this time was short in clones SF-E 412 and SF-E 657 of *L. decidua*. However, during March 1966 this development was slower in these clones of *L. decidua* than in *L. sibirica*. The temperature conditions following the initiation of development from diplotene will determine the rate of development. This means that great variations in the time needed for this development will occur from year to year. For *L. decidua* CHRISTIANSEN (1960) claimed that the minimum temperature needed for a continuation of the division was 1—2° C lower during late prophase than during metaphase I—anaphase II.

To sum up the data from the winter 1966—67, it may be stated that only the clones SF-E 412 and SF-E 657 fulfilled the three requirements A—C above. The Japanese larch clone L 1003 did not fulfil any of these requirements, whereas the Siberian larch clones Z 1001, Z 1002, and Z 1003 as well as the dubious clone SF-E 655 only fulfilled requirement A.

For general statements concerning the probabilities for low or high pollen fertility it is necessary to test the temperature curves for the time of the year during which the sensitive part of the meiosis takes place, which is mainly constituted by the development diakinesis—telophase II. Owing to the early initiation of the development from diplotene in *L. sibirica* growing in southern Sweden, the PMC of this species will in most cases be exposed to low temperatures. Therefore, they will suffer severely from frost damage and the pollen fertility will, with high probability, mostly be relatively low, rarely exceeding 50 per cent. Also in *L. decidua* and *L. leptolepis* the initiation of further development from diplotene can take place at the wrong

time, as was observed during March 1966 in Stockholm, when the sensitive stages were exposed to low temperatures shortly after the initiation of the development from diplotene (cf. EKBERG and ERIKSSON 1967).

The size of the regression coefficients for the relationships between sensitive cells, low temperatures and irregularities must also be considered when an anticipation of the pollen sterility is made, as these coefficients when compared for the same variables express the relative sensitivity to induction of a particular irregularity. As the regression coefficients were larger in *L. decidua* than in *L. sibirica* for the variable—aberration area minus polyspory—the presence of low temperature during sensitive phases are of greater importance in *L. decidua* than in *L. sibirica*. The situation might be reversed if polyspory could be compared between these two species.

From the discussion carried out above it can be concluded that the forecast for good pollen formation cannot be regarded as favourable.

It might be debated what practical implications high pollen sterility has for a good seed setting. As this was discussed in the introduction (cf. page 10) it will here only be stated that high pollen fertility is probably needed for proper seed formation for the following reasons:

A. The short range distribution of pollen in Larix (cf. DYAKOWSKA 1936 and DYLIS 1948).

B. The reduction in seed setting due to lack of pollen reported by SARVAS (1952, 1955, 1957 and 1958).

Therefore, a belief that high pollen sterility has no influence on seed setting is probably not true, at least in *Larix*.

In this connection it might be discussed whether the estimate of sterility should be based on the sample of pollen released from anthers at maturity or if it should be related to primarily formed primordial cells in the male germ line. The first method is a convenient one and would be accurate enough if large samples of pollen were available. Quite another situation is met with if the number of pollen grains is limited. This is best illustrated by an example from the paper of EKBERG *et al.* (1968) where the pollen sterility of *L. leptolepis* at Brunsberg was reported to be 36.9 per cent. From the fixation carried out during the end of January it was observed that about 90 per cent of the buds tested (total 112) contained only damaged PMC. This means that the sample used for estimation of the pollen quality at maturity consisted of only 10 per cent of the originally formed primordial cells. The amount of fertile pollen grains in this special case seems to be sufficiently high for a successful pollination. However, it must be remembered that the fertile pollen grains constitute about 6 per cent of the originally formed primordial cells. In the light of this the probability for a high seed

setting will be reduced considerably, especially as the pollen dispersal in *Larix* is limited.

According to KIELLANDER (1966 A and B) the most promising results concerning cultivation of *Larix* under Swedish conditions originated from interspecific hybrids. Therefore, the seed orchards are mostly composed of two different species. The hybrid seeds will be obtained following artificial crossings. The practical crossing work in the seed orchards could be guided by the aid of cytological examination during the active division from diplotene to microspores in the PMC, as there has been shown to be a direct relationship between irregularities during meiosis and pollen sterility (cf. Figs. 57—58). In the case of a high percentage of irregularities in a presumptive father tree, the planned crossing must be postponed to another year. Therefore, much labour will be saved by making use of random tests of meiotic irregularities during the phase diakinesis—telophase II. The moment for a test fixation can also easily be estimated once the temperature response for the initiation of further development of the diplotene cells has been clarified. This has not been possible to achieve exactly in the outdoor experiments performed. However, for such random tests as outlined above the present knowledge of the response is probably accurate. With the aid of the amount of irregularity on such an occasion it should be possible to predict pollen sterility with a relatively high degree of accuracy. The crosses planned should be based on these predictions.

It is interesting that the knowledge of temperature-conditioned male sterility can save still more practical work in the seed orchards. Thus, in the case of observed or predicted 100 per cent pollen sterility in a clone, all filled seeds formed in the grafts of this particular clone have originated from crossings. This could be used advantageously for interspecific crossings if the seed orchards were composed of one clone of one species and about 20 clones of another species as is frequently the case (cf. ANDERSSON and ANDERSSON 1962). If the single clone is male-sterile, exclusively interspecific hybrid seeds will be obtained. Furthermore, this will be obtained without any labour. Also in the case of somewhat lower pollen sterilities than 100 per cent of the single clone, the production of inbred seeds can probably be neglected, as they will mainly give rise to seedlings of reduced vitality owing to inbreeding depression (cf. LANGNER 1951—1952; DIEKERT 1964) and can easily be selected against in the nursery. The siting of seed orchards in the future should take advantage of the knowledge of the temperature-conditioned male sterility.

Finally it will be discussed whether the data obtained for *Larix* are of any value for the important Swedish forest trees, Scots pine and Norway spruce.

Above it was pointed out that the long duration of meiosis in PMC of *Larix* was not responsible for the frequently poor pollen quality. Therefore, this unique meiotic pattern in *Larix* does not constitute a limitation for the universal applicability of the *Larix* data. It has also been shown by ANDERSSON (1954, 1965) that low temperatures (below -4° C) were responsible for induction of irregularities in the PMC of the native spruce in Sweden. For this species ANDERSSON (1965) obtained a positive correlation between the percentage of empty seeds and the number of days with a minimum air temperature of -4° C or lower during a period of 45 or 50 days preceding the flowering. Although the correlation coefficients were regarded as uncertain, it is somewhat astonishing that these correlations could be established. Thus, during such a long time the sensitivity of the PMC and microspores against low temperatures must have varied tremendously if parallels with *Larix* exist.

It can be concluded that disturbances of the meiotic development due to low temperatures also exist in spruce. As meiosis takes place later in spruce than in larch the probability for exposure of the sensitive cells to low temperatures must be lower in spruce than in larch.

The temperature limit below which damage is induced is also of importance for an evaluation of the probability for induction of irregularities. From literature it is known that this limit varies among the conifers from $+2$— $+4^\circ$ C for *Pinus edulis* (CHIRA 1967) to -4° C for *Picea abies* (ANDERSSON 1954). A complete freezing of the PMC will probably not take place at any of these temperatures. This suggests that some physiological process(es) is (are) disturbed within a certain temperature range. The exact temperature response of the PMC of *Larix* could not be made. However, irregularities have been observed at temperatures higher than -4° C (see also CHRISTIANSEN 1960). Thus, in this respect also the situation in the native spruce is more favourable than in larch. However, knowledge about the generative development in the introduced spruce is relatively limited (ANDERSSON personal communication). As foreign provenances in some cases have shown extremely promising growth in southern and middle Sweden (cf. LANGLET 1960, 1964; KRUTZSCH 1968) a seed supply of them will in future be needed for further plantations. The seed material can probably not be obtained in all cases from the native stands or plantations. Therefore, seed orchards of foreign spruce provenances have to some extent been established in Sweden. For future siting the experience from *Larix* may be of some value. As the generative fitness in *Larix* was poor it means that great care must be taken in the siting of seed orchards, especially as little is known about the generative fitness of the introduced provenances of spruce. Therefore, one of the most important tasks for the Swedish forest research

of today is to analyse the generative development in the vegetatively most promising foreign spruce provenances.

It is rather probable that pollen banks will be established to a great extent in the near future. It is also possible that the pollen will be produced under artificial conditions, for instance, in plastic green houses. Obviously, a high pollen quality is required for the pollen banks. However, nothing is known about the meiotic development and the pollen formation under the conditions prevailing in a plastic green house. In particular, high temperatures occur frequently in such green houses. As it has been stated that high temperatures also can induce irregularities during meiosis in conifers (CHIBA and WATANABE 1952; ANDERSSON 1966) it is necessary to test the meiotic development under these extraordinary conditions to avoid disastrous drawbacks. One of the most detailed investigations concerning heat treatment during meiosis emanates from STRAUB (1939), who used *Gasteria* as test plant.

The high sensitivity of meiosis also indicates that great care should be taken concerning artificial flowering stimulation. This means that parallel to the stimulation experiments, tests of the generative development following stimulation should be carried out. It is useless to induce flowering unless the gamete quality is guaranteed to be at a high level.

Summary

In the present paper the meiotic development in the PMC of the three larch species *Larix decidua*, *L. leptolepis*, and *L. sibirica* was studied. Emphasis was paid to the analysis of the conditions of importance for breaking the dormancy of the diplotene stage as well as the factors responsible for the initiation of further development from diplotene. On the basis of a detailed analysis of meiosis in a few clones 1966—67 as well as in larger number of clones in 1967—68, the following characteristics of the meiotic development of the three species were observed:

L. decidua: Diplotene is reached relatively early. The initiation of the further development from diplotene takes place late.

L. leptolepis: Diplotene is reached relatively late. The initiation of the further development takes place somewhat earlier than in *L. decidua.*

L. sibirica: Diplotene is reached early. The initiation of the further development from diplotene takes place early, even during the autumn.

During the course of the investigation irregularities appeared frequently. For an understanding of the origin of the irregularities a distinction was made between different categories. Chromosomal irregularities and irregularities in cell division were the two main categories. The appearance of the various irregularities was demonstrated by photomicrographs. In *L. decidua* and *L. leptolepis* stickiness and degeneration predominated, whereas polyspory, stickiness and degeneration occurred most frequently in *L. sibirica.*

It was shown that irregularities did not appear until sensitive cells were exposed to low temperatures. All phases of active division during meiosis were believed to be highly sensitive to low temperatures.

An attempt was made to obtain quantitative relations between low temperatures and sensitive cells on one hand and irregularities on the other hand. Good approaches for such relations were observed in several cases. However, the exact temperature response could not be revealed, owing to the impossibility of achieving an artificial control of the outdoor temperature conditions.

From this investigation, as well as previous ones, the prerequisites for proper pollen formation could be stated in the following way:

1. Reaching of diplotene before frost appears during autumn.

2. Stability of the resting diplotene stage to short temperature fluctuations around 0°C (− 5—+ 5°C).

3. Rapid reaching of the tetrad stage when development from diplotene is initiated.

On this basis it could be concluded that the probability of obtaining proper pollen formation was not promising as *L. leptolepis* did not fulfil any of these requirements, whereas *L. sibirica* only fulfilled the first one. For the Stockholm area the best chance of obtaining a high pollen fertility should be given by *L. decidua*.

In agreement with this forecast, the lowest pollen sterility during spring 1967 was observed in *L. decidua* where the percentages were mostly close to or less than 10 per cent. The highest sterilities were observed in clone Z 1002 of *L. sibirica*. In some cases almost complete male sterility was registered. The average sterility in the other Siberian larch clones tested amounted to about 50 per cent. Also in *L. leptolepis* almost complete male sterility was observed in some grafts. Measurements of microspores and pollen grains were performed to elucidate further the pollen formation.

A direct relationship between the maximum percentage of irregularities during meiosis and pollen sterility was demonstrated. This means that cytological examinations during meiosis could advantageously be performed in order to predict the pollen sterility. With the aid of such tests the crossing programmes in seed orchards should be planned.

Other practical implications of the observations for the work in seed orchards were discussed as well. Some outlines for future work along these lines were briefly touched upon.

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Sammanfattning

Temperaturens betydelse för pollenbildningen hos lärk.

Meiosutvecklingen hos pollenmoderceller (PMC) från de tre lärkarterna *Larix decidua* (europeisk lärk), *L. leptolepis* (japansk lärk) och *L. sibirica* (sibirisk lärk) har studerats och presenterats i det föreliggande arbetet. Tyngdpunkten hos denna del av undersökningen har varit att utreda vilka faktorer som är ansvariga för brytande av viloperioden (dormancy) hos PMC och för igångsättningen av den fortsatta utvecklingen från diploten. Särdragen för meiosutvecklingen hos de tre arterna kan sammanfattas enligt följande:

L. decidua: Diplotenstadiet uppnås relativt tidigt under hösten. Initieringen av den fortsatta utvecklingen från diploten äger rum sent, oftast under mars.

L. leptolepis: Diplotenstadiet uppnås relativt sent under hösten. Initieringen av den fortsatta utvecklingen från diploten äger rum något tidigare än hos europeisk lärk.

L. sibirica: Diplotenstadiet uppnås tidigt. Initieringen av den fortsatta utvecklingen från diploten äger rum tidigt, ibland redan under hösten.

Oregelbundenheter hos PMC var vanligt förekommande. För att förstå uppkomstsättet för oregelbundenheterna gjordes en indelning av dem i två huvudgrupper, kromosomala oregelbundenheter och oregelbundenheter vid celldelningen. Utseendet hos de olika kategorierna framgår av planscherna 1—5.

Undersökningen visar att oregelbundenheterna uppträdde först i och med att känsliga celler exponerades till låga temperaturer. Alla faser under meiosen då aktiv delning förekom förmodades vara starkt känsliga för exponering till låga temperaturer.

Ett av syftena med undersökningen var att fastlägga kvantitativa samband mellan låga temperaturer och känsliga celler å ena sidan samt oregelbundenheter å den andra sidan. Dylika samband kunde påvisas i vissa fall. Den exakta temperaturtröskeln för uppkomst av skador kunde emellertid inte bestämmas beroende på att undersökningen utfördes på material som växte utomhus där det inte finns några möjligheter att reglera de temperaturer som PMC exponerades till.

Egenskaper som är av stor betydelse för en god pollenutveckling kan med ledning av föreliggande resultat sammanfattas på följande sätt:

1. Uppnående av diplotenstadiet innan höstfrosterna gör sig kännbara.
2. Stabilitet hos diplotenstadiet gentemot kortvariga temperaturfluktuationer kring 0°C (– 5— + 5°C).
3. Snabb utveckling diakines—tetrader när initieringen av den fortsatta utvecklingen från diploten ägt rum.

Baserat på detta kan det konstateras att möjligheterna för en god pollenutveckling inte ter sig särskilt ljusa för den japanska lärken, vilken inte uppfyller några av ovanstående punkter. Likartat är förhållandet för den sibiriska lärken, som endast uppfyller det första kravet. Hos den europeiska lär-

ken däremot ter sig möjligheten till en god pollenutveckling åtminstone inom stockholmsregionen något gynnsammare.

I överensstämmelse med detta erhölls våren 1967 den lägsta pollensteriliteten hos *L. decidua* där procenttalen sterila pollenkorn ofta var nära eller under 10 %. Den högsta steriliteten observerades hos klon Z 1002 av *L. sibirica*, där nära nog fullständig hansterilitet iakttogs hos vissa ympar. Den genomsnittliga steriliteten i övriga sibiriska lärkkloner uppgick till cirka 50 %. Även hos *L. leptolepis* observerades i vissa fall nästan fullständig hansterilitet. För att erhålla ytterligare informationer rörande pollenbildningen bestämdes mikrospor- och pollenstorleken vid ett par tillfällen.

Ett direkt samband mellan pollensteriliteten och det maximala procenttalet av oregelbundenheter under meiosen kunde fastläggas. Detta innebär att cytologiska undersökningar under meiosen med fördel kan utföras för att erhålla en förutsägelse om pollenkvaliteten. Dylika test kan därför vara till stor hjälp vid planeringen av korsningsarbetena i fröplantagerna.

Andra praktiska tillämpningsmöjligheter som kan utdragas ur de erhållna resultaten diskuterades även. Dessutom gjordes några utblickar för framtida arbeten längs de forskningslinjer som ovan presenterats och diskuterats.

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