

# The Formation of Pollen, the Pollination Mechanism, and the Determination of the Most Favourable Time for Controlled Pollinations in *Larix*

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During our investigations to determine the best time and method for the execution of controlled pollinations in *Larix* it soon became evident that the pollination mechanism in this genus must be different from that in other *Gymnosperms*. Inter alia ungerminated but apparently live pollen could be found embedded on the stigmatic flap of the ovule several weeks after pollination, but germinated pollen grains or pollen tubes were never found on the flap or in the micropyle.

A review of the literature on the subject showed, that according to GOEBEL (3) a period of 6–8 weeks elapses between pollination and fertilization in *Larix*. He commented this unusual procedure as follows:

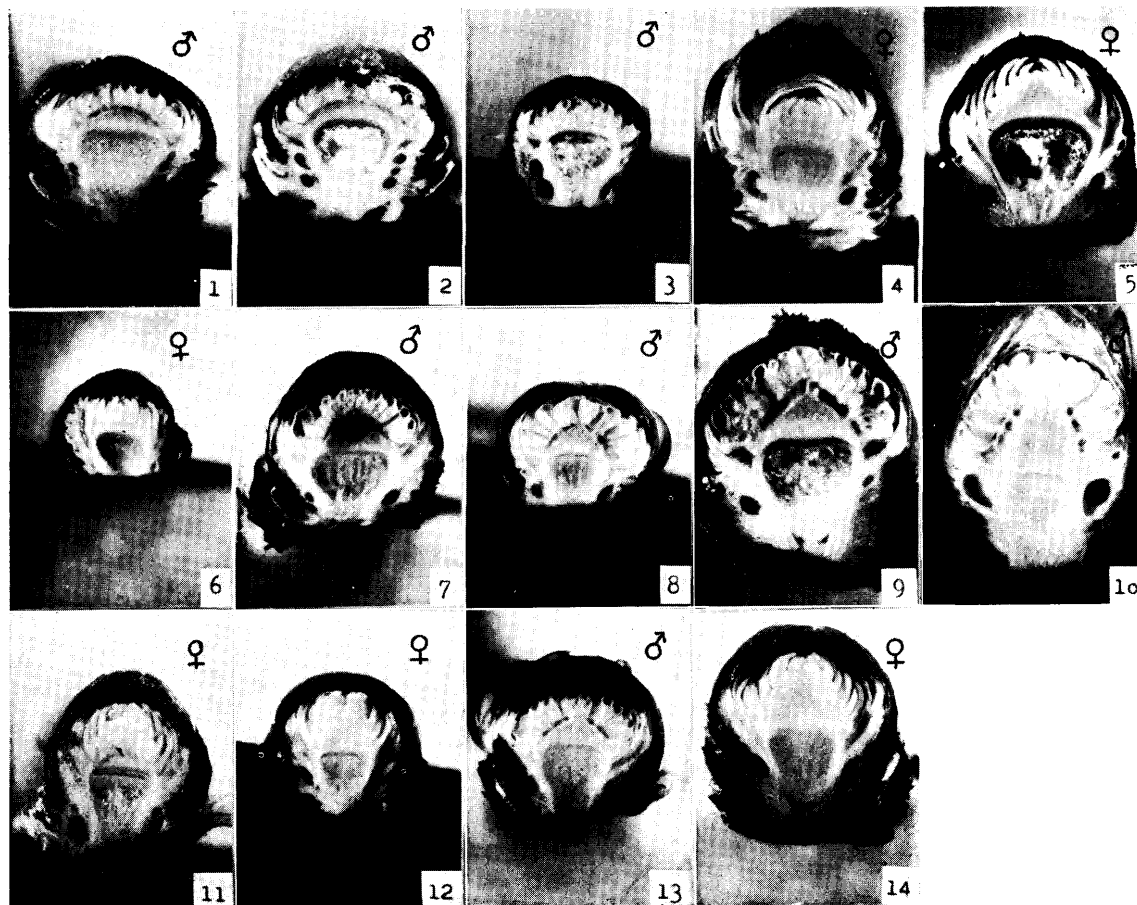
„Leider sind diese merkwürdigen Vorgänge experimentell so gut wie gar nicht geprüft, wir wissen also nicht, was die Ruheperiode der Pollenschläuche bedingt und wodurch sie wieder zur Weiterentwicklung veranlaßt werden.“

DOYLE and O'LEARY (2) have in several extremely interesting, but apparently little known, works described the

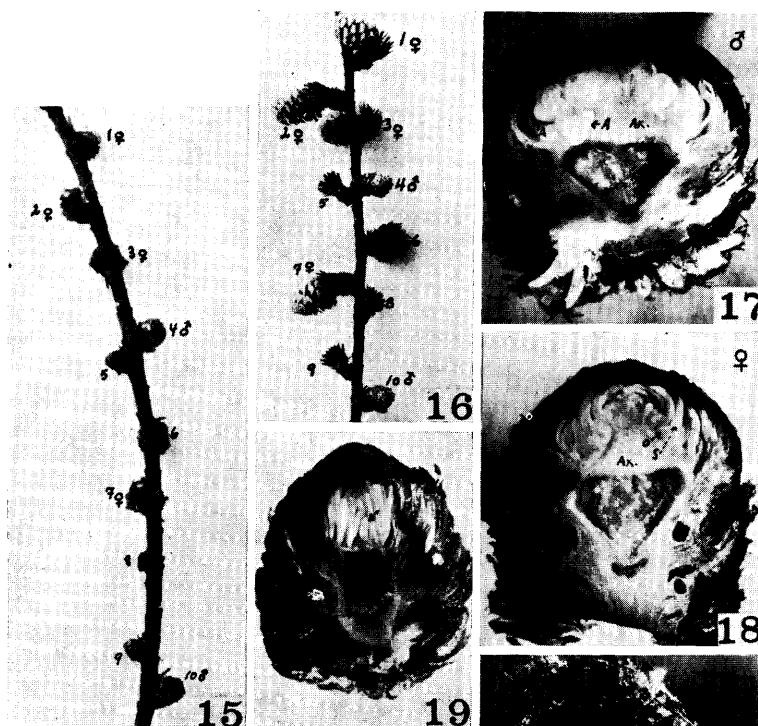
pollination mechanism in a considerable number of *Gymnosperms*. They describe this mechanism in *Larix* as follows:

„. . . . . the only germinated pollen found, or recorded from other accounts, appears on the nucellus. The grains clearly have to pass the micropylar canal in an upward direction, although they are large and wingless and sink readily in water. Unfortunately we have not been able to decide how that is brought about. The following mechanism is tentatively suggested pending further observations: Fluid frequently may be found apparently filling the micropylar canal. This fluid may loosen the grains and, then retracting, bring these with it, held by the strength of the surface film; but the mechanism does not seem a very satisfactory one. It is astonishingly common, even if grains are on the nucellus, to find other grains left behind at the top of the micropyle, and, in many cases, two months or more after pollination numerous swollen but ungerminated grains may be still adhering to the unturned rim but none on the nucellus. No slightest protrusion has ever been noticed coming from these, contact with the nucellus is clearly necessary for pollen-tube growth.“

The *Larix* pollen itself also presented certain difficulties. The pollen frequently showed irregularities which pointed to disturbances during the reduction division,



Figs. 1–14: — Longitudinal sections of inflorescences of *Larix* species (first week of October 1959).  $\times c. 5.5$ . — *L. decidua* ♂: Fig. 1; ♀: Fig. 4. — *L. sibirica* ♂: Fig. 2; ♀: Fig. 5. — *L. laricina* ♂: Fig. 3; ♀: Fig. 6. — *L. leptolepis* ♂: Fig. 7; ♀: Fig. 11. — *L. gmelini* ♂: Fig. 8; ♀: Fig. 12. — *L. occidentalis* ♂: Fig. 9. — *L. griffithii* ♂: Fig. 10. — *L. decidua*  $\times$  *leptolepis* ♂: Fig. 13; ♀: Fig. 14.



Figs. 15—20. — Fig. 15: Twig of *L. leptolepis* with male (4, 10), female (1—3, 7) and leaf buds (5, 6, 8, 9) photographed on the tree at time of meiosis (March 19, 1956).  $\times c. 0.6$ . — Fig. 16: Same twig as shown in Fig. 15 at pollination time (May 6). The buds have same numbers and signatures on both pictures.  $\times c. 0.4$ . — Figs. 17—19: Longitudinal sections of buds of *L. decidua* at time of meiosis. — Fig. 17: Male bud slightly more flat on top than female and leaf buds; anthers and loose pollen are visible. — Fig. 18: Female bud, distinguished from male and leaf buds by a more rounded top and the cone shaped axis. — Fig. 19: Leaf bud, slightly smaller than male and female buds, and the top is a trifle more pointed.  $\times c. 8$ . — Fig. 20: Longitudinal section of male bud of *L. leptolepis*, April 7, 1956 (about two weeks after meiosis). The axis of a male bud of *L. decidua* fixed the same day had the usual rounded appearance shown in Fig. 17. — Note the difference in shape of the axis.  $\times c. 7$ . — Abbrev.: A = anther; Ax = axis; N = needle; O = ovuliferous scale; S = bract.

which might perhaps be the cause of unsuccessful controlled pollinations. To control the viability of *Larix* pollen by germination in vitro proved impossible, the pollen did not germinate. KÜHLWEIN (5) arrived at the same result. The forcing of twigs and the extraction of pollen has been described elsewhere (1).

As an intimate knowledge of the biology of pollination and fertilization is of paramount importance to the plant breeder we have during the last four years attempted to solve, or at least elucidate, the most important of the above mentioned problems. At the same time we tried, mainly by means of photos, to show the practical worker what is actually taking place inside the buds, inflorescences and cones at various stages of development, so as to make it easier for him to recognize the stages which are of importance to his work.

The investigations mainly comprise *Larix decidua* MULL. and *Larix leptolepis* (SIEB. & ZUCC.) GORD., and when it has been possible we have examined both species at the same time.

The inflorescences in *Larix* are formed already in August—September of the year preceding the flowering year. This permits, at least about the end of September or beginning of October, to make a forecast of the flowering to be expected next year. After a few hours study of sectioned buds with the aid of a preparation microscope, unsectioned flower buds can be distinguished from leaf buds with the naked eye. Flower buds are considerably larger than leaf buds, and often — but not always — the former are glossy-yellow on the top, while the leaf buds as a rule are dark grey. The size of the buds of different species varies considerably (cf. Figs. 1—14), and so may the size of the buds of the same species, but there is always a noticeable difference in size between flower buds and leaf buds.

As the percentage of female inflorescences in relation to male ones may vary considerably, it is of interest to be able to distinguish between male and female buds, inter alia to avoid isolations on trees, which may later on turn out to have very few female inflorescences. Female buds cannot with certainty be distinguished from male ones by shape or size; the buds must be sectioned longitudinally with a razor blade (or better a Gillette surgical blade, shape E), and the blades must be changed frequently.

The axis of the male buds of *L. decidua*, *L. sibirica* and *L. laricina* is rounded, approximately as an arc of a circle with center in the middle of the bud (Figs. 1—3), while the axis of the female buds is cone-shaped (Figs. 4—6). Using these characteristics, male buds can on sections be distinguished from female buds with the aid of a lens or by the naked eye.

The male buds of *L. leptolepis*, *L. gmelini* and the Hybrid *L. decidua*  $\times$  *L. leptolepis* cannot be distinguished from female buds without a preparation microscope. In these species the axis of both male and female buds is cone-shaped (Figs. 7, 8, 13 and 11, 12, 14). This probably also applies to *L. occidentalis* (Fig. 9) and *L. griffithii* (Fig. 10), but in these species no female buds

could be found.

The appearance of a sectioned leaf bud is approximately the same as shown in Fig. 19, but the bud is of course smaller in October.

The sections of buds shown in Figs. 1—14 have all been photographed to the same scale during the first week of October 1959, and as far as possible buds of average size have been used.

It is interesting to note that the male flowers of *L. laricina* (North Eastern and Northern part of North America), *L. decidua* (Europe) and *L. sibirica* (Northern Russia and Western Siberia) have all rounded axis (Figs. 1—3), while *L. gmelini* (Eastern Siberia), *L. leptolepis* (Japan), *L. occidentalis* (N.W. part of USA) and *L. griffithii* (Himalaya) (Figs. 7—10) have all cone-shaped axes. The axis of the male flowers of the hybrid *L. decidua*  $\times$  *L. leptolepis* (Fig. 13) is approximately intermediate between the two parents.

Meiosis in the pollen mother-cells of *Larix* has been described by SAX and SAX (9) and KNABEN (4). — SMOLSKA (11)

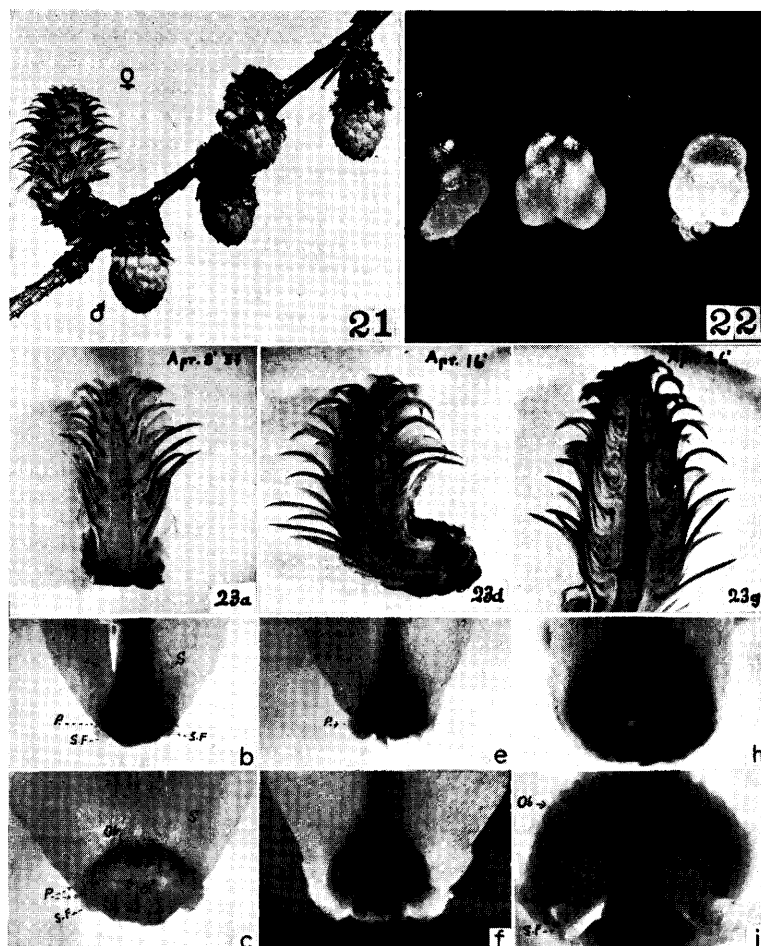
has in detail described the formation of the archegones and the fertilization. Neither of these processes will, therefore, be treated in the following text, except when it is possible to supplement the information given by the above mentioned authors.

According to TISCHLER (12), NEMEC (Czechoslovakia) considers it possible that meiosis in *Larix* may start in the autumn and be completed in the course of the winter. SAXTON (10) (England) states that pollen mother-cells in *Larix* may start division ultimo Sept. and after a month's rest complete the divisions. In Denmark we have not observed meiosis in *Larix* earlier than Febr. — March, presumably because the temperature here is lower during the winter. A number of male buds from different species of *Larix* examined during the first week of October 1959 were at the early prophase stage.

We have, however, found that divisions of the pollen mother-cells in *Larix decidua* may stop if the temperature drops below  $+4$  to  $+2^{\circ}\text{C}$  and remain immobile until the temperature rises, and meiosis will consequently often proceed on sunny days and stop during cold nights. For instance medio March 1956 meiosis was practically stopped at the stages Diakinesis-Metaphase I for 5–6 days in a *Larix decidua* in the garden of The Royal Veterinary and Agricultural College at Copenhagen, and when it was resumed, a considerable number of irregularities were observed in the P.M. cells and in the resulting pollen. Evidently the extremely complicated processes of the heterotypic and homotypic divisions had been disturbed by the interruption. Inflorescences pollinated with this pollen yielded no seeds. This may perhaps have significance for the fructification of *Larix* in regions where alternating periods of relatively warm and cold weather are frequent in the early spring.

Fig. 15 shows a twig of *Larix leptolepis* with male and female inflorescences and with leaf buds photographed on the tree at the time of meiosis (March 19th 1956). Fig. 16 shows the same twig at pollination time (May 6th 1956). The buds have the same numbers and signatures on both photos. — Figs. 17, 18 and 19 shows longitudinal sections through male, female and leaf buds of *L. decidua* respectively. The male bud is a little more flat on the top and a trifle larger than the female buds and the leaf buds. Fig. 20 shows a longitudinal section of a male bud of *L. leptolepis* fixed April 7th 1956 (about two weeks after meiosis). The axis has extended considerably, while male buds from *L. decidua*, fixed the same day, still had the characteristic rounded appearance shown in Fig. 17. However, about a week later the axes of the male buds of *L. decidua* also began to extend, and at the time of pollen shedding, the axes of the two species seem to be much alike. Fig. 22 shows 3 anthers in sideview, abaxial and adaxial view respectively.

*The Shedding of Pollen and the Pollination.* The development of the inflorescences after meiosis and the shedding of pollen are to a certain degree

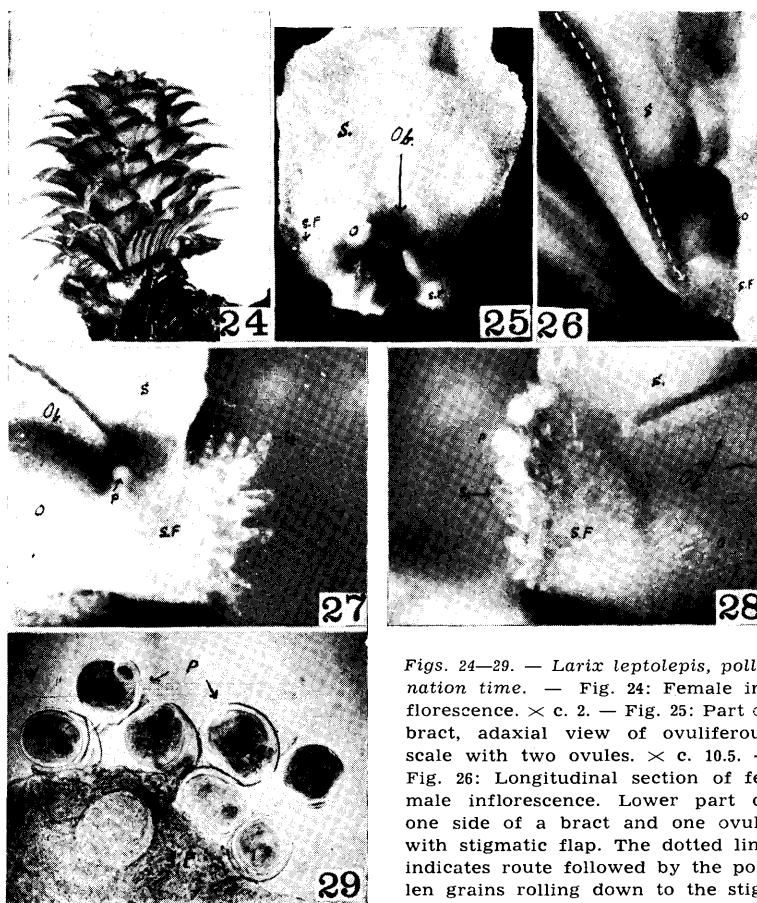


Figs. 21–23. — Fig. 21: Male and female inflorescences of *L. leptolepis*, pollination time. Males directed downwards, female upright. — Fig. 22: three anthers at time of reduction division. From left: side-view, abaxial and adaxial view.  $\times c. 22.5$ . — Fig. 23: a, d, and g: Longitudinal sections of female inflorescences fixed April 8, 16 and 26, 1957, respectively. b, e, h: abaxial; c, f, i: adaxial view of lower part of bracts with ovuliferous scales from the inflorescence above each. The inflorescences were isolated in pergamyne bags and before fixation dusted with pollen to ascertain whether the flaps were stigmatic. Although the inflorescence fixed April 8 was only slightly open, some flaps held pollen (a, b, c). On April 16 (d) the inflorescences were probably at the most receptive stage (e, f). On April 26 (g) the inflorescence was closed and the stigmatic flaps collapsed (h, i). Note the gradual broadening of the base of the bracts. In b and e the stigmatic flaps are protruding from behind the bracts, in h they are covered by the bract. In b–c and e–f pollen grains are adhering to the flaps, in “i” the flaps have collapsed. — a, d, g =  $\times c. 2$ ; b, e, h =  $\times c. 7.5$ ; c, f, i =  $\times c. 11.5$ . — Abbrev.: O = ovule; Ob. = ovuliferous scale; P = pollen grain; S = bract; S. F. = stigmatic flap.

dependent on the weather conditions and may be delayed a week or more by cold and moist weather. Normally, however, the shedding of pollen takes place in Denmark during the last days of April or first days of May. When the anthers burst, the pollen is caught by the wind and transported to the female inflorescences. Fig. 21 shows a twig of *Larix leptolepis* at pollination time, male flowers are turned downwards, female inflorescences are upright.

Figs. 23 a, 23 d and 23 g show longitudinal sections through female inflorescences fixed on the 8th, 16th and 26th of April 1957 respectively. “a” is fixed during the first part of the receptive period, “d” when probably most of the stigmatic flaps were receptive and “g” when the flaps had collapsed and the inflorescence was closed. Under each inflorescence is shown the lower part of one of its bracts with ovuliferous scales in abaxial and adaxial view.

The bracts close irrespective of whether the inflorescence



Figs. 24—29. — *Larix leptolepis*, pollination time. — Fig. 24: Female inflorescence.  $\times$  c. 2. — Fig. 25: Part of bract, adaxial view of ovuliferous scale with two ovules.  $\times$  c. 10.5. — Fig. 26: Longitudinal section of female inflorescence. Lower part of one side of a bract and one ovule with stigmatic flap. The dotted line indicates route followed by the pollen grains rolling down to the stigmatic flap.  $\times$  c. 12. — Fig. 27: Part of ovuliferous scale and ovule with stigmatic flap. Note the stigmatic protuberances. Pollen grains are caught on inner part of stigmatic flap probably after rolling down the bract. Adaxial view.  $\times$  c. 32. — Fig. 28: Ovule and stigmatic flap as in Fig. 27, but pollen grains are here caught on the stigmatic protuberances.  $\times$  c. 32. — Fig. 29: Part of collapsed stigmatic flap with embedded pollen grains. The flap was cut off and "squashed" a week after pollination. By squashing the pollen grains were pressed out of the tissues; they have swelled and started germination but rest at this stage 5—7 weeks.  $\times$  c. 125. — Abbrev.: O = ovule; Ob = ovuliferous scale; S = bract; S. F. = stigmatic flap; Sp = stigmatic protuberance; P = pollen grains.

ces have been pollinated or not. The closing of the inflorescences seems mainly to be caused by the enlargement of the ovuliferous scales (cf. p. 9).

Fig. 24 shows a female inflorescence at pollination time. The ovuliferous scales carry two ovules (Fig. 25), each one surrounded by an integument with an orifice at the apex, the micropyle (Fig. 30). The adaxial side of the orifice has an extension with stigmatic projections which serves as a pollen-catching device, the stigmatic flap, abbreviated S. F. (Fig. 27—28). When the ovule is receptive, the S. F. is swollen and stigmatic and protrudes beyond the rim of the ovuliferous scale, and on its abaxial side a narrow slit opens to the micropylar canal.

If the inflorescences are receptive i.e. the bracts are open and the S. F. swollen and stigmatic, the pollen grains brought by the wind roll down the two cavities formed by the upper sides of the bracts and land on the stigmatic flaps (cf. Fig. 26 showing one side of a bract and one ovule). It may happen that the pollen roll directly into the micropyle, but pollen has never been observed on the top of nucellus at the time of pollination.

According to DOYLE and O'LEARY (2) the S. F. collapses after pollination, whereby the pollen is embedded on the

micropylar side of the collapsed tissues. The S. F. collapses however also without pollination, and when collapsed there is externally no visible difference between pollinated and unpollinated S. Fs.

A few days after the receptive period the apex of the ovule has the appearance shown in Fig. 32 (without the drop). A longitudinal section through the apex of the ovule in Fig. 30 is shown in Fig. 31, pollen grains are adhering to the collapsed tissues of the S. F. Fig. 33 shows a transverse section through the apex of another ovule.

During the first week after pollination the embedded pollen swell and often begin to germinate, the pollen tube forming a bulge of the intine of up to  $\frac{1}{2}$  of the pollen diameter (Fig. 29), but at this stage the germination stops for a resting period of 5—7 weeks.

According to SMOLSKA (11) the archegones are not formed until about 44 days after pollination, which may be one of the reasons for the resting period of the pollen.

After the resting period the pollen is transferred to the nucellus top (abbreviated N. T.) where it germinates. Fig. 36 shows a longitudinal section through a nucellus. Two pollen grains have germinated on its top, and traces left by the growth of their pollen tubes through the tissues are visible. In Fig. 37 the end of a pollen tube containing the male nucleus has started to penetrate the archegonial tissues in order to reach the egg cell. The penetration is presumed to be effected by enzymatic action, and a cavity has already been formed in the tissues. When the male nucleus (which has 12 chromosomes) reaches the egg cell, it fuses with the female nucleus (which has also 12 chromosomes), and from the resulting nucleus containing 24 chromosomes the embryo, i.e. the new individual develops. Fig. 38 shows a metaphase of the first division of the egg cell after fertilization, and

Fig. 39 depicts the 24 chromosomes contained in all somatic cells of a *Larix decidua*.

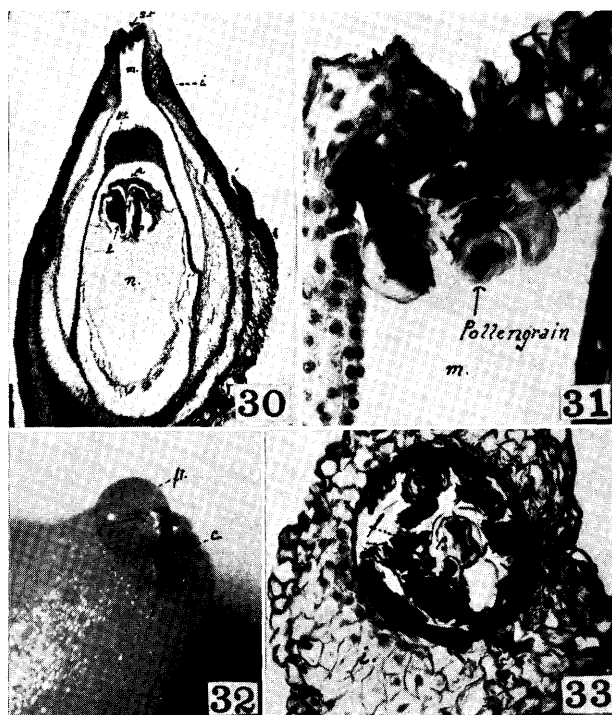
As mentioned above we have tried to elucidate the ways and means of the pollen transport from the collapsed stigmatic flap to the top of the nucellus in *Larix*. We have examined a considerable number of ovules on "Squash" or microtome and held as many trees as possible under observation. There are, however, substantial difficulties. Fixed material will of course show nothing about presence or movements of fluid in the micropylar canal, and the bracts permit no direct observations of the ovules without destroying the inflorescences. For these reasons our observations do not claim a high degree of exactitude, nor do they solve all the problems mentioned.

To examine pollen on the S. F., the latter were removed with a very sharp and pointed knife under a Zeiss stereomicroscope, macerated in 9 parts acetic Lacmoid + 1 part 1N HCl [Tjo (13)] and squashed in acetic Lacmoid. To examine the nucellustop for pollen, the integument was cut through at level with the N. T., and the cone-formed top with the S. F. tipped over (Fig. 34). Pollen on the nucellus top, and fluid and loose pollen in the micropylar canal are easily seen in the microscope. Twigs with inflores-

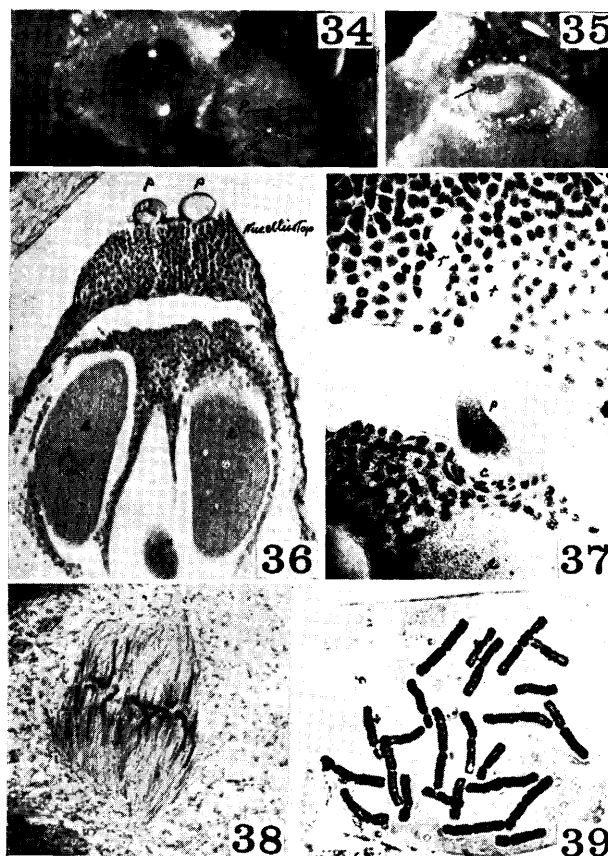
cences for examination were wrapped in moist filter paper, enclosed in plastic bags and immediately brought to the laboratory. As an extra check, the microscope was one night placed near the trees under investigation and the ovules were examined with 1—2 hours intervals from 8 o'clock in the evening till 9 o'clock in the morning. The time interval between removal of the inflorescences from the tree till they were placed under the microscope was thus reduced to a few minutes. A number of ovules were fixed in NAWASCHIN's fixative and cut on microtome. These investigations refer mainly to Japanese Larch, but the processes seem to proceed in the same manner in European Larch.

During the resting period, i. e. in Denmark usually from the beginning of May till about the second week of June, the pollen remained unchanged on the S. F. and was never found in the part of the micropylar canal near the nucellus top or on the nucellus top, nor was fluid found in the canal. This seeming inactivity does, however, not apply to the nucellus, where the archegones are formed. According to SMOLSKA the time elapsing between the differentiation of the archegones and the fertilization is about 17 days. This is well in accordance with our findings of the first archegones about the first of June, and fertilization middle or ultimo June.

During the middle or last part of June, probably when the egg cells are ready for fertilization, the nucellus swells and its top is often pressed so hard into the micropylar canal, that it is difficult to remove the upper part of the



Figs. 30—33. — *Larix leptolepis*, after pollination. — Fig. 30: General view of ovule, longitudinal microtome section. — a = archegones; e = egg cell; i = integument; m = micropylar canal; n = nucellus; N. T. = nucellus top; S. F. = stigmatic flap, collapsed.  $\times$  c. 15. — Fig. 31: Longitudinal section through top of integument and collapsed stigmatic flap. Pollen grains adhering to the filaments of the latter. — m = micropylar canal.  $\times$  c. 125. — Fig. 32: Upper part of ovule with cavity left by collapsed stigmatic flap (c) and drop of fluid (fl) pressed out from the filled micropylar canal. Medio June.  $\times$  c. 45. — Fig. 33: Transverse section (microtome) through top of integument and collapsed stigmatic flap. i = integument; f = filaments of stigmatic flap; p = pollen grain.  $\times$  c. 105.



Figs. 34—39. — *Larix*, fertilization (Fig. 34—38: *L. leptolepis*; Fig. 39: *L. decidua*). — Fig. 34: Drop of fluid on nucellus top (fl). O = ovule; ot = cut off and overtipped top of integument with collapsed stigmatic flap; P = pollen on underside of collapsed stigmatic flap.  $\times$  c. 25. — Fig. 35: Nucellus top after removal of top of integument with collapsed stigmatic flap. The drop of fluid shown in Fig. 34 exudes from the dark area in the middle, at the arrow's point.  $\times$  c. 20. — Fig. 36: Two germinated pollen grains (p) on the nucellus top. Traces of two pollen tubes (t) through the tissues of the top. Below: archegones with two egg cells (e) and a fragment of a third.  $\times$  c. 40. Microtome section. — Fig. 37: Traces of two pollen tubes (t) in the tissues of the nucellus top. End of pollen tube (p) has started to penetrate the tissues of the archegonium to reach the egg cell (e). Note the cavity (c) already formed presumably by enzymatic action.  $\times$  c. 125. Microtome section. — Fig. 38: Division (Metaphase) of egg cell after fertilization. The pollen tube nucleus (male) with 12 chromosomes has fused with the egg cell nucleus (female), also with 12 chromosomes. From the resulting cell with 24 chromosomes; the embryo — i. e. the new individual — develops.  $\times$  c. 500. Microtome section. — Fig. 39: The 24 somatic chromosomes of *Larix decidua* after colchicine treatment and squash. As far as known at the present moment, the chromosomes of *Larix leptolepis* and *Larix decidua* are morphologically identical.  $\times$  c. 1000.

integument with the S. F. without injury to the top. This swelling is not seen on fixed material, as the nucellus immediately shrinks by fixation. The micropylar canal which has up to now been dry is filled with fluid, evidently under pressure, as fluid is often pressed out through the S. F. forming a drop on the top of the latter (Fig. 32). The fluid filling the micropylar canal seems to be pressed through the tissues of the nucellus top, at any rate we have sometimes found a drop of fluid on the nucellus top although the micropylar canal was dry (Fig. 34). When this drop was experimentally removed, a new one was formed in a couple of minutes; at night this could usually be repeated 3—4 times, during daytime only once or twice. Fig. 35 shows the area of the top of the nucellus, from which the drop of fluid shown in Fig. 34 was exuded.



When the fluid has filled the micropylar canal it dissolves the substance in which the pollen is embedded on the S. F. or, sometimes, it seems to cause the pollen to swell and break out of the exine, whereafter it floats in the fluid. The filling of the micropyles of a tree, or even of an inflorescence, with fluid is not synchronized. Out of 7 ovules from the same inflorescence 1 had fluid in the micropylar canal, 5—6 were dry. Often 1 ovule on a scale was dry, the other "wet".

After the pollen has been disengaged from the collapsed S. F. it is transported upwards to the nucellus top. According to DOYLE and O'LEARY *Larix* pollen does not float on water, hence it is not probable that it floats up by itself. The fluid in the canal may, of course, have another specific gravity than water, but in any case it might be expected that if the pollen floats up by itself, we would often have seen loose pollen floating on the surface of the fluid in the cut-off and overtipped tops. No floating pollen on the surface of the fluid in overtipped tops has, however, been found, but we have often seen loose pollen grains lower down in the fluid. We are, therefore, inclined to agree with DOYLE and O'LEARY that the pollen does not float up by itself, and that it is more probable that the fluid, in retracting, carries the pollen with it. In view of the fact that the nucellus top is pressed tightly into the micropylar canal, the explanation presents itself, that the fluid is retracted the same way it was exuded, and that the pollen is thereby deposited on the nucellus top, the latter acting as a filter.

If this is correct there would be no pollen on the nucellus top before the fluid has been retracted. On the other hand we have at the same time found pollen on the nucellus top and fluid in the canal, which seems to indicate that the fluid has been retracted and again exuded, perhaps more than once.

We have not been able to determine how long time the exudation and retraction of fluid in one ovule may continue, but it would seem probable that it continues until all egg cells are fertilized or inactive.

Fixed and cut material show that there may be up to 6 egg cells in one ovule, and that in ovules with fluid in the micropylar canal some of these may be unfertilized, while others are at the beginning of proembryo formation. Although as a rule only one embryo survives, they may all be fertilized and start development. This may seem a waste, but actually it increases the chances that a seed is eventually produced.

The pollen transport from the stigmatic flap to the nucellus top in the ovules of a single tree has been observed to take about 5—6 days. At least, this period elapsed from the time we found the first fluid in a micropylar canal of the tree, till only dry canals could be observed, but probably the length of the period varies with the environmental conditions.

While the hypothesis that the pollen is filtered from the retracting fluid by the porous nucellus top is supported by certain facts, viz: that the fluid can be seen exuding through the nucellus top and that there seems to be no other way for retraction, we are much in the dark as to the mechanism governing these processes.

In this connection a short recapitulation of the pollination mechanisms in a few other gymnosperms may perhaps be of interest. — According to DOYLE and O'LEARY *Pinus* has two mechanisms for retraction of fluid, one that starts the retraction after the introduction of pollen into the fluid and deposits the pollen on the nucellus top in less than ten minutes, and another one, which regularly

retracts the fluid from all unpollinated canals in the morning (the canals are dry during the day, but filled during the night). With regard to the former retraction mechanism they remark: "The cellular physiology underlying the phenomena presents a pretty problem, as yet unfaced." The regular exudation in the evening and the retraction in the morning are, they think, possibly due to variations in the water contents of the whole plant tissue. — The pollination mechanism in *Picea* resembles, according to the above cited authors, that of *Pinus* in so far as the fluid is retracted, although more slowly, after introduction of pollen; but there are no day and night changes, and fluid may be found in unpollinated ovules at all hours. The pollination mechanism in *Pseudotsuga menziesii* (Douglas fir) is of interest in this connection because, according to DOYLE and O'LEARY, the ovule and stigmatic flap morphologically resemble those of *Larix* to a high degree, and in both genera the pollen grains are embedded in the collapsed tissues of the stigmatic flap for a resting period. There, however, the resemblance ceases. In *Larix* the pollen grains are transferred to the nucellus top, where they germinate, but in *Pseudotsuga* they germinate in situ sending their pollen tubes through the lumen of the micropyle to the nucellus.

In *Larix* the existence of a retraction mechanism, started by the introduction of pollen into the fluid, does not seem probable. The pollen is much more firmly embedded in the collapsed tissues of the stigmatic flap of *Larix* than on the stigmatic arms of *Pinus* and *Picea*, and furthermore exudation and retraction in *Larix* seem to take place more than once. A day and night mechanism as in *Pinus* seems also out of the question, as we found fluid in the canals of *Larix* at all hours.

Notwithstanding the possibility of more complicated intracellular activities governing the exudation and retraction mechanisms in *Larix*, we are inclined to prefer the simpler explanation suggested, but not followed up, by DOYLE and O'LEARY, viz: that the said processes may be the sequel of respectively high and low sap pressure in the tree's tissues. According to LADEFOGED (6) the water consumption of for instance a Birch tree reaches a distinct maximum about noon and a minimum not far from zero during the night. On sunny days with low air humidity there is a considerable difference between maximum and minimum, on cloudy days with high air humidity the difference is smaller, and on rainy days the water consumption is about zero. — If the water contents of the soil is sufficient, the sap pressure will presumably vary inversely with the water consumption and reach a maximum when the consumption is lowest. Although nothing is known about the water consumption in *Larix*, it may probably be assumed that it follows approximately the same pattern. In practice this would presumably mean, that if the N. T. is porous and the water contents of the soil adequate, fluid will be exuded into the micropylar canals when the transpiration of the tree is low, for instance at night and on rainy days, and retracted when transpiration is high, for instance on hot and windy days. If the canals are filled and emptied in this way, these functions will, of course, be less spectacular and more irregular than those described in *Pinus*, but they might leave more time for loosening of the embedded pollen from the collapsed S. F. tissues, especially if the period of pollen transport is rainy. On the other hand it is possible that drought in this period may result in more or less dry canals with the consequence that few

pollen or none are transferred to the nucellus tops, and fertilization and seed setting may be impaired.

In any case it would seem that the pollen transport stage in *Larix* is no gain from a fertilization point of view. Besides the fact that many pollen grains left on the S. F. get no chance to germinate and participate in the fertilization the transport itself may expose the grains to perils, to which pollen grains in other *Gymnosperms*, germinating on the place on which they were deposited during pollination are not subjected. —

DOYLE and O'LEARY's observation that: "two months or more after pollination numerous swollen but ungerminated grains may be found still adhering to the unturned rim, but none on the nucellus" probably refers to ovules, whose development for some reason has been stopped or delayed. An examination of later stages often shows that only one of the two ovules of an ovuliferous scale has developed normally. Apparently good pollen on the collapsed stigmatic flap and germinated pollen on the nucellus top is often found as mentioned by the above cited authors. The reason for this probably is that the pollen has not been loosened or disentangled from the S. F. before the canal had dried up.

*Test of the Effectiveness of Controlled Pollinations During the Resting Period.* The long resting period of the pollen on the collapsed stigmatic flaps in *Larix* may be turned to advantage for a precheck on the effectiveness of controlled pollinations. During this period the pollen on the S. F. may be examined and counted in the following way: The top of the integument with the embedded pollen is cut off with a sharp razor blade (or better a Gillette surgical blade, shape E) under a good preparation microscope, macerated in a drop of 1 part 1N HCl + 9 parts of acetic Lacmoid or acetic Orcein on a slide, and slightly heated and squashed. By this method we found for instance by checking 60 S. F. after controlled pollination, that 10 S. F. (17%) were pollinated with a total of 25 pollen grains (an average of 2.5 pollen grains per S. F.), while 50 S. F. (83%) were without pollen. A similar check on 60 S. F. after free pollination showed, that 37 S. F. (62%) were pollinated with a total of 112 pollen grains (average 3 pollen grains per S. F.), while 23 S. F. (38%) held no pollen. The maximum number of pollen grains on one S. F. was 6 after controlled pollination and 11 after free pollination. The results indicate that the controlled pollination had not been as effective as free pollination.

*The most favourable time for Controlled Pollinations* would seem to be the time when the highest possible number of ovules are receptive, i. e. the micropylar flaps are swollen and stigmatic and the bracts open.

The determination of the stage of development of the inflorescences is, therefore, of paramount importance, not only for the execution in due time of isolation, pollination and removal of bags, but also for the utilization of the whole period of receptiveness for pollination.

To find out when the inflorescences are receptive would not seem to present serious difficulties, but anyone who has tried to decide whether a small 8—10 mm long inflorescence is receptive or not, will know that the task is by no means easy. — As we felt that the usual indicator for receptiveness viz., the position of the bracts, was not as

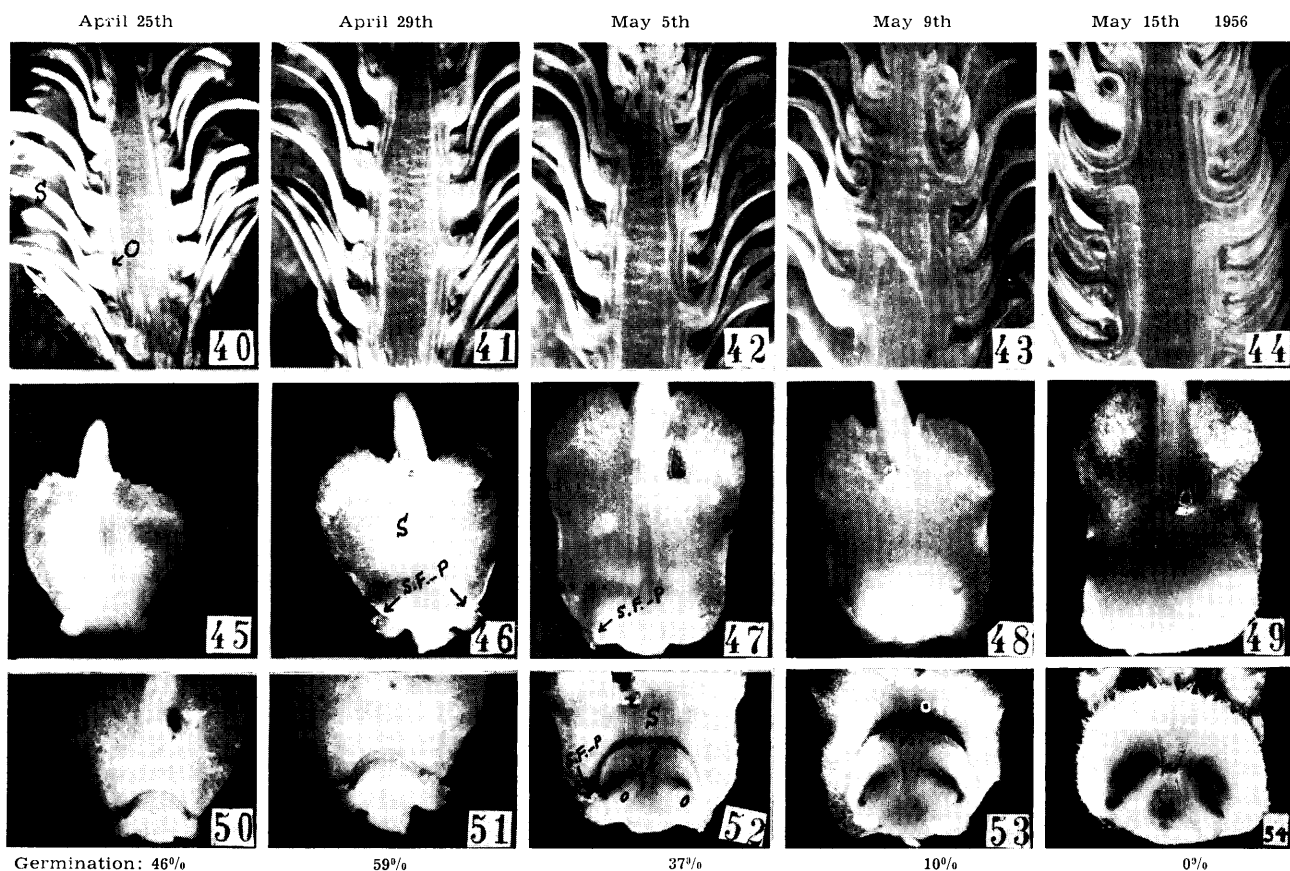
accurate as might be desired, we have tried by experiments to find more reliable criteria. These experiments were carried out in the years 1956—57 and 1958. In 1957 scant flowering and frost injury reduced the material, which was therefore discarded. In 1956 and 1958 the working conditions were satisfactory, and two clones of *L. decidua* marked V. 418 and V. 618 as well as two clones of *L. leptolepis* marked V. 831 and V. 1376 were used. The experiments were carried out as follows: a series of controlled pollinations were performed on the four clones during a period calculated to cover the receptive period plus a number of days before and after. A number of bags were left unpollinated to control the effectiveness of the isolation. Contemporaneously with each pollination 2—3 female inflorescences from the bags, as far as possible of average type and at the same stage of development as the pollinated inflorescences, were fixed in 70% alcohol. — The fixed inflorescences were sectioned longitudinally, one half part was photographed, the other half dissected and examined. Thus the shape, position and size of the bracts and ovuliferous scales and the state of the stigmatic flaps were registered for each pollination date. — Although the pollinated inflorescences and the fixed ones were not identical, and although there is some variation in the development of the inflorescences, a comparison of the registered results of the above examinations with the percentage of germinated seeds from the corresponding pollinations gives a rather good idea of the significance which the development and state of the various organs had for fertilization.

During the autumn the cones emanating from the pollinated inflorescences were collected separately from each clone and for each pollination date. The seeds were extracted from the cones and the percentage of viable seed determined (in *Larix* unfertilized ovules develop into empty seeds, which can only be distinguished from the viable seeds by cutting or by germination test). The results of the germination tests are tabulated below (Table 1).

For various reasons we were not able to start pollination early enough to cover the beginning of the receptive period.

Table 1. — Germination test of seeds emanating from different pollination dates

Dates of Pollination	Percentages of Germination							
	European Larch				Japanese Larch			
	Clone No. V. 418		Clone No. V. 618		Clone No. V. 831		Clone No. V. 1376	
	1956	1958	1956	1958	1956	1958	1956	1958
	%	%	%	%	%	%	%	%
25/4	48		46		17		25	
26/4	60		54		56		33	
27/4	66		62		75		59	
28/4	67		57		63		69	
29/4	62		59		60		64	
30/4		36		53		56		45
1/5	65	49	52	41	53	51	24	49
2/5	60	19	47	44	52	61	23	38
3/5		23		40				27
4/5	64	38	57	30	17	21	53	10
5/5	63		37		1		44	
6/5								
7/5	39	3	31	36	0	2	12	16
8/5								
9/5	4		10	10	0	0	0	0
10/5								
11/5	0		0		0		0	
12/5		0		2	0	0		0
15/5	0	0	0		0		1	
Control	0	0	0	0	0	0	0	0



Figs. 40—54. — Criteria for receptiveness (Expl. see text). — Figs. 40—44: Longitudinal sections of inflorescences of *L. decidua* V. 618 1956 fixed on the dates heading the photos. 40—42 are open and receptive (41 and 42 are slightly more open than 40). 43 and 44 are closed. On account of the spiral arrangement of the bracts the photos show gaps where the section was made between two bracts.  $\times$  c. 4. — Figs. 45—49: Abaxial view of bracts from the inflorescences shown above. Note the change in the shape of the bracts from inverted heart-shaped in 45 to rectangular in 49. In 45, 46 and 47 the stigmatic flaps are more or less receptive (note the adhering pollen) and protrude from behind the bracts; in 48 and 49 the stigmatic flaps have collapsed and are hidden behind the now rectangular bracts.  $\times$  c. 6. — Figs. 50—54: Adaxial view of lower part of the bracts shown in 45—49. The area of the ovuliferous scale in 54 has increased to about 7 times the area in 50. — Under each figure is shown the germination percentage of seeds originating in corresponding experimental pollination on the date in question (cfr. explanation in text).  $\times$  c. 6. — Abbrev.: b = ovuliferous scale; o = ovule; S.F.-P = stigmatic flaps with adhering pollen grains; s = bract.

but although a few days are thus missing, the experimental pollination period nevertheless comprises not only the main part of the period when the inflorescences were receptive but also the end thereof, which it is very important to know.

To illustrate the connection between the position and shape of the bracts and of the stigmatic flaps on one side, and the germination percentages on the other, Figs. 40—54 show 5 typical stages of development of female inflorescences from clone V. 618 (*L. decidua*) during the experimental period April 25th — May 15th 1956, and also germination percentages of seeds emanating from the corresponding pollinations made on the dates when the 5 inflorescences were fixed. The 5 inflorescences were selected from the 13 fixations made during the experimental period (Table 1); the intervals between them are 4—7 days. Each inflorescence was sectioned longitudinally, one half was photographed (shown in Figs. 40—44); from the other half a typical bract with ovuliferous scale was removed and photographed from both sides. Figs. 45—49 show an abaxial, Figs. 50—54 an adaxial view of these bracts. According to the germination test one of the days when the inflorescences were most receptive was April 29th (59%); on this date the bract shown in Figs. 46 and 51 was inverted heart-shaped, and the stigmatic flaps were protruding from

behind the bract with pollen adhering. On May 5th the germination percentage had dropped to 37; on this date the bract shown in Figs. 47 and 52 was more rectangular, the stigmatic flap, although still receptive, was almost hidden behind the bract. May 9th the germination percentage was only 10; the bract shown in Figs. 48 and 53 was more or less rectangular, the stigmatic flaps were collapsing and probably not receptive. On May 15th the bract shown in Figs. 49 and 54 was almost rectangular, the stigmatic flaps were hidden and collapsed. — While there is only little alteration in the position of the outer parts of the bracts (cf. Figs. 40—44 and Fig. 23, a, d, g) during the experimental period, there is a very conspicuous change in the shape of the base of the bracts and in the size of the ovuliferous scales.

During the period of receptiveness the length of an inflorescence is approximately doubled. During the first part of the period the bracts are more or less inverted heart-shaped (Fig. 45), but towards the end of receptiveness the width of the base of the bract has increased, making the latter almost rectangular (Fig. 49). At the end of the period the area of the ovuliferous scale has increased approximately 6—7 times as compared with the beginning (Figs. 50 and 54), and it is our impression that the closing of the inflorescences is in a much higher degree due to the growth



of the ovuliferous scales than to a decrease of the space between the bracts.

The stigmatic flaps, when receptive, are swollen and stigmatic and retain pollen grains conveyed to them (Fig. 28). According to our investigations, the stigmatic flaps of the ovules Figs. 45—47 (and 50—52) were receptive, and when viewed from the abaxial side they are all seen protruding from behind the bracts. On the contrary, in Fig. 49, where the flaps have collapsed and consequently are not receptive, they are not visible beyond the rim of the bract, and this also seems to be the case at the very early stages. Examination of a considerable number of inflorescences has confirmed these observations.

The investigation has shown that on a series of photos of inflorescences at various stages of development there is a distinguishable difference between the position of the outer part of the bracts at earlier and later stages. But although the position of the bracts gradually changes from more or less upright at the beginning of the receptive period to more or less backwards curved after the receptiveness has ceased (Fig. 23, a, d, g) the differences during the said period are so small that when, as in practice, only inflorescences of approximately the same stage of development are available, the position of the bracts alone is not a satisfactory indicator for receptiveness.

In view of the above it is our opinion that the shape of the bracts and the protrusion of the stigmatic flaps from behind them are more reliable criteria for receptiveness than the position of the bracts. Using these characteristics it should in practice be possible at any time to determine the stage of development of an inflorescence as follows: From the middle of a typical inflorescence, a bract with the attached ovuliferous scale is removed *without injury to the stigmatic flap*. The bract is examined under a lens from the side facing outwards from the inflorescence (the abaxial side). If the bract is inverted heart-shaped and the flaps are visible, the inflorescence is just before or in the first part of the receptive period (Figs. 45 and 50; 46 and 51). If the bract is more or less rectangular and the flaps are visible (Figs. 47 and 52), the inflorescence is in the second or last part of the receptive period. If the bracts are rectangular and the flaps are not — or only slightly — visible, the latter have collapsed, and the inflorescences have ceased to be receptive (Figs. 49 and 54).

Among the illustrations the very earliest stages of receptiveness are missing. We had hoped to get the necessary material in 1959, but unfortunately the flowering of *Larix* failed. Judging however from the almost upright bracts, and by comparing the base of the bracts and the stigmatic flaps with the illustrations shown, the identification of the early stages ought not to present difficulties.

According to Table 1 the pollinations effected on and between April 27th and May 4th 1956 have given almost equal results. This seems to indicate that the receptiveness of the inflorescences has remained practically unaltered during the 8 days comprised by the period (the fact that the inflorescences of clone V. 618 were receptive during this period is confirmed by the pollen grains adhering to the stigmatic flaps shown in Figs. 46 and 51, 47 and 52). The relatively high germination percentages from pollinations on April 25th—26th show that the receptive period had started before that date, and taking into account that the termination of receptiveness took place between May 7th and May 11th it may be assumed that the total period

during which receptiveness occurred covered 2—3 weeks. According to ROHMEDE and SCHÖNBACH (8) it is possible that pollen, conveyed to the inflorescences before the receptive period, may settle on the stigmatic flaps when the latter become receptive. Pollinations carried out before the receptive period may consequently also be effective; and it is important that the isolation of female inflorescences and the removal of male flowers before isolation is carried out as early as possible.

In view of the above, it would seem that a period of 3 weeks, during which varying numbers of ovules are receptive, includes about one week (perhaps more) with a gradually increasing number of receptive ovules, one week with a stationary number and one week with a gradually decreasing number of receptive ovules. On account of the scant material and because too little is known about the variations in receptiveness from tree to tree and within the inflorescences, this is, of course, only a rough estimate and, furthermore, since the influence of air temperature and moisture must be considered, the length of the periods will no doubt vary from year to year.

It seems however clear that there is a number of days without much variation in the number of receptive ovules, and it would no doubt be preferable to effect pollination during this more or less stationary period, especially if only one or two pollinations are carried out. — At present our knowledge of the first part of the receptive period is, however, not sufficient to determine accurately the beginning of the stationary period, but if pollination is started when the bracts have just opened and the stigmatic flaps are clearly protruding from behind the inverted heart-shaped bracts (cf. Figs. 45 and 50) this will probably be approximately correct. If only two pollinations are to be effected the next one should be carried out when the bracts begin to assume a more rectangular shape with the stigmatic flaps clearly visible (a little later than Fig. 46, but earlier than Fig. 47); if the stigmatic flaps are disappearing, it will be too late to pollinate.

It is, however, questionable whether pollination of all (or nearly all) ovules can be secured by only two pollinations. Much depends of course on the pollination technique applied, on weather conditions and on the viability of the pollen, which cannot be tested by germination in vitro. But pending more thorough investigations of the problems involved, it is no doubt advisable, where circumstances permit, to pollinate not only during the stationary period but as often as possible, at intervals of a couple of days or more, from the beginning of the receptive period until the inflorescences close, and to use pollen from more than one extraction.

As already mentioned in the text most of the investigations described in the present work have been made by the aid of a Zeiss Stereo Microscope on which also the photographs (except Figs. 29—31, 33, 36—39) have been taken. In this connection we would like to point out the immense value the possession of a good preparation (low power) microscope would have for the personnel engaged in plant breeding, nursery work, seed control etc., not only for investigation work, but even more so in the daily routine for identification of insect pests, diseases, examination of buds and inflorescences in connection with controlled pollinations, examination of seeds, control of germination etc. etc. — The modern preparation microscope, which is now being used in the daily routine work of the industry on a rapidly increasing scale, is highly effective, easy to handle, and it

ought to belong to the standard equipment of every forest district.

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### Summary

A brief description is given of the development of the male and female inflorescences of *Larix* from the formation of buds in the year preceding the flowering year till fertilization. The most important stages are illustrated by photos.

Interruption of meiosis by low temperature and resumption of divisions several days later by rising temperature were observed; resulting irregularities of the meiotic divisions are described, and it is suggested that this occurrence may have significance for the fructification of *Larix* in regions where alternating periods of cold and warm weather are frequent in early spring.

The unique pollination mechanism in *Larix* is described, viz. the pollen grains are caught and embedded on the stigmatic flap, where they rest for 5—7 weeks. They are thereafter transported to the top of the nucellus (on which they germinate) by fluid exuded into the micropylar canal and retracted through the nucellus top. Exudation and retraction of fluid is presumed to be governed by high and low sap pressure in the trees' tissues and may, therefore, be influenced by moist weather and drought. Not all pollen grains caught on the stigmatic flap are transported to the nucellus top, for which reason the mechanism seems less effective than when pollen germinate in situ.

A short description is given of the pollination mechanisms in *Pinus*, *Picea* and *Pseudotsuga* after DOYLE and O'LEARY.

A method for testing the effectiveness of controlled pollinations in *Larix* by counting the pollen grains caught and embedded on samples of the stigmatic flaps is described.

The most favourable time for the execution of controlled pollinations in *Larix* is discussed, and it is recommended that if possible all inflorescences should be pollinated several times with one or two days' intervals during the whole period of receptiveness, with pollen from different extractions; if only one or two pollinations are possible, these should be carried out during the middle of the receptive period. The development of the female inflorescences during the receptive period and at the end of the latter is described and illustrated by photos; and a method to ascertain the stage of development of an inflorescence is submitted. It is suggested that the shape of the bracts and the state of the stigmatic flaps are more reliable criteria for receptiveness than the position of the bracts.

It is recommended that the isolation of the female inflorescences is carried out as early as possible.

### Zusammenfassung

Titel der Arbeit: Die Pollenbildung, der Bestäubungsmechanismus und die Bestimmung des günstigsten Zeitpunktes für kontrollierte Bestäubungen bei *Larix*.

Die Entwicklung der ♀ und ♂ Blütenstände bei *Larix* wird vom Beginn der Knospenbildung im Jahre vor der

Blüte bis zur Befruchtung kurz beschrieben und durch Photos belegt.

Die Unterbrechung der Reduktionsteilung bei niedrigen Temperaturen und die Wiederaufnahme der Teilungen mehrere Tage danach bei steigender Temperatur wurden beobachtet. Daraus resultierende Unregelmäßigkeiten bei der Meiose werden beschrieben. Es wird angenommen, daß diese Tatsache für die Samenbildung bei der Lärche in den Gebieten von Bedeutung sein kann, wo (im Frühjahr) kalte und relativ warme Umweltbedingungen häufig wechseln.

Der außergewöhnliche Bestäubungsmechanismus der Lärche wird beschrieben: Die Pollenkörner werden festgehalten und in der geschrumpften Narbe eingebettet, wo sie 5 bis 7 Wochen im Ruhezustand verharren. Danach werden sie durch eine Flüssigkeit, die durch die Nucellusspitze ausgepreßt und wieder aufgesogen wird, von der Narbe auf die Nucellusspitze transportiert, wo sie dann keimen. Es wird angenommen, daß die Ausscheidung und das Wiederaufsaugen dieser Flüssigkeit durch die wechselnde Saftspannung im Gewebe geregelt werden, und daß es darum auch möglich sein kann, daß diese Vorgänge von trockenen und feuchten Witterungsverhältnissen beeinflussbar sind. Nicht alle Pollenkörner werden dabei von der Narbe auf die Nucellusspitze transportiert, und es scheint darum der Bestäubungsmechanismus weniger effektiv zu sein als bei einer Pollenkeimung in situ.

Die Bestäubungsmechanismen bei *Pinus*, *Picea* und *Pseudotsuga* werden kurz nach DOYLE und O'LEARY beschrieben.

Die Effektivität von kontrollierten Bestäubungen läßt sich dadurch untersuchen, daß man während der Ruheperiode die in den Narben eingebetteten Pollenkörner in Stichproben auszählt. Die Methodik dazu ist angegeben worden.

Der günstigste Zeitpunkt für die Durchführung kontrollierter Bestäubungen wird diskutiert. Es wird empfohlen, daß alle ♀ Blütenstände, wenn möglich, während der gesamten Periode der Empfängnisfähigkeit mehrmals in Intervallen von 1 bis 2 Tagen polliniert werden. Sind nur 1 bis 2 Bestäubungen möglich, so sollten diese am besten im mittleren Abschnitt der Periode der Empfängnisfähigkeit durchgeführt werden. Die Entwicklung der ♀ Blüten im Verlaufe dieser Periode und am Ende derselben ist beschrieben und durch Abbildungen belegt worden. Eine Methode zur Bestimmung des Entwicklungsstadiums einer Blüte wird mitgeteilt. Dabei wird die Ansicht vertreten, daß die Form der Schuppen und der Entwicklungszustand der Narben zuverlässigere Kriterien für die Empfängnisfähigkeit sind als die Position der Schuppen.

Um weitgehend die Fehlerquelle auszuschließen, daß sich bereits Pollen frühblühender Bäume in den Blütenständen der Versuchsbäume befinden, die später zur Befruchtung kommen können, wird empfohlen, die Isolierung so früh wie möglich vorzunehmen.

### Résumé

Titre de l'article: Formation du pollen, mécanisme de la pollination et détermination de la date optimum des pollinations contrôlées pour les mélèzes.

L'article commence par une brève description du développement des inflorescences mâles et femelles des mélèzes depuis la formation des bourgeons dans l'année qui précède la floraison jusqu'à la fertilisation. Des photographies

illustrent les stades des plus importants de ce développement.

Des observations ont été faites sur l'interruption de la méiose par les basses températures et la reprise des divisions cellulaires plusieurs jours plus tard lorsque la température s'élève, ainsi que sur les irrégularités des divisions méiotiques qui en résultent; on pense que cela peut avoir une influence sur la fructification des mélèzes dans les régions où il se produit, fréquemment, au début du printemps, des alternatives de périodes chaudes et froides.

On a décrit un mécanisme unique de la pollination chez le mélèze: les grains de pollen sont captés et se fixent sur l'extrémité du stigmate où ils restent en repos pendant 5 à 7 semaines. Ils migrent ensuite vers le sommet du nucelle sur lequel ils germent; cette migration se fait dans le liquide exsudé dans le canal micropylaire, liquide qui se concentre à travers le sommet du nucelle. On pense que l'exsudation et la concentration de ce liquide sont commandées par les variations de pression de la sève et peuvent, par conséquent, être influencées par l'humidité et la sécheresse atmosphérique. Tous les grains de pollen captés sur le sommet du stigmate ne sont pas transportés vers le nucelle et pour cette raison son mécanisme semble moins efficace que la germination des grains de pollen en place.

On donne une brève description des mécanismes de pollination, d'après DOYLE et O'LEARY pour des genres *Pinus*, *Picea*, et *Pseudotsuga*.

L'efficacité de la pollination contrôlée des mélèzes peut être testée par le comptage des grains de pollen captés et fixés sur les stigmates.

L'époque la plus favorable pour l'exécution des pollinations contrôlées des mélèzes fait l'objet d'une discussion; on recommande chaque fois que cela est possible de polliniser toutes les inflorescences plusieurs fois à 1 ou 2 jours d'intervalle pendant toute la période où les fleurs sont

réceptives, et cela, avec des pollens provenant de différentes extractions; s'il n'est possible de faire qu'une ou deux pollinations, celles-ci doivent être exécutées au milieu de la période de réceptivité. Des photographies illustrent le développement des inflorescences femelles au cours de la période de réceptivité et après celle-ci. On propose une méthode pour contrôler le stade de développement d'une inflorescence. On pense que la forme des bractées et l'état des stigmates sont des critères de réceptivité plus valables que la position des bractées.

Il est nécessaire d'isoler les inflorescences femelles aussitôt que possible.

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## Provenance Study of Douglas-Fir in the Pacific Northwest Region

### I. Nursery Performance

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#### Introduction

Indiscriminate transfer of seed from collection area to nursery or to planting site and, more recently, from collection area to direct seeding site long has been of concern to agencies interested in forest regeneration. Results of such practices in other countries (1, 2, 22), and of early projects on provenance testing in the western part of the United States (15, 25) have indicated such transfer is risky. Reforestation of lands with trees poorly adapted to the new environment may well result in an inefficient use of the productive capacity of the land. The errors may be considered even more costly since the result may not become evident until 30 — 50, or even 80 years after establishment of the stand.

The general policy of planting or seeding plants or seeds

from local sources of comparable altitude has been adopted in recent years. This procedure, while minimizing gross errors, also imposes great limitations on collection of seed from a species which produces only an intermittent crop varying widely in annual abundance and locality. Strict adherence to the "local seed" policy may leave a cut-over area without a proper seed source for several years — the very years, in fact, which probably are the most important for re-establishment of a new stand.

As a result of several meetings of foresters concerned with this situation, the Oregon Forest Research Center began planning in 1954 a region-wide provenance study of Douglas-fir (*Pseudotsuga menziesii* [M. RB.] FRANCO var. *menziesii*). Objectives of the study were to detect the genetic variation of this widely distributed variety, and to