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On the Effect of Low Temperature on Meiosis and Pollen Fertility in Larix decidua Mill.

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In connection with routine examinations of pollen of larch used in 1954 in controlled pollinations at the Tree Improvement Station, it was frequently observed that samples of pollen contained a considerable number of irregular pollen grains (BARNER and CHRISTIANSEN [1]), viz: pollen with large vacuoles, giant pollen, micropollen, twin-pollen, apparently normal pollen of varying size and dead pollen. Giant pollen and pollen of varying size have been observed in Larix decidua MILL. and Larix occidentalis NUTTAL by SYRACH LARSEN and WESTERGAARD (12), and twin-pollen is reported in L. leptolepis SIEB. et Zucc. by SAXTON (9) and MÜLLER-STOLL (7). In 1954 little could be done to find the cause of these irregularities; larch pollen does not germinate in vitro, at least not by ordinary methods, and the results of the controlled pollinations did not give any clue, firstly because part of the pollen always looked normal and secondly because there could be many other reasons for unsatisfactory seed setting than the irregular pollen.

It was observed, however, that two samples of pollen mother cells (abbreviated: PMCs), taken from the same tree at an interval of six days, were at the same stage of meissis (Diakinesis-Metaphase I); the air temperature was about zero. It was further found that PMCs at the meiotic metaphase I (abbrev.: M,) in male buds of twigs of larch stopped divisions when placed in a refrigerator at a temperature of 0^0 — $+3^0$ C, but when, several days later, they were moved to the laboratory at about $+22^0$ C, the "frozen" divisions started again, and aftsr a few hours only metaphase, (abbrev.: M,) and pollen tetrads could be found.

This of course gave rise to the question whether the extremely complicated processes of meiosis (cf. LANGNER [5]) could endure such prolonged inhibitions without detrimental effects. — As mentioned by TISCHLER (13), NEMEČ (Tschekoslovakia) considers it possible that meiosis in larch may start in autumn and be completed in the course of the winter. SAXTON (9) (England) states that PMCs in larch may start divisions ultimo September and complete them after a month's rest. According to these statements, an extension of meiosis over several months is not unusual in larch, and it would imply that this tree should also be able to undergo fractionated meiosis without detriment to the resulting pollen. In Denmark we have, however, never observed meiosis in larch earlier than February-March and as, furthermore, the temperature during winter and early spring in this country is probably iower, and the variations more extreme, than in Great Britain or Tschekoslowakia, it might be supposed that injuries to the PMCs, which have not been observed in more southern regions, might occur here.

In the winter 1955-56 flower buds were abundant, and considerable variations in air temperature, from rather high in the months November-January to unusually low in February-March ofiered an opportunity to study the reaction of the PMCs. — November and December 1955 and January 1956 had 27, 17 and 8 days respectively on which the maximum temperature was $+5^0$ C or higher, and on which the meiotic processes could be assumed to have proceeded, although slowly. During February the maximum temperature of the month was $+3.5^0$ C (only one day), the minimum $-17{,}3^0$ C. On 26 days of February the temperature did not rise above $+1^0$ C, and the nieiotic processes were no doubt completely inhibited. The period March 1st -- March 24th had only three days with max. temperature above $+5^0$ C. Minimum temperatures were on 17 days below zero and varied between $+1.3^0$ and -8.1^0 C. — The temperatures quoted are those registered by the Meteorological Institute at Copenhagen (3).

On March 16th, at the time when the reduction division could be expected to take place, the male buds of two trees of L. decidua in the garden of the Royal Veterinary and Agricultural College at Copenhagen were at the prophase stage of meiosis. In the hope that no sudden rise in temperature would take place, in which case the investigation would probably have had to be postponed till next years flowering of larch, it was decided to follow the progress of meiosis in those two trees.

Material and Methods

The two larch trees were about 50 years old and grow so close together that the branches touch. They are placed approximately North-South to each other. The northern tree is in the following named the N-tree, the southern the S-tree. Although as mentioned the twc trees stanc! close together, it was soon found that meiosis in the S-tree proceeded considerably faster than meiosis in the N-tree, and detailed examination was, therefore, limited to the latter. The investigation started on March 16th, when the PMCs of both trees were in early prophase, and terminated on May 6th when the shedding of pollen had ceased. From March 20th, when the first PMCs at the stages diakinesis-M, were found, till March 28th, when about 79% of the PMCs were at the stages telophase, — pollen tetrades, at least 50—60 PMCs from different parts of the N-tree were daily examined (Table 1, col. *6)*. On account of the difficulties in determining the stages of the irregular PMCs and because of a certain variation in stages in dif-

Table 1.

Date 1956		*L. decidua N-tree*														*L. decidua S-tree*
		Max. temper. during the 24 hours C°	Min. temper. during the 24 hours C°	Daily number of hour's sunshine	Max. rel. air humidity during 24 hours %	Number of PMCs examined	Percentages of PMCs at the stages: Prophase	Diakinesis	Metaphase I	Anaphase I	Telophase I	Metaphase II	Anaphase II	Telophase II	Pollen tetrads	Estimated % % of PMCs at various stages: S-tree
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
March	16	0.7	·/.1.7				100									Prophase 100%
	17	0.7	·/.1.3				100									"
	18	1.9	·/.1.1	3.2			100									"
	19	1.3	·/.1.1	7.6			100									"
	20	2.9	·/.2.3	7.5		[1]	70	25	2	1	1					Diak., M_1-T_1 c. 80%
	21	3.1	·/.1.1	4.7		[1]	70	25	2	1	1					"
	22	4.1	·/.0.7	7.8		51	24		42	4	15	14		1		"
	23	3.9	0.1	1.1	88	66	54	17	27	2						T_1-M_2 c. 80%
	24	3.9	0.3	6.4	80	56	46	11	39	2	2					Pollen tetrads c. 80%
	25	5.5	0.1	8.1	85	56	55		30	6	9					" c. 100%
	26	7.1	·/.0.1	8.3	94	60	45	8	40	2	3			2		"
	27	7.5	·/.0.7	9.6	92	61			30	3	46	16	2	3		"
	28	8.9	·/.0.9	7.4	93	63					11	8	2	31	48	Poll. tetrads, Poll. grains
	29	10.1	·/.1.3	10.0												
	30	9.1	1.7													
	31	12.1	2.9	8.7	a few pollen tetrads & pollen											Pollen, a few tetrads
April	1	5.3	2.7													
	2	10.4	0.5	2.3												May 5th-May 6th: pollen discharge.
	3	6.1	1.7													
	4	4.7	·/.0.9	4.9												
	5	3.9	·/.0.7	1.4												
	6	3.1	·/.1.1	4.8												
	7	4.7	·/.3.7	11.4												
	8	7.7	·/.4.5	10.2	first mitotic poll. grain div. observed											
	9	7.5	1.0													
	10	10.1	4.9	0.2												
	11	9.1	1.3	9.8												
	12	8.1	1.9	8.1												
	13	6.9	0.7	1.3												
	14	9.1	1.5	7.6												
	15	5.3	0.5													
	16	3.9	0.7	0.4												
	17	3.9	0.5													
	18	8.1	0.1	3.7												
	19	11.7	1.1	10.2												
	20	13.5	2 3	8.3												
	21	10 3	0.7	9.3												
	22	9.9	2.9													
	23	11.5	2.5	9.5												
	24	4.7	2.7													
	25	8.1	2.5	0.8												
	26	9.3	2.9	2.5	pollen grain divisions completed.											
	27	12.0	4.2	2.3												
	28	9.9	3.9	2.2												
	29	12.9	3.3	3.1												
	30	5.1	3.9													
May	1	6.2	2.3													
	2	8.6	4.1													
	3	12.0	5.3	1.0												
	4	18.3	5.1	8.4												
	5	19.1	8.1	4.3	pollen discharge											
	6	19.7	10.5	1.4	"											

[1]) Percentages shown March 20th and March 21st are estimates.

ferent inflorescences, the figures are only approximate. Of the S-tree a daily estimate of the percentage of PMCs at the various stages was made *(Table 1, col. 16)*. An attempt to determine the percentage of normal and damaged PMCs had to be abandoned on account of the impossibility to decide whether some slight irregularity of a division was of any importance and, furthermore, serious internal injuries to cells or chromosomes might be present without showing.

The study of the mitotic pollen grain divisions had to be limited to a few cells on account of the difficulty of staining and preparation. Pollen from both trees was tested by artificial pollinations at the Tree Improvement Station and no seeds were produced. In the fall cones from the N- and S-tree were examined for viable seeds and none were found; evidently all the pollen was sterile.

Daily readings of maxima and minima of air temperature were made from a shaded thermometer placed on one

of the lower branches of the N-tree. During the period when divisions were going on, the temperature was recorded continuously by a thermograph placed under the N-tree. As these readings, however, only differed by 1—2° C from the official daily readings of the maximum and minimum temperatures carried out in another part of the College garden by the Meteorological Institute (3) only the latter are used (cf. *Table 1, col. 2—3). Table 1, col. 5,* shows daily readings of the maximum percentages of the relative air humidity during the period March 23rd — 28th; the percentages were practically unaltered during the period. *Col. 4* represents the daily number of hours of sunshine registered by the Meteorological Institute. The sunshine hours are of interest because the temperature inside the dark brown buds may be considerably higher than the air temperature when the sun shines. The maximum temperatures shown in *col. 2* may, therefore, not always be the maxima to which the PMCs have actually been exposed. During the period March 23rd — 28th the daily number of hours, during which the air temperature was higher than +3° C, was practically the same as the daily number of hours of sunshine. During this period the weather was sunny with only slight cloudiness; the maximum temperature always occurred during the day, the minimum during the night.

Progress of Meiosis

N-Tree: On the 16th of March the PMCs were as mentioned above in early prophase *(Fig. 1).* The exact stage could not be determined, but the nuclear membrane and the nucleolus were still present, and in many nuclei paired threads could be discerned, suggesting pachytene. If this is correct the zygotene pairing of the chromosomes had already taken place. During the period March 16th — 19th the maximum air temperature was about +1 — 2° C, and no change in the stage of the PMCs could be found. On March 20th the max. temperature reached c. +3° C during the day. In some of the inflorescences examined the PMCs were still at prophase, while in others ab. 28% were at diakinesis. Evidently the slight rise in temperature had been sufficient to enable some of the PMCs to go through to diakinesis, but not sufficient for the functioning of the spindle mechanism. — On the 21st both the max. temperature and the stages were unaltered, but on the 22nd the max. temperature was c. 1° C higher, and about 42% of the PMCs examined were at M_1, and 34% at later stages. The 34% at later stages were, however, not typical of the general situation, the cells being probably from a single anther considerably ahead of the majority; in effect the position was practically unaltered during the period March 22nd — 26th, although the daily maximum of air temperature had reached approx. +7° C. — On the 27th — 28th the stages anaphase$_1$ — telophase$_2$ and pollen tetrads were predominant. On the 31st only pollen and a few pollen tetrads were found.

On April 8th the first mitotic pollen grain divisions were observed, and on about April 26th the divisions were completed. On May 5th — 6th pollen was discharged on both trees.

On March 22nd a number of twigs from the N-tree with PMCs in the stages diak.-M_1 were placed at a temperature of +7 — 15° C; after 32 hours only pollen tetrads could be found. This showed that the spindle mechanism was able to function more or less normally, and that the inhibition was caused by the low temperature.

The PMCs of the S-tree were at the same stage as those of the N-tree (presumably pachytene) on March 16th, but on March 20th about 80% were at diak.-T_1, and on the 25th practically all had reached the pollen tetrad stage. According to *Table 1, col. 7—11* and *col. 16* the PMCs of the N-tree apparently developed at the same rate as those of the S-tree until they reached the stages where spindle activity begins (March 20th). Henceforth the S-tree took the lead and reached the pollen tetrad stage 3—4 days before the N-tree. — The most manifest reason for the difference in duration of meiosis in two trees growing so close together is, of course, that the S-tree is more exposed to the sun. As larch, however, has no leaves at this time of the year, the branches do not throw much shade, and no higher difference in temperature than c. 1° C could actually be measured in the crowns of the trees. This small difference in temperature may, of course, be the sole cause, but in that case it ought to be expected that the S-tree had also reached the diak.-M_1 stage before the N-tree. As it is, it seems probable that physiological dissimilarity of the trees may be at least a contributary cause.

The mitotic pollen grain divisions in the S-tree were not examined.

Irregularities Observed During Meiosis

The irregularities were similar in both trees. Those most frequently observed were: stickiness, pycnosis, chromosome breaks and fragmentation, chromatin bridges at anaphase$_1$ (abbrev.: A_1) and anaphase$_2$ (abbrev.: A_2), abnormal contraction of the chromosomes, irregular cell wall formation, deformities of PMCs and unequal size of nuclei, and abnormal chromosome numbers. The bivalents at the stages diplotene-M_1 were so strongly contracted that it was often difficult to determine the stage. — The irregularities may of course, have started some time before the observation was made, but it was often impossible to determine from which stage they developed. In the following an account is given of the irregularities most frequently observed at the different meiotic stages and — where possible — of their most probable origin.

Prophase: As mentioned above the stage of the PMCs when the investigation was started, seemed to be about pachytene and nothing can, therefore, be said about the earlier stages. Even at pachytene an analysis was not possible on account of the tangled and diffuse chromosomes. The diplotene stage *(Fig. 2, 3, 4)* was rarely observed, perhaps because this stage seems to be able to proceed at lower temperature than the stages M_1—A_2 and such cells therefore had carried on to the diakinesis stage. A considerable number of prophase cells were observed in which the contents of the nucleus were either "pulverized" e. g. reduced to tiny fragments *(Fig. 5),* or no chromatin could be seen. In both instances the cells were no doubt dying.

Diakinesis and Metaphase$_1$. The chromosomes were very strongly contracted and in the majority of the PMCs stickiness, fusion of bivalents *(Fig. 6, 8),* fragmentation *(Fig. 7),* pycnosis *(Fig. 9)* or other deformities were observed. In fact it was difficult to find a PMC with 12 apparently normal bivalents *(Fig. 10* shows one of the most normal M_1). In many PMCs two or three bivalents were fused together, but often several bivalents were fused together into a lump that could not be analysed. PMCs with the diploid number of chromosomes were not found at these stages. It is possible that part of the fusion and lumping of bivalents may emanate from interlocking during the pairing at zygotene. It is well known that stickiness and

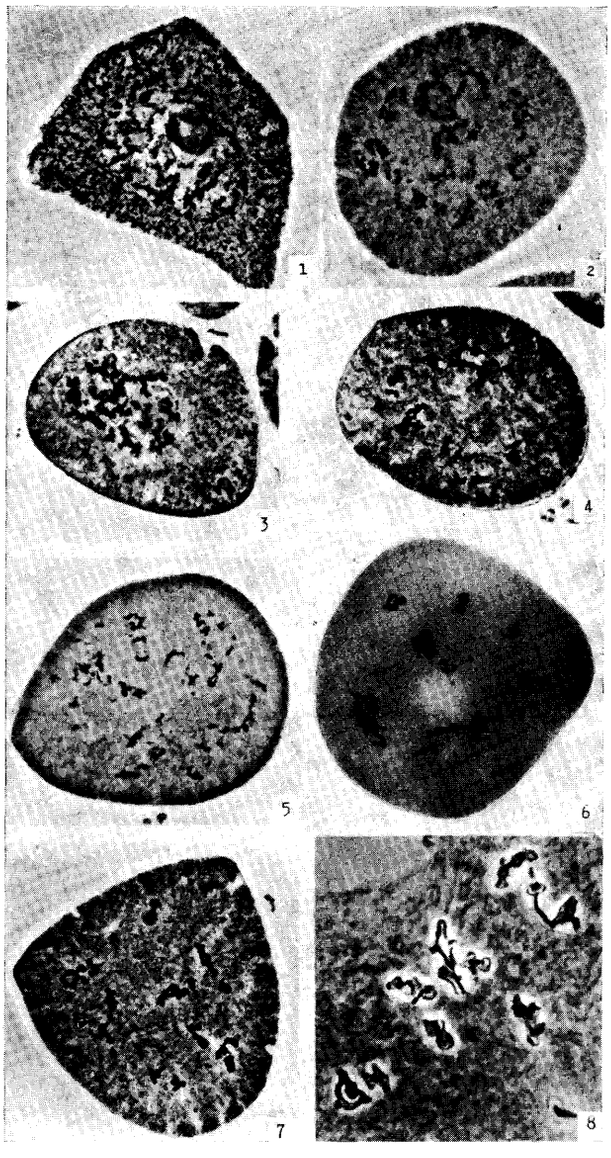
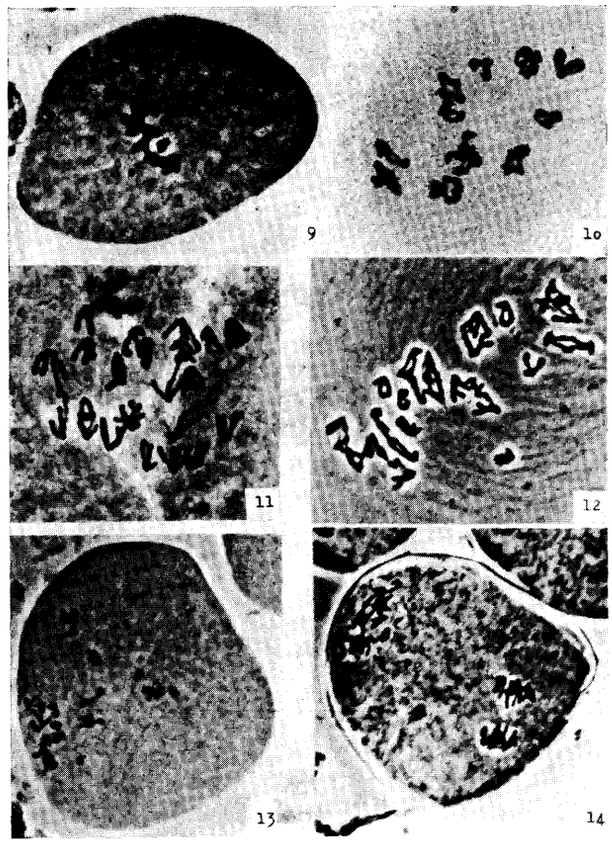


Fig. 1—8. — Irregularities in pollen mother cells of *Larix decidua* caused by low temperature. — Fig. 1: PMC, probably at pachytene stage. Fixed March 16th 1956. — Fig. 2: Diplotene stage, possibly normal. Fix. March 23rd. — Fig. 3: Diplotene stage, nucleus contracted, probably defect. Fix. March 26th. — Fig. 4: Diplotene stage, Fusion of bivalents, fragments. Fix. March 26th. — Fig. 5: Prophase, stage undeterminable, "pulverized". Fix. March 26th. — Fig. 6: Diakinesis, fusion of bivalents f. inst. two nethermost groups. Fix. March 23rd. — Fig. 7: Diakinesis, fragmentation. Fix. March 26th. — Fig. 8: Metaphase$_1$, at top two (or more) bivalents fused. Fix. March 24th. — (Figs. 1—7: × c. 450; Fig. 8: × c. 1000.)



Figs. 9—14: Irregularities in pollen mother cells of *Larix decidua* caused by low temperature. — Fig. 9: Metaphase$_1$, pycnosis. Fix. March 26th. — Fig. 10: Metaphase$_1$, probably normal. Fix. March 24th. — Fig. 11: Anaphase$_1$, left: chromatin bridge; middle: bridge and other irregularities. Fix. March 24th. — Fig. 12: Anaphase$_1$, disjunction difficulties. Fix. March 24th. — Fig. 13: Anaphase$_1$, spindle probably paralyzed, stickiness. Fix. March 25th. — Fig. 14: Left: sticky metaphase$_2$; right: telophase$_2$. — (Figs. 9, 13, 14: × c. 450; Figs. 10—12: × c. 600.)

fusion may be induced in mitosis by chemicals and low temperature, and it is most likely that low temperatures may have the same effect on meiotic chromosomes. Most of the cytologically abnormal cells seem to be able to proceed at least to telophase$_1$; they are rarely found unaltered among later stages.

Anaphase$_1$. Some of the chromatin bridges or nondisjunction found at A$_1$ *(Fig. 11, 12)* are probably due to stickiness or fusions at earlier stages. In some of the cells the chromosomes are strongly contracted, and it happens that they are scattered over the cell as if the spindle has been paralyzed *(Fig. 13).* Mononucleate telophase$_1$ cells have not been observed, but it is most likely, that part of the diploid cells fund at later stages and some of the diploid pollen grains may originate from M$_1$ cells which after separation of the chromosomes have formed a restitution nucleus and later undergone mitosis at the M$_2$ stage.

Telophase$_1$ — Pollen Tetrads. In M$_2$—A$_2$ stickiness and fusions of chromosomes are frequently found *(Fig. 14),* but whether they are newly formed, or they have merely been passed on from earlier stages could not be determined. Probably both alternatives occur. — A considerable number of T$_2$ cells and pollen tetrads, with 3 nuclei were observed *(Fig. 15, 16),* often two nuclei are normal while one is about twice the normal size and later may form a giant pollen grain. Sometimes such giant nuclei seem to start from a sticky M$_2$ nucleus which has been unable to divide *(Fig. 14);* pollen grains arising from such cells are not viable. Many cells at the stages M$_2$—T$_2$ are deformed, and the spindles, which are normally arranged tetrahedrally, Devisé (4), seem to extend in the direction in which there is most space. Consequently the nuclei of A$_2$—T$_2$ and of the resulting pollen tetrads are arranged in different patterns ranging from the normal 4 nuclei at the corners of a quadrate *(Fig. 17)* to all 4 nuclei in a row *(Fig. 18—19).* The irregular positions of the T$_2$ nuclei evidently interfere with the cell wall formation, and often pollen tetrades with unequal quantities of cytoplasm in the four cells are seen *(Fig. 16).* — Examination of single PMCs under the microscope has shown that these deformities are

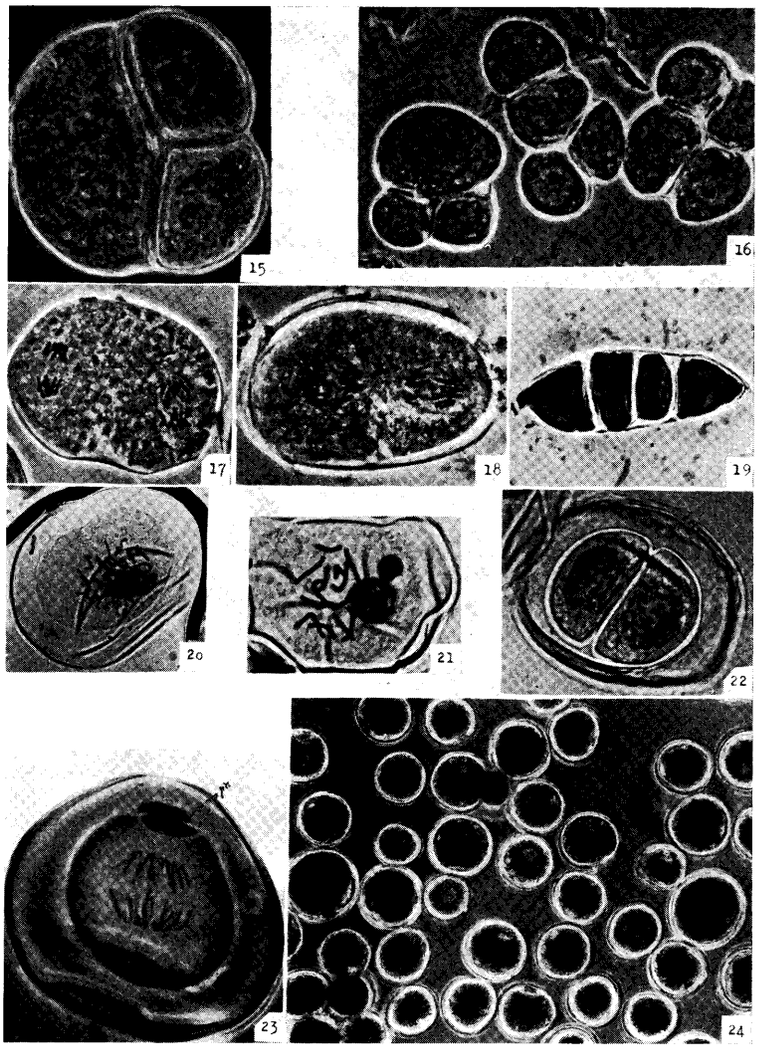


Fig. 15—24: Irregularities in pollen mother cells and pollen grains of *Larix decidua* caused by low temperature. — Fig. 15: Pollen "tetrad": left: two nuclei in one giant cell; emanates possibly from cell like Fig. 14. — Fig. 16: Left: pollen "tetrad" with one giant cell and two normal cells. The giant cell may emanate from a cell like Fig. 14 (if the chromatin mass remained in one cell), or from a restitution nucleus; middle: irregular tetrad (cf. text); right: tetrad resulting in pollen of unequal size. Fix. March 31st. — Fig. 17: Anaphase$_2$: possibly normal. — Fig. 18: Anaphase$_2$: the two spindles in a row; the telophase$_1$ from which it originated was deform, probably due to too viscid cytoplasm. — Fig. 19: "Pollen tetrad": emanates from a A_2 like Fig. 18. — Fig. 20: Giant pollen grain, diploid (24 chromosomes), second pollen grain division. Fix. April 16th. — Fig. 21: Pollen grain, haploid (12 chromosomes), second pollen grain division. Fix. April 16th. — Fig. 22: Twin pollen, formed by nondegeneration of a prothallium cell at first pollen grain division. Fix. April 17th. — Fig. 23: Pollen grain, anaphase of third pollen grain division. Note the two degenerating prothallium cells (pr.) Fix. April 16th. — Fig. 24: Mature pollen from N-tree; in some of the pollen grains two degenerating prothallium cells are visible, f. inst. below, at the arrows point. Fix. May 6th. — (Figs. 15, 17, 18: × c. 600; Figs. 16, 19—23: × c. 350; Fig. 24: × c. 120.)

present already at the prophase stage, and apparently the viscidity (DE ROBERTIS [8]) of the cytoplasm caused by the low temperature has prevented the cells from adopting the normal oval shape. — As far as is known, the wall formation of the four cells of a pollen tetrad of *Larix* normally takes place simultaneously. In the material under investigation the wall formation is highly irregular. Sometimes a wall is formed in T_1, M_2 or A_2 with resulting dyads, and sometimes a T_2 is found with 2 of the nuclei in separate cells and the two other nuclei included in one cell *(Fig. 15)*. In some cells the walls are straight and regular, in others they are more or less waved, probably due to the deformed shape of the PMCs.

The Mitotic Pollen Grain Divisions. The first divisions were observed on April 3th, and on April 26th the divisions were apparently finished. On May 5th — 6th the pollen was discharged. The divisions are not synchronized, each pollen grain starts when it is ready. — During the period April 4th — 26th pollen grains at all stages of 1st, 2nd and 3rd mitotic division were found, but it may be that this resulted from the relatively cold weather in April, and that the divisions will be finalized earlier if the temperature is higher. *Fig. 23* shows a pollen grain at anaphase of the 3rd mitotic division.

Many of the mitotic divisions of the pollen grains were irregular, but as the minimum temperature of April 4th — 8th, when the first pollen mitoses were found, was below zero, it was most often not possible to decide with certainty whether the irregularities were new or were derived from defective PMCs. In some instances, when the size of the pollen grain is abnormal, a sticky metaphase no doubt result from a sticky PMC, but when the size of the pollen grain is normal and the stickiness not very pronounced, it may be caused by the low temperature during the pollen division itself.

Pollen Grains of Varying Size may, as mentioned above, arise from irregular wall formation in pollen tetrads *(Fig. 16)*, in which case the nucleus is probably normal and the pollen may be viable; many pollen grains of varying size underwent the three pollen divisions. Some pollen grains seem, however, to start from PMCs in which the nuclei were deformed into lumps of fused chromosomes of varying size. Such lumps could be seen at metaphase with the matrix partially decayed but they were, of course, unable to divide, and the pollen grain probably died.

Micropollen was rarely observed and evidently degenerated quickly.

"Giant" Pollen Grains are of interest because they are often diploid and may be effective in the production of triploid plants. In the pollen under investigation, 4 — 8% were of the "giant" type (the pollen diameter was about 30% larger than the diameter of normal pollen). Many of them underwent the three mitotic divisions and in several giant pollen grains 24 chromosomes could be counted *(Fig. 20* shows a diploid, *Fig. 21* a haploid pollen grain). Giant pollen grains may emanate in several ways: — (1) from a defective PMC at M_2, in which the chromosomes (or the majority of them) are fused together into a lump and therefore cannot divide. The resulting pollen grain is not viable. — (2) if the spindle mechanism is inhibited, a PMC at M_2 may form a nucleus with the diploid chromosome number. The resulting pollen grain may be viable. — (3) if the spindle mechanism is inhibited, a restitution nucleus may be formed at $M_1 — A_1$ with the diploid chromosome number. This nucleus may later divide and form a dyad; the resulting pollen may be viable. Restitution nuclei have

Table 2. — Diameter in µ of 100 mature pollen grains from *L. decidua* (N-tree and S-tree, May 6th 1956).

Pollendiameter in µ	55	57	59	62	64	66	68	70	73	75	77	79	81	84	86	88	90	92	95	97	99	101	103	106	108	110	112	114	117	119	121	Total
N-tree	2	1	3	1		9	6	7	20	7	20	6	8	4	1	1			2	1				1								100
S-tree						2		1	1	2	16	13	20	4	8	15	2	2	4	2	6					1					1	100

N-tree: The diameter of the majority of the pollen grains is between 66 µ and 84 µ min.: 55 µ, max.: 106 µ.
S-tree: The diameter of the majority of the pollen grains is between 75 µ and 88 µ; min.: 66 µ, max.: 121 µ.

not been observed, but PMCs at the stage $M_1 - A_1$ with separated but not orientated chromosomes were found and point to this possibility *(Fig. 13)*.

Twin Pollen i. e. pollen grains with two nuclei separated by a wall were observed *(Fig. 22)*. According to MÜLLER-STOLL (7) one of the nuclei is probably a prothallium cell which has failed to degenerate after a mitotic pollen grain division. Such pollen is probably not viable.

The Size of Pollen from N-Tree and S-Tree is shown in *Table 2*. About 82% of the pollen grains were apparently normal, although of varying size (they stained in Iron-aceto-Carmine); c. 4 — 8% were "Giant" pollen, c. 7% dead pollen (unstaining) and twin pollen. The state of the rest could not be determined.

Pollen Fertility

Although most of the pollen seemed normal, it nevertheless proved to be ineffective when used for controlled pollinations. In the fall a number of cones from the trees were examined, but no viable seeds were found. The pollen must, therefore, have had internal deficiencies which could not be detected. *Fig. 24* shows mature pollen grains from the N-tree.

Discussion and Results

The present investigation has shown that the meiotic divisions in *L. decidua* stop when the air temperature reaches a certain minimum, and that they are resumed again when the temperature rises.

There is some evidence that this minimum, or threshold temperature, is not exactly the same in all trees, and that it is somewhat lower (1—2° C) at the stages from late prophase to diakinesis than at the stages from to $M_1 - A_2$ when the spindle mechanism is involved.

The relation between the temperature inside the flower buds and the air temperature is unknown, and it is therefore not possible to determine the actual threshold temperature. Moreover the air temperature usually varies throughout the 24 hours, thus exposing the PMCs to different temperature influences in periods of different duration. Refrigerator-experiments with PMCs of *L. decidua* at the stage diak.-M_1 seem to indicate however, that the threshold temperature at the latter stage is about +2—3° C when continuously applied.

The primary reason for the inhibition of the meiotic divisions is, of course, the low temperature, but how the reaction occurs is not known. It may be that the delicate physiological and chemical processes which govern the chromosome movements are more or less suppressed, but the possibility exists that the viscosity of the cytoplasm becomes so high that the chromosomes simply cannot move.

Whether an inhibition of meiotic divisions is detrimental or not probably depends on the duration of the inhibition and on the temperature. It might f. inst. be supposed that a short inhibition, f. inst. of a few hours, by a temperature slightly below the threshold, will pass without damage, while a longer inhibition, f. inst. of several days, by the same temperature, may have serious consequences. According to DARLINGTON and LA COUR (2) low temperature restricts the supply of nucleic acid available for mitotic metaphase and anaphase chromosomes in *Trillium*, whereby stickiness and chromatin-bridges may be produced. Perhaps the irregularities of the same kind found in the meiotic divisions of *Larix* are brought about in the same way. The abnormal contraction of the chromosomes of the larch material may, of course, be supposed to cause difficulties, but in view of the fact that mitotic chromosomes contracted by colchicine treatment are able to divide normally, it does not seem probable that the abnormal contraction is the principal cause of the irregularities.

The effects of low temperature on meiosis are, however, not always solely detrimental. After low temperature during meiosis in hyacinth, DE MOL (6) found irregularities in the PMCs and numerous pollen grains with more than the haploid number of chromosomes. He states that: „In der Entstehung mehrchromosomiger Pollenkörner infolge besonderer äußerer Verhältnisse ist die Anfangsursache der ungeheuren Größenzunahme der holländischen Hyazinthenvarietäten gelegen."

The ovules of the two trees were not examined. In view of the fact that the archegones are not formed until the beginning of June (SMOLSKA [11]), damage to the ovules would not seem probable, but the possibility, of course, exists.

It is not known whether sterility of *Larix* pollen due to meiotic disturbances caused by low temperatures is a common occurrence. Usually flowering trees of Larch produce an abundance of pollen, and even if the greater part of it were sterile, the fact would presumably pass unnoticed. Moreover all trees at the same locality do not undergo meiosis at exactly the same time (or may not have the same threshold temperature), and as perhaps only a couple of days with different temperatures are sufficient to cause disturbances, the result may be, that the pollen of some trees is damaged and that of others not.

If the completion of the mitotic pollen grain divisions so near upon the pollen discharge is a frequent occurrence, it may explain some of the difficulties often met with in the forcing of male inflorescences for pollen extraction. If the twigs are cut off, while the pollen grains are dividing, the divisions will no doubt stop, and the pollen derived (if any) will be more or less sterile. — This ill effect may, however, be avoided if the pollen is examined in aceto-carmine before twigs are taken in for forcing. If the divisions are completed, 2 degenerated prothallium cells will be seen at the edge of many of the pollen grains (cf. *Fig. 24*).

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Summary

Inhibition and resumption of meiosis in *L. decidua* due to low temperature is described. Irregularities of pollen mother cells and pollen grains were observed; the resulting mature pollen was sterile. The threshold temperature, at which inhibition of meiosis takes place was by refrigerator-experiments found to be about $+2 - 3^0$ C continuously applied, and it seems that the threshold is $1 - 2^0$ C lower at the stages late prophase-diakinesis than at the stages metaphase$_1$ — anaphase$_2$, and that there is some variation from tree to tree. — The mitotic pollen grain divisions were not synchronized, and they were not completed until about a week before the discharge of pollen. If this is a normal occurrence, it limits the period during which male buds can be forced for pollen extraction.

Zusammenfassung

Titel der Arbeit: *Über den Effekt von niedriger Temperatur auf die Meiosis und die Pollen-Fertilität bei Larix decidua Mill.*

Die Unterbrechung und Wiederaufnahme der Reduktionsteilung bei *L. decidua* unter Einwirkung von niedriger Temperatur wird beschrieben. Es wurden Unregelmäßigkeiten während der Meiosis und der Pollen-Mitose beobachtet; der entstandene reife Pollen erwies sich als steril. — Die kritische Temperatur, bei welcher die Reduktionsteilung unterbrochen wird, wurde bei Kühlschrankversuchen mit konstanter Temperatur mit ca. $+2$ bis 3^0 C ermittelt Die kritische Temperatur scheint für die Stadien späte Prophase-Diakinese um 1 bis 2^0 C niedriger zu sein als für die Stadien Metaphase$_1$ — Anaphase$_2$, eine gewisse Variation zwischen den individuellen Bäumen ist wahrscheinlich. — Die mitotischen Teilungen der Pollenkörner waren nicht synchronisiert, und sie waren erst circa eine Woche vor dem Ausstäuben beendet. Wenn dies eine normale Erscheinung ist, bedeutet sie eine starke Begrenzung der Periode, während der männliche Blütenknospen für die Pollengewinnung angetrieben werden können.

Résumé

Titre de l'article: *Influence de températures basses sur la méïose et la fertilité du pollen de Larix decidua Mill.*

L'article décrit l'inhibition et la reprise de la méïose chez *Larix decidua* sous l'influence de basses températures. Des irrégularités ont été observées chez les cellules mères des grains de pollen et chez les grains de pollen; le pollen mûr était stérile. Le seuil de température qui déclenche l'inhibition de la méïose a été déterminé par des expériences en réfrigérateur: il correspond à une température continue de $+2^0$ à $+3^0$ C et il semble que ce seuil est inférieur de 1^0—2^0 C pour les stades finaux de la prophase — diakinèse que pour les stades de la métaphase 1 à anaphase 2; de plus, il existe certaines variations d'arbre à arbre. Les divisions mitotiques de grains de pollen n'étaient pas synchronisées et ne furent achevées qu'environ une semaine avant la dispersion du pollen. S'il s'agit d'un phénomène normal cela limite la période pendant laquelle les bourgeons mâles peuvent être forcés en vue de l'extraction du pollen.

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Pollen Dispersion of Slash Pine (Pinus elliottii Engelm.) with Special Reference to Seed Orchard Management

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Introduction

The successful production of tree seed in a seed orchard depends on the effective control of pollen contamination. All extraneous pollen should be eliminated, and the free dispersion and ample supply of desired pollen should be maintained within the orchard to assure the quantity production of a crop with the desired genetic constitution.

In connection with the forest genetics research program of the University of Florida, 15 seed orchards of slash pine *(Pinus elliottii* ENGELM.) and loblolly pine *(Pinus taeda* L.) were established by cooperating wood-using industries in Alabama, Florida, Georgia, Mississippi and South Carolina. The management plans for these seed orchards provide for either wind pollination or hand pollination of the selected trees within the orchard. Provision must be made to pre-

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