The Formation of Pollen, the Pollination Mechanism, and the Determination of the Most Favourable Time for Controlled Pollination in Pseudotsuga Menziesii

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In connection with the work performed at the Tree Improvement Station to determine the most favourable time for controlled pollinations in the more important forest trees, an investigation similar to that previously reported for Larix *decidua* (2) has been made in *Pseudotsuga Menziesii* (MIRBEL) FRANCO var. *viridis* SCHWER.

In the course of this investigation it was found that, contrary to the general conception, the pollen in *Pseudotsuga* does not germinate on the stigmatic flap but on the nucellus top, to which it is transferred after a resting period just like in *Larix*. Although there is a close resemblance between *Larix* and *Pseudotsuga* as regards the ovules and the pollination mechanisms (4a, b) there is, however, an interesting difference. At the time of pollination the pollen grains of both genera are spheroidal, but while the pollen of Larix remain spheroidal during the resting period and germination, the pollen grains of *Pseudotsuga* elongate to such an extent that they measure about 550 μ by 120 μ when they germinate on the top of the nucellus.

Material and Methods

The investigation and pollination experiments were carried out in 1960 when the flowering of *Pseudotsuga* was abundant. For the pollination experiments 3 Clones (V. 659, V. 661 and V. 847) were used. At intervals of one to two days a number of isolated female inflorescences were pollinated. Later on the germination percentage of the seeds, obtained from the pollinated inflorescences of each clone at each pollination date, was determined and compared with the stage of development of the inflorescences at the corresponding dates (*Fig.* 42—61). For the investigation of the pollination mechanism mainly the same trees were used, but when possible, the findings were checked on other trees. — It was intended to repeat the experiments in 1961, but flowering failed almost completely, and this plan had thus to be abandoned. About 3 weeks after the time of flowering in 1961, it came to our notice that a few trees of *Pseudotsuga* Menziesii var. *glauca* and var. viridis in private gardens at Hornbaek carried female inflorescences. They were used to check the pollen development during the resting and germination period; no noticeable difference between the development in var. *glauca* and var. *viridis* was found.

The material was fixed in Carnoy and transferred to 70% alcohol. The main part of the investigation of the pollination mechanism was made by means of the squash method. Under a preparation microscope the pollen grains were picked or scraped out of the micropylar canal or off the stigmatic flap into a drop of acetic orcein or carmine on a slide. The preparations were frozen and made permanent in euparal. Pollen grains were also dissected from the top of the nucellus, but here a fine and very sharp needle or a gilette surgical blade shape E, was used. — In examining nucellus tops for pollen a preparation microscope and ordinary light was used, but the adjustment to render the transparent empty pollen grains visible was somewhat critical. Care must be taken that the pollen grains do not dry and shrink; if this happens, they are usually lost. For the study of pollen grains in the canal or on the nucellus top (except of course in connection with the movements of fluid in the canal) it is preferable to use material stored in 70% alcohol and to do the dissecting in this fluid without exposing the pollen to the air.

Part of the material was dehydrated, embedded in paraffin and sectioned on a microtome at 25 μ. The microtome slides were particulary used to trace the male cells on their route through the nucellus top and the archegonium, and to check the results of the examinations made by the squasi methods.

The study of the structure and development of the microspores is difficult on account of their thick exine and dense contents of granules, which often practically obscure the interior.

The exine and intine of young pollen grains may, however, be made expand and be more transparent (and sometimes the exine may rupture) by heating in acetic carmine to which afterwards glycerine is added; the slides were pressed gently with filterpaper and examined with a phase contrast microscope.

Mature pollen was placed in water for a couple of hours, whereby the exine was ruptured and cast off, then heated in acetic carmine or, if a heavier stain was desired, in propionic carmine, to which glycerine was added. By addition of glycerine the pollen grains are made more transparent and will keep for a considerable time. — To make mature pollen grains more transparent the following procedure also gives good results: 1) put the dry pollen in a small glass tube with rounded bottom, add destilled water and keep for one or two hours to allow the pollen to expand and the exine to rupture; the pollen will sink to the bottom, and the following changes of fluid are done with a smsll pipette; 2) fix in Carnoy over night, thereafter fix in Nawaschin 5-6 hours; 3) dehydrate in alcohol-Xylen series as for embedding in paraffin. On account of the slow rate of penetration through the membranes of the pollen grains, the duration of the usual stages of the series must at least be doubled. From the Xylen stage small quantities of pollen grains may by means of a pipette be transferred to a drop of canada balsam on a slide, covered by a cover glass and examined with a phase contrast microscope. If desired the pollen grains rnay after Xylen be embedded in paraffin, sectioned on microtome, stained in Feulgen or Hematein (Orange G may be used as a counterstain) and examined with an ordinary microscope. We found it, however, virtually impossible to locate more than one section of a pollen grain fit for use; the following sections were either lost or damaged, or they could not with any degree of certainty be distinguished from the sections of the many other pollen grains present on the slide.

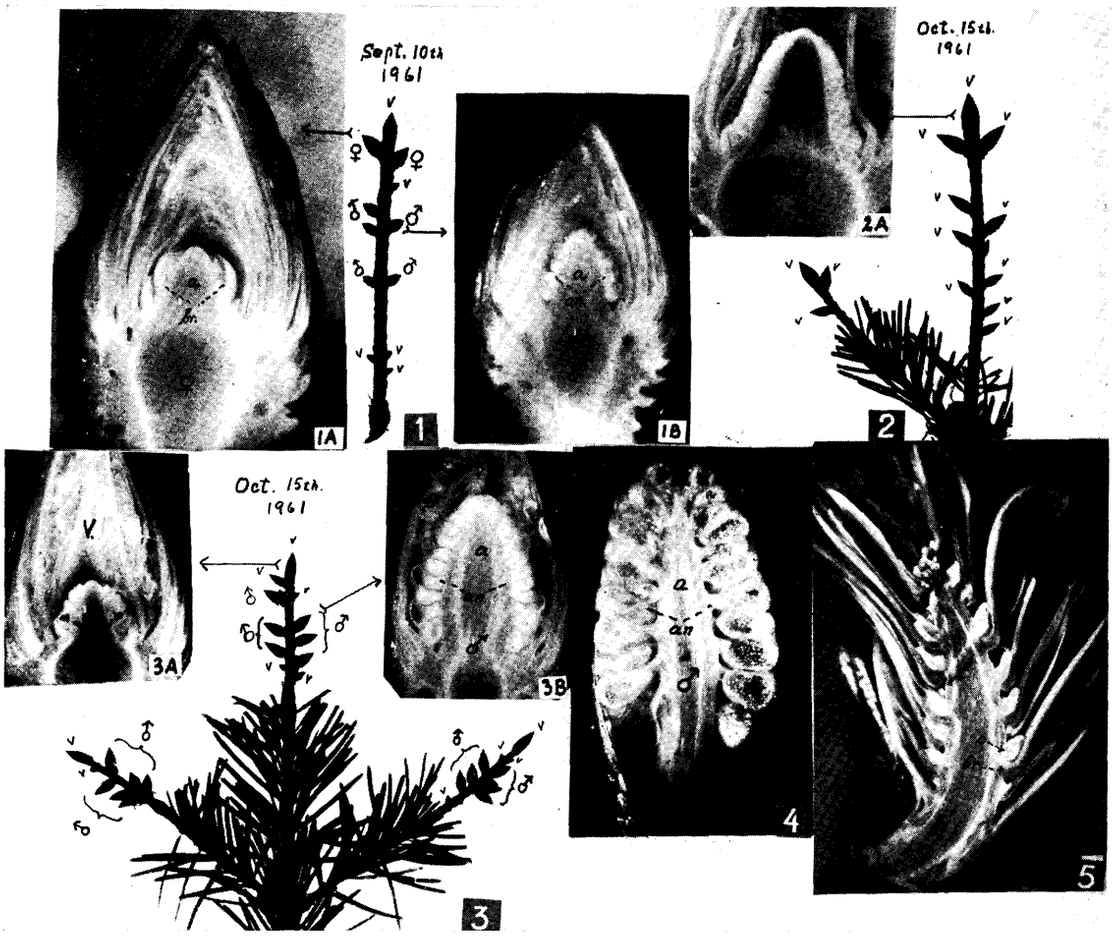

Fig. 1. — Terminal shoot of twig of *Pseudotsuga Menziesii* with female, male and vegetative buds; needles removed; × ca. 0.6. — *Fig. 1 A.* — Longitudinal section of left female bud, note coneshaped axis; × ca. 7. — *Fig. 1 B.* — Longitudinal section of upper male bud, note oval axis; fixed September 10, 1961 × ca. 8. —

Fig. 2. — Shoots of large and fast growing tree, all buds are vegetative; × ca. 0.6. — *Fig. 2 A.* — Longitudinal section of axis of top vegetative bud; × ca. 8; fixed October 15, 1961. —

Fig. 3. — Twig of slow growing and prolific tree with male inflorescenses and vegetative buds, needles removed from outer part of shoots; × ca. 0.6. — *Fig. 3 A.* — Longitudinal section of vegetative terminal bud, note slender axis; × ca. 5. — *Fig. 3 B.* — Longitudinal section of upper male bud, note oval axis; × ca. 8.; fixed October 15, 1961. —

Fig. 4. — Longitudinal section of male inflorescence at time of pollination; × ca. 5; fixed May 1, 1960. —

Fig. 5. — Longitudinal section of female inflorescence at time of pollination; × ca. 3; fixed May 1, 1960. —

Abbreviations: a axis; *an* anther; *br* bract; *n* needles; *o* ovule; *p* pollen; *v* vegetative buds. —

As the exine gradually thickens during the development of the pollen grain, the most expedient treatment should be tried out in each individual case, but the best results were always obtained by the use of a phase contrast microscope and weak staining, and sometimes without any staining at all.

The Inflorescences of Pseudotsuga

The inflorescences of *Pseudotsuga* are formed in the year preceding the flowering year. ALLEN (1a) gives a detailed account of the reproductive chronology of *Pseudotsuga* near Victoria, British Columbia. According to this account, the differentiation of the male strobilus takes place in August, meiosis in March, pollination medio March to medio April and fertilization in the beginning of June. If we add 2—3 weeks to each of the aforementioned dates, ALLEN's timetable will probably also apply to conditions in Denmark. Thus in 1960 meiosis in *Pseudotsuga* at Humlebaek took place ultimo March, pollination early May and fertilization ultimo June.

According to ROHMEDER and SCHÖNBACH (12) female buds are pointed and resemble the vegetative buds more than the male buds, yet they are considerably larger than the latter. The male buds are smaller than the female buds and more rounded, but trees with more slender and pointed male buds are also found. *Figs. 1—3* demonstrate the appearence of female, male and vegetative buds in September-October of the year preceding the flowering year, and *Figs. 4—5* male and female inflorescences at the time for pollination in early May. As will be seen from *Fig 1* and *2* there is not much difference between the exterior of female buds and of terminal vegetative buds in the autumn, but on sections they may readily be told apart (*Figs. 1A, 2A* and *3A*). This also applies to the difference between male buds and the smaller vegetative buds. If a flowering forecast for the next year is wanted in the autumn, one may normally get a general impression from the number of male inflorescences present. It must however be borne in mind that even if the forecast is promising, it sometimes happens that very few female inflorescences are found the

following year; the reason for this is not known. It must also be remembered that some trees predominatingly carry male inflorescences and very few female ones. If flowering is abundant the forecast is comparatively easy because the male buds are then occurring in clusters *(Fig. 3)*, but if the flowering is mediocre or scant the shoots look like those shown in *Figs. 1* and *2*, where it is practically impossible without sectioning to tell the male buds in *Fig. 1* apart from the small vegetative buds in *Figs. 2*. On sections, however, the buds are easily distinguished as they have the appearance shown in *Figs. 1B* and *2A* and *Figs. 3B* and *3A*. It is however, imperative that a *new* razor blade is used for sectioning (otherwise the differences are obscured), and that a strong hand lens or a good preparation microscope is available.

The female buds are usually found near the terminal bud of the shoot *(Fig. 1)* one or two together while the male buds, when the flowering is abundant, sit in clusters mainly on the distal half of the shoots *(Fig. 3)*; when they turn yellow and open they may in good flowering years be quite a remarkable sight. *Fig. 4* show a male and *Fig. 5* a female inflorescence at the time of pollination.

The female inflorescences are not erect at the time of pollination as in *Larix decidua* and *L. leptolepis*, but stand at any angle between erect and horizontal. During the second and third week after pollination, the young cones gradually assume the drooping position characteristic of the older cones (4b, p. 200).

The structure of the female inflorescences of *Pseudotsuga* seems symmetrical than that of *Larix*, and often the axis is curved to such a degree that pollination of the concave side of the inflorescences seems obstructed (cf. *Fig. 5* and *46a)*. The axis itself is, at least at the time of pollination, slender and fragile, wherefore bagging and pollination work must be carried out cautiously in order not to break off or injure the inflorescences. Longitudinal sectioning is also difficult at this stage for the same reason.

Examination of a considerable number of female inflorescences has shown, that there are two unproductive regions, one at the top and one at the base, each about ¼ of the length of the inflorescence. In these regions most of the ovules do not develop beyond the initial stage.

Meiosis, Embryogeny and Chromosomes of Pseudotsuga Menziesii

Meiosis and embryogeny have been described in detail by ZENKE (16) and ALLEN (1b) respectively and we have little to add.

The haploid chromosome number is 13 (SAX and SAX [13]), which is interesting since most of the conifers have 12, including *Larix* to which *Pseudotsuga* in many respects has a strong resemblance. According to SAX and SAX, *Pseudotsuga* has 6 heterobrachial chromosomes, 6 with approximately median constriction and one chromosome (the shortest) with terminal attachment. — A number of plates from colchicine treated root tips of our material gave not the same results as we found two pairs (the shortest) with terminal attachment. The four telocentric chromosomes show no constrictions, but each of them has two short terminal threads *(Fig. 6C)*. Furthermore chromosomes Nos. 14 and 15 have a peculiar secondary constriction close to the centromere *(Figs. 6A* and *6B)*.

SAX and SAX mention the possibility that the extra chromosome is a duplication, but as no meiotic divisions were observed they left the question open. — Another possibility is that each of the two shortest pairs of chromosomes are derived from a long chromosome with median or submedian constriction by breakage. — *Fig. 6* presents a microphoto of the 26 somatic chromosomes in a root tip metaphase of *Pseudotsuga*. — *Fig. 7* is an actual idiogram of the metaphase shown in *Fig. 6; Fig. 8* shows a hypothetical idiogram with two long chromosomes constructed from the two shortest pairs in *Fig. 7* and inserted into the idiogram (stippled) according to their length; the idiogram thereafter shows 12 long chromosomes and 12 short ones like in *Larix*. — *Fig. 9* is an actual idiogram of the chromosomes of *Larix occidentalis* according to KNABEN (7); it has a strong resemblance to the hypothetical idiogram of *Pseudotsuga (Fig. 8)*. This resemblance is interesting in view of the fact, that the two genera have so many characteristics in common.

There is some evidence pointing to a certain instability of the meiotic pairing and of the anaphase separation of some of the chromosomes. Lagging chromosomes and bridges are sometimes seen in ZENKE's excellent illustrations. We have observed similar irregularities in our material *(Fig. 10)*, and even in somatic cells from root tips, chromosomes were occasionally seen in the cytoplasm outside the spindle regions *(Fig. 11—15)*.

Our material is not sufficient to motivate an opinion as to the origin of these irregularities, but it seems probable that the chromosomes involved are preferably the telocentric chromosomes and perhaps the pair with the peculiar centromere, No. 14—15. The two prematurely separated univalents in the metaphase I shown in *Fig. 10* are probably two of the telocentric chromosomes whose centromeres have been too weak. The two mitotic chromosomes shown in *Fig.* 11 (arrows) are perhaps the same as seen in *Fig. 10;* they have evidently passed undivided to one pole during the preceding mitotic division and have not developed into prophase as the other chromosomes in the nucleus. The chromosomes in the cytoplasm of *Figs. 12* and *13* show a certain resemblance to each other; they also descend from a previous division and have not followed the prophase chromosomes. These chromosomes are obviously not telocentric and they may possibly be identical with chromosomes Nos. 14 and 15. The lagging chromosome in the telophase in *Fig. 14* (which is probably identical with that seen in the cytoplasm in *Fig. 15)* has evidently not divided at the anaphase stage and will probably remain in the cytoplasm. Its shape is peculiar and having a median constriction it may perhaps be an isochromosome derived by misdivision of one of the shortest telocentric chromosomes.

The irregularities observed were not very frequent; most cells were normal. It would however seem that they call for a more thorough cytological investigation, not only of var. *viridis* but also of the other varieties and species of *Pseudotsuga*, of which even the chromosome numbers are not known.

The Pollen of Pseudotsuga Menziesii

According to WODEHOUSE (15) the pollen grains are "approximately spheroidal without trace of bladders, pore or furrows, rather uniform in size, 90—100 μ in diameter, closely resembling those of *Larix*. Exine thin and quite smooth. When the grain is moistened it swells, the exine generally splits wide open and is frequently thrown completely off". ERDTMAN ([5] p. 40) states that the pollen grain has a tenuitas (i. e. a thin aperturoid area functioning as

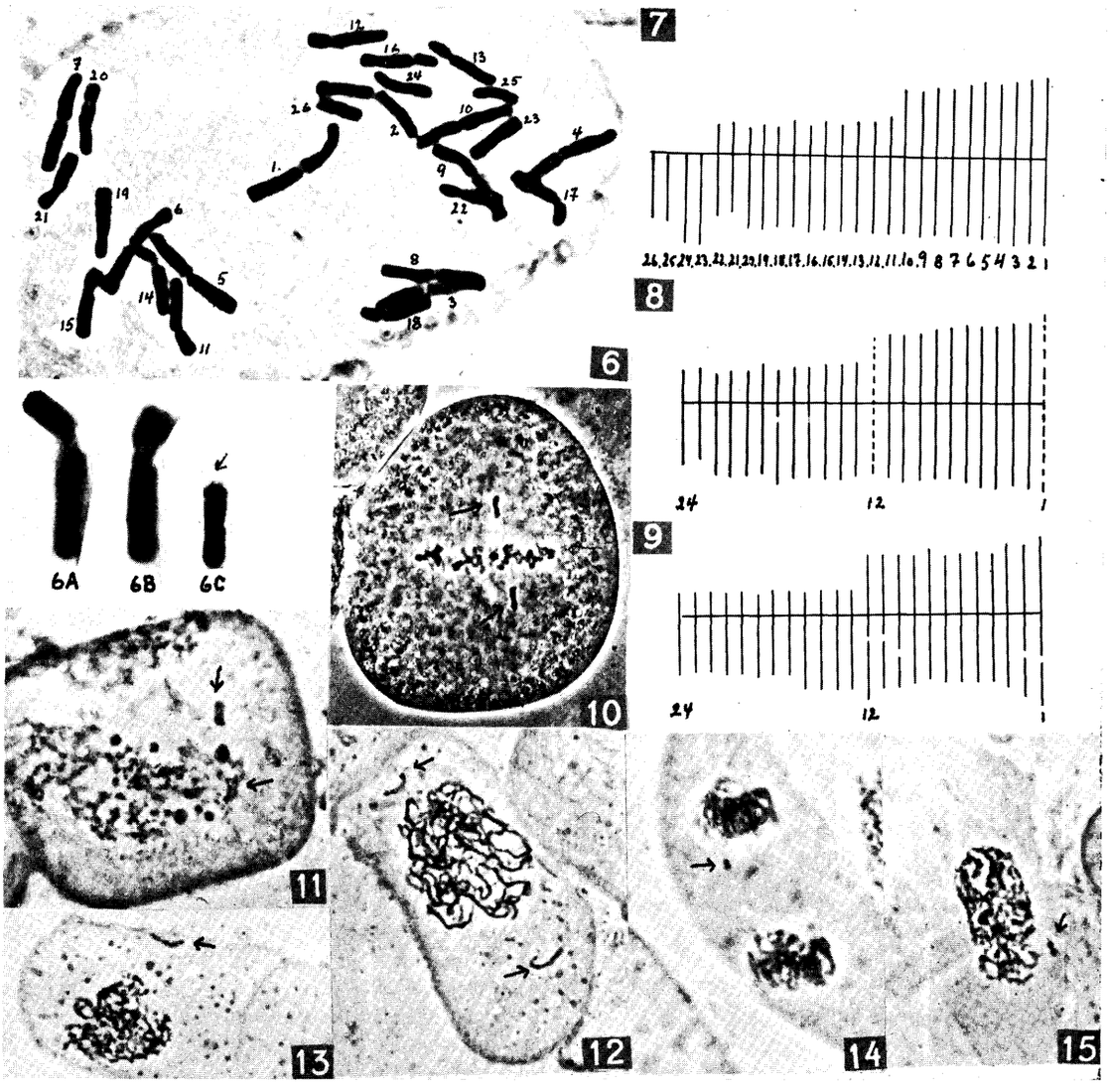

Fig. 6. — Root tip metaphase (colchicine treated) of *Pseudotsuga Menziesii*, 26 chromosomes; × ca. 1550. — *Figs. 6 A and B.* — Enlargement of chromosomes no. 14 and 15, note peculiar constrictions; × ca. 3600. — *Fig. 6 C.* — Enlargement of longest telocentric chromosome, no. 23, note the two small ends of chromosome threads (at arrow), no constrictions; × ca. 2200. —

Fig. 7. — Idiogram of chromosomes shown in *Fig. 6.* —

Fig. 8. — Hypothetical idiogram showing *Fig. 7* but with two long chromosomes (no. 1 and 12) constructed of chromosomes no. 23 + 24 and 25 + 26 respectively, making a total of 12 long and 12 short chromosomes like in *Larix.* —

Fig. 9. — Idiogram of needle metaphase chromosomes of *Larix occidentalis* (after G. KNABEN), note the resemblance to *Fig. 8.* —

Fig. 10. — Irregular meiotic metaphase I, two univalents prematurely separated, probably weak centromere; × ca. 500. —

Fig. 11. — Mitotic root tip prophase (from germinated seeds), one chromosome (or part of chromosome) outside nucleus and a similar inside nucleus resembling the univalents in *Fig. 10;* × ca. 1000. —

Fig. 12. — Mitotic root tip prophase, two chromosomes outside nucleus, perhaps the pair with the peculiar centromeres shown in *Figs. 6 A* and *B;* × ca. 1000. —

Fig. 13. — Mitotic root tip prophase, one chromosome outside nucleus, resembles the two in *Fig. 12;* × ca. 1000. —

Fig. 14. — Mitotic root tip telophase, lagging chromosome with median constriction; × ca. 1000. —

Fig. 15. — Mitotic root tip prophase, one chromosome outside nucleus resembling lagging chromosome in *Fig. 14,* perhaps isochromosome derived from misdivision of one of smallest telocentric chromosomes; × ca. 1000. —

Abbreviation: chr chromosome(s). —

an aperture gradually merging into the surrounding exine) at the distal pole, that is the pole farthest from the prothallium cells. Most of the above details are in accordance with our observations, but we found other interesting characteristics of the pollen grains which will be described in the following.

With regard to pores, none seem to be present in the exine. At the proximal pole of the pollen grains there is, however, an aperture surrounded by a thickening of the intine *(Fig. 16). Fig. 17* shows the exine (or perhaps an inner layer of the exine, the endexine) covering the pore. Two small discs, a flat one and a more concave one, are

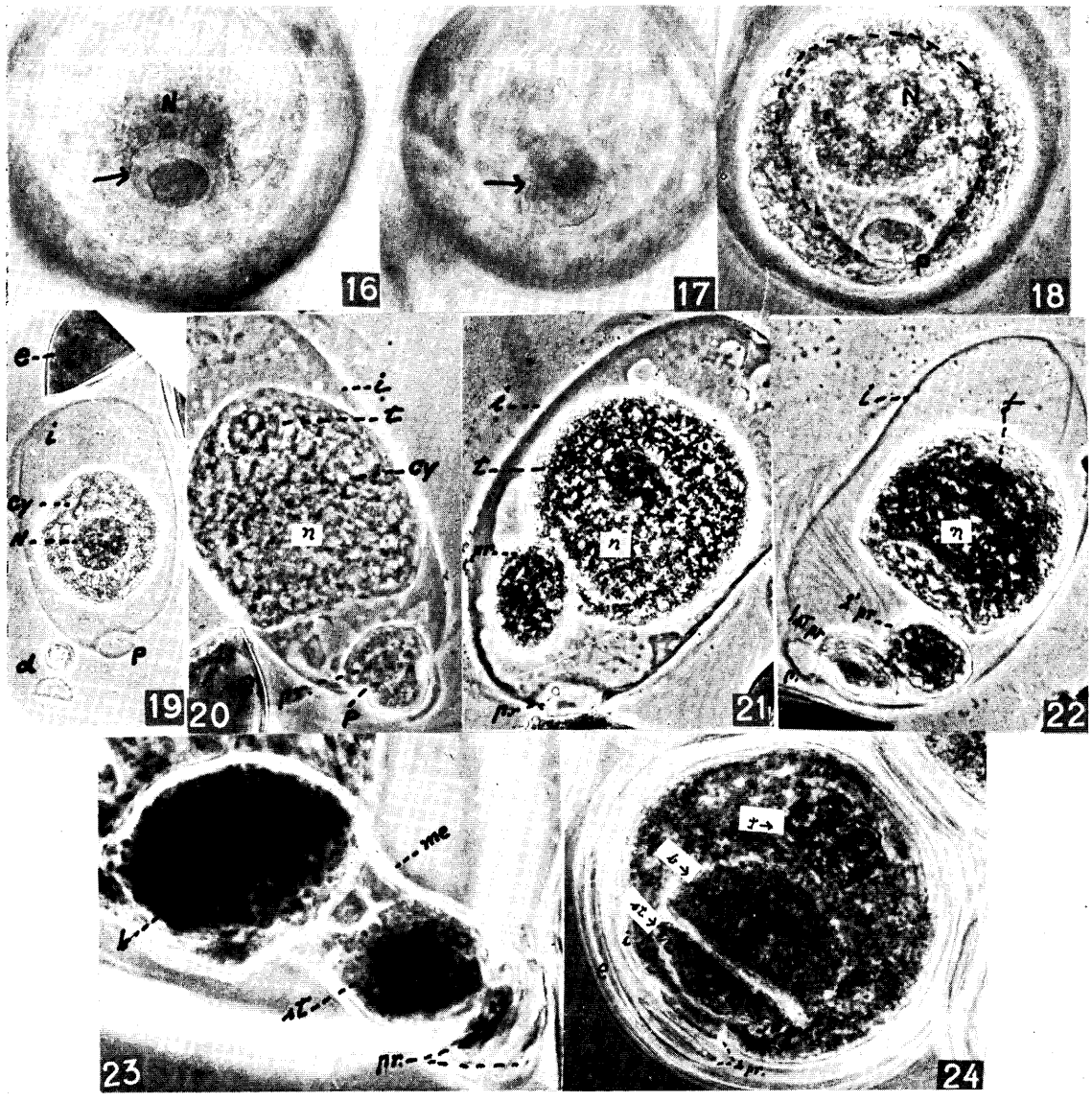

Fig. 16. — Mature pollen grain at time of pollination (May 1st). Pore surrounded by thickening of intine (arrow). —

Fig. 17. — Pollen grain in *Fig. 16*, higher focus. Puckered exine (perhaps endexine) covering pore. —

Fig. 18. — Pollen grain 10 days before pollination, probably degenerating. Pore connected with membrane surrounding micro-spore cells (within stipled line). —

Fig. 19. — Pollen grain April 21, „inflated„ without exine. Two discs *(d)* outside pollen grain near pore have probably been covering the pore opening. —

Fig. 20. — Pollen grain April 21, after first (perhaps second) division. Prothallium cell stuck in pore opening, note "*t*" which strongly resembles tube nucleus in *Fig. 24*. —

Fig. 21. — Pollen grain April 21, first prothallium cell "cut off". —

Fig. 22. — Pollen grain April 21, second prothallium cell "cut off". —

Fig. 23. — Proximal part of mature pollen grain. Body cell and stalk cell pressed out of position showing special common membrane enclosing both cells and terminating at pore (cf. *Figs. 25—27*). —

Fig. 24. — Mature pollen grain, body/stalk cell stage, stalk cell disorganizing; next division about two months later (cf. *Fig. 38*). —

Figs. 16—18, 24; × ca. 500; — *Figs. 19—22;* × ca. 300; — *Figs. 23;* × ca. 700. —

Abbreviations: *b* body cell; *cy* cytoplasm; *d* discs probably covering pore; *e* exine; *i* intine; *me* membrane enclosing body cell and stalk cell; *n* nucleus; *p* pore; *pr* prothallium cell; *st* stalk cell; *t* tube nucleus or (in *Figs. 20—22*) body resembling tube nucleus. —

often found near the pore after squashing *(Fig. 19)*, they may be remnants of the two degenerated prothallium cells or, perhaps, membranes covering the pore. *Figs. 28* and *29* show the structure of the pore, and it appears from *Figs. 18, 23, 25—27* that the pore is the orifice of a sackformed membrane enclosing the body cell and stalk cell. The time when this membrane was formed could not be determined.

When a mature pollen grain is moistened (in water or during its stay on the stigmatic flap, in the micropylar canal or even on the external surface of the ovule) it swells and throws off the exine as mentioned by Wodehouse, but thereafter it elongates very considerably, and in the canal it may attain a length of up to 550 μ before it germinates (the word germination in the present work

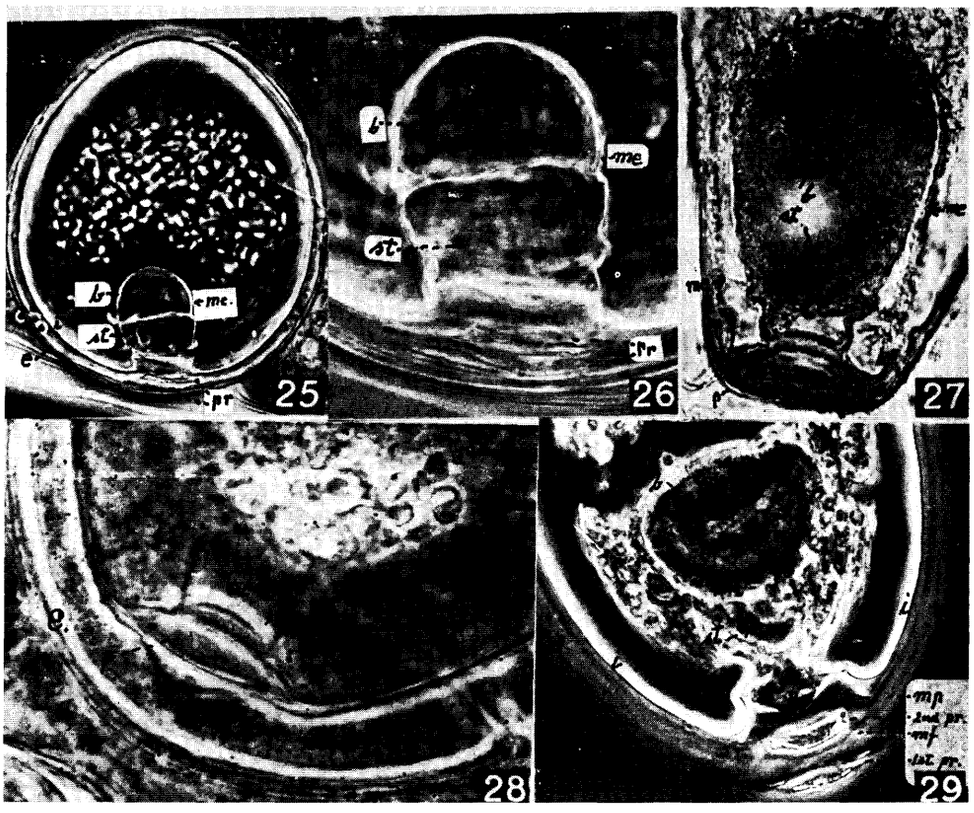

Fig. 25. — Mature pollen grains, most of cytoplasm dissolved; membranes of body cell, stalk cell and remains of two prothallium cells; b and st enclosed in a common membrane *(me)* connected to pore; × ca. 300. —

Fig. 26. — Microspore cells in *Fig. 25* enlarged; × ca. 800. —

Fig. 27. — Proximal pole of pollen grain about 5 weeks after pollination; *b* and *st* enclosed in common membrane connected to pore *(me)*; × ca. 500. —

Fig. 28. — Proximal part of mature pollen grain after water treatment about a week, most of cytoplasm dissolved; showing pore *(p)* and short tube like connection between pore and microspore cells *(tu)* (cf. *Figs. 25—27)*; × ca. 800. —

Fig. 29. — Mature pollen grain after water treatment 3 days, without exine, showing pore region and membranes separating prothallium cells; × ca. 600. —

Abbreviations: *b* body cell; *e* exine; *i* intine; *me* membrane enclosing *b* and *st*; *mp* membrane separating second *pr* from pore; *mf* membrane separating first and second *pr*; *n* nucleus; *p* pore; *pr* prothallium cell. —

denotes the stage, when the pollen grain discharges the male cells). If mature pollen is placed in a drop of water or, if quicker action is desired, in a drop of a weak ammonical liquor (cf. 10, p. 630) most of the grains will rupture or throw off the exine and start to elongate within half an hour. If left in the water for a couple of days, some of the grains will attain approximately the same length as grains found in the micropylar canal, but the microspore cells remain stationary at the proximal end of the pollen grain. This applies also to pollen stored in the laboratory for more than a year without any precautions taken and therefore no doubt dead. The elongation of the pollen seems therefore to be a purely mechanical process, and as a criterion for viability it is of no value. In spite of many endeavours we have never been able to attain actual germination in vitro of *Pseudotsuga* or *Larix* pollen, probably because the pollen grains need some special stimulus produced by the nucellus when the egg cells are receptive. The elongation of the pollen grains takes place at the pole opposite the degenerated prothallium cells, i. e. the distal pole, evidently by means of the tenuitas at this pole (Erdtman [5]). The intine of the pollen grains is according to Müller-Stoll (10) "differenziert in eine äußere, leichter verquellende Schicht und eine innere, sehr dünne, substanzreichere Schicht". Müller-Stoll states that the two degenerated prothallium cells are enclosed in the outer layer of the intine and that the partition-walls between the prothallium cells, and between the latter and the interior of the microspore, consist of later formed membranes of intine. These membranes are seen in *Fig. 29*, which also shows that the two degenerated prothallium cells at this stage (body cell/stalk cell stage) lie outside the pore; that they can pass through the pore (at least when the slide is pressed) is seen in *Fig. 20*, where a prothallium cell (probably the first) is halfway through the pore. As the membranes enclosing the prothallium cells are situated between the exine and the inner intine containing the pore, they must have been formed after the exit of the prothallium cells; the latter are probably pushed out as soon as they have shrunk to a size permitting them to pass the pore

The Development of the Male Gametophyte in Pseudotsuga

According to Lawson (9) the development proceeds along the same lines as reported in *Pinus* and *Picea*, that is, two degenerating prothallium cells are cut off by two consecutive divisions; by the third division a tube nucleus

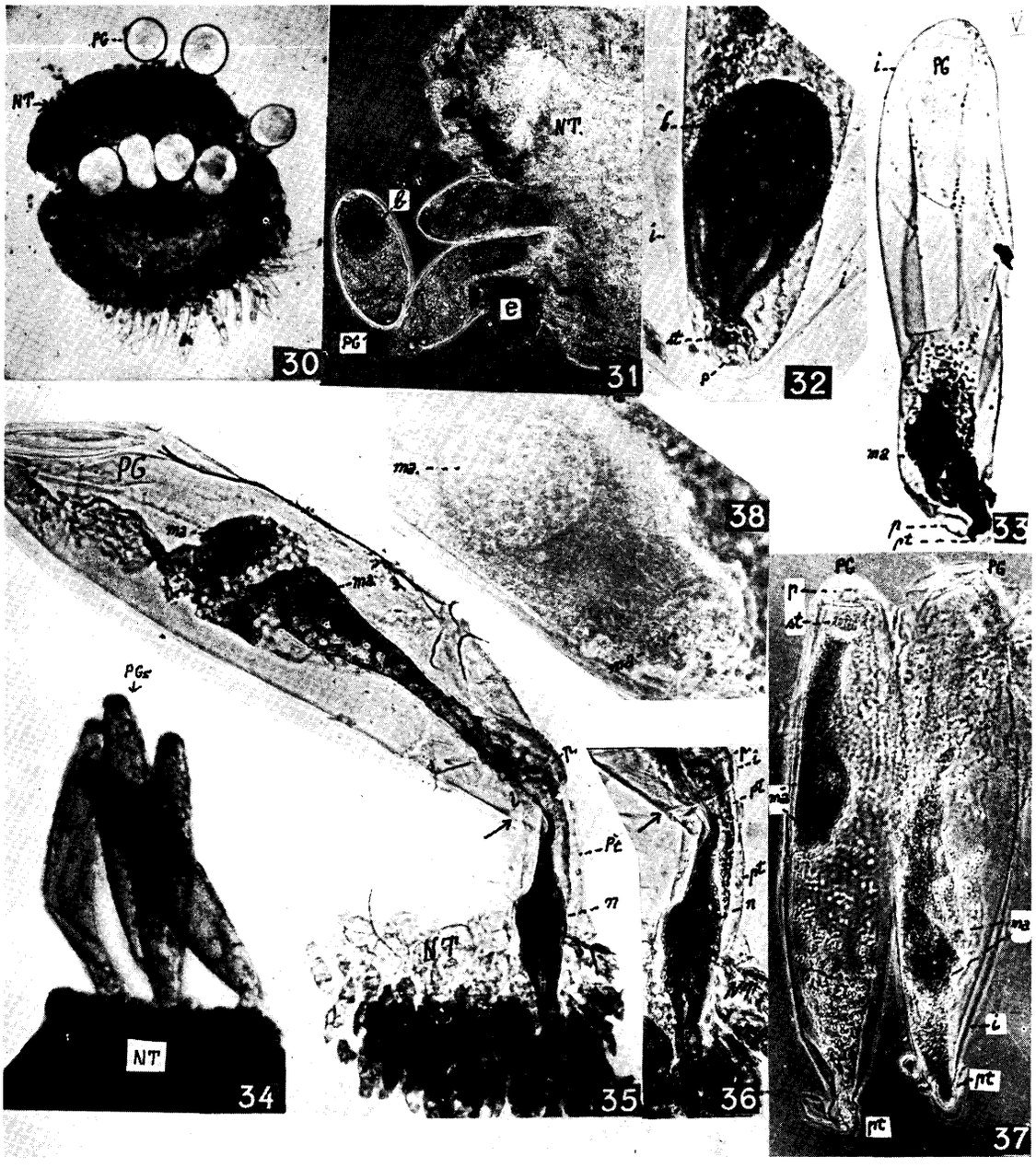

Fig. 30. — Pollen grains on excised and squashed *NT*, exine ruptured and cast off, still spheroidal; × ca. 60; fixed May 13, 1960. —

Fig. 31. — Pollen grains on excised and squashed *NT*, exine cast off, elongating, *b* and *st* still at proximal pole; × ca. 100; fixed June 2, 1960. —

Fig. 32. — Proximal part of pollen grain fixed June 19; *b* moving toward distal pole, note the suspensor like connection to proximal pole; × ca. 250. —

Fig. 33. — Germinating elongated pollen grain broken off from *NT*, fixed June 23; proximal pole downward, pollen tube *(pt)* has proken through intine near pore *(p)*; × ca. 200. —

Fig. 34. — Four elongated empty pollen grains on *NT*, all with distal pole on *NT*; × ca. 75; fixed June 28. —

Fig. 35. — Germinated elongated pollen grain on *NT*, proximal pole on *NT*, pollen tube *(pt)* ruptured intine near pore *(p)* and penetrating into *NT* tissue; two nuclei are still in pollen grain, one in pollen tube (cf. text); fixed June 7, 1961; × ca. 200. —

Fig. 36. — Enlargement of proximal pole and pollen tube of pollen grain *Fig. 35*, note ruptured intine at arrow. —

Fig. 37. — Two germinating pollen grains probably fallen off *NT*, fixed June 23, 1960; body cells have divided and formed male cells; pollen tubes rupturing intine at distal poles (downward); left pollen grain: male cells have still connection to pore at proximal pole; right pollen grain: connection to pore severed; × ca. 150. —

Fig. 38. — Two male nuclei from left pollen grain *Fig. 37*, smallest nucleus nearest distal pole; × ca. 500. —

Abbreviations: *b* body cell; *e* exine; *i* intine; *ma* male nuclei; *n* nucleus; *NT* nucellus top; *p* pore; *PG* pollen grain; *pt* pollen tube; *st* stalk cell. —

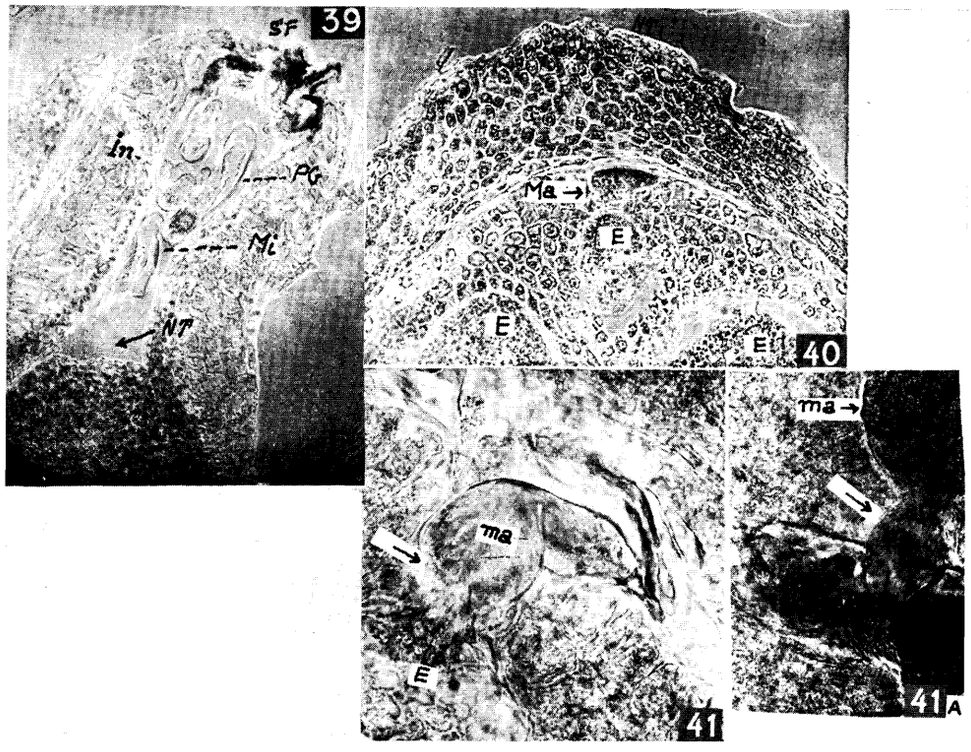

Fig. 39. — Longitudinal section of stigmatic flap *(SF)*, micropylar canal and nucellus top (NT), one whole pollen grain and fragments of other; × ca. 70; fixed June 2. —

Fig. 40. — Nucellus top, apex of three archegones, male nuclei *(ma)*near uppermost; fixed June 28; × ca. 100. —

Fig. 41. — Male nuclei *(ma)*, which seem enclosed in a membrane, contacting apex of egg cell *(E)*, blunt end of nuclei is front, pointed end "tail"; the blunt end is sending out contacting body (at arrow), which is seen more clearly in *Fig. 41 A;* fixed June 28; × ca. 300. —

Abbreviations: in integument; *E* egg cell; *Mi* micropylar canal; *ma* male nuclei; *NT* nucellus top; *PG* pollen grain; SF stigmatic flap —

and a generative cell are formed, whereafter the body cell and stalk cell are derived from the generative cell by a fourth division. LAWSON mentions (p. 165) that he did not see the actual dividing stages. — In our material we found ample evidence of the first microspore division *(Fig. 21)*, the second microspore division *(Fig. 22)* and of a division from which body cell and stalk cell are derived *(Fig. 24)*. A body resembling the tube nucleus present at the body cell stage *(Fig. 24)* was regularly found already during the first and second microspore divisions *(Figs. 20—22)*. Our material did, however, not contain any of the actual dividing stages of the microspore cells, neither is it extensive enough for proper elucidation of the sequence of the nuclear divisions, wherefore the above description of the development of the male gametophyte is not difinite.

When pollination takes place, i. e. when the pollen grains are lodged on the hairlike processes of the stigmatic flap *(Fig. 58—59)*, they were normally, as mentioned above, at the body cell/stalk cell stage *(Fig. 24—27)*. According to STRASBURGER (14) the situation in *Larix* is exactly the same. About a week later, the pollen grains have swelled, and when the stigmatic flap is squashed, they slip out of the exine *(Fig. 30)*, but they are still spheroidal. Three weeks later, the pollen grains have started to elongate *(Fig. 31)* the body cell and stalk cell remaining at the proximal pole.

During the following two weeks the pollen grains continue to elongate, and at the same time the body cell moves from the proximal pole toward the middle of the pollen grain *(Fig. 37)*. Although the body cell moves quite a considerable distance, it does not at once loose connection with the proximal pole. Sometimes this connection reminds of the suspensors of an embryo *(Fig. 32)*. While the elongation of the pollen grains and the movement of the body cell proceed, the pollen grains have thrown off the exine; some are hanging from the filaments of the stigmatic flap, some are lying in the micropylar canal, but none were found on the nucellus top *(Fig. 39)*. About a week later i. e. 7—8 weeks after pollination, the pollen grains are transferred to the nucellus top, where they are firmly planted in an upright position *(Fig. 34)*. We were unable to determine how the pollen grains were transferred from the stigmatic flap to the nucellus top, but it probably takes place in the same way as in *Larix*, i. e. that fluid is exudated through the nucellus top, and when the fluid is retracted it deposits the pollen on the nucellus top. At this stage (probably when the pollen grains come into contact with the nucellus top) the body cell divides forming two male cells one of which, the one nearest to the distal pole, is smaller than the other *(Figs. 37—38)*. Simultaneously a pollen tube is formed either from the membrane of the body cell or from an inner layer of intine *(Figs. 35—36, 37)*. The pollen tube is always formed at the pole of the pollen grain which is in contact with the nucellus top. If the distal pole is in contact, the pollen tube ruptures the intine at that pole and continues into the tissue of the nucellus top. *Figs. 33, 35—36* show, that when the proximal pole of the pollen grain is in contact, the tube is formed at this pole, but it does not leave by the pore as might be expected, it breaks through the intine near the pore. — In *Fig. 35* two male nuclei are still in the pollen grain, but furthermore there is a larger nu-

cleus in the pollen tube. The origin of this nucleus is not clear, but the most plausible explanation is that the largest nucleus (which is considered the active nucleus and is situated next to the proximal pole) has divided an extra time, and that the sister cell derived from the division is the one seen in the tube. In view of the fact that so many pollen grains found with the proximal pole in contact with the nucellus top are empty, it seems probable that the two remaining nuclei will follow the first in due course.

As regards the pollen tube, we have only been able to trace it a short way into the tissue. *Figs. 40—41* show the male nuclei, which seem to be enclosed in a membrane, penetrating the neck of the archegonium. We can confirm ALLEN's statement (1b) that nothing substantiates LAWSON's account of a breakdown of the nuclear apex (the nucellus top) in advance of the pollen tube; apart from the mucilaginous layer on the nucellus top, the tissue is normal.

The number of nuclei discharged into the egg could not be determined, but ALLEN (1b) states that usually all four nuclei, i. e. two male nuclei, stalk cell nucleus and tube nucleus pass into the egg. According to ALLEN the larger one of the male nuclei moves rapidly toward the egg nucleus and syngamy takes place, while the other three nuclei divide or fuse in various ways and finally seem to disintegrate.

Although an abundance of empty pollen grains were found on the nucellus tops, very few were found dividing or forming pollen tubes; it is therefore possible that a more thorough investigation may uncover new details of interest for the elucidation of this unusual method of pollen germination.

The present account elucidates, it is hoped, the main points of the pollination mechanism of *Pseudotsuga,* but certain phases of the development are still unexplained. One of these is the transport of the long pollen grains from the stigmatic flap to the apex of the nucellus and the spectacular upright position of the pollengrains on the latter. That the pollen grains are floated to the nucellus top seems beyond doubt, although the navigation in the narrow canal must be rather difficult; but how they are planted on end is puzzling. It is, however, possible that the distance from the lower end of the canal to the nucellus top is shorter in living material than in fixed, and that the upper ends of the pollen grains are still in the canal when the other end touches the nucellus top. After germination they are firmly fixed by the pollen tube and the mucilaginous layer of the nucellus top.

Without going deeper into the phylogeny, it is interesting to note the resemblance of the long grains of *Pseudotsuga* to the "pollen tubes" of certain cycads, f. inst. *Dioon edule* (COULTER and CHAMBERLAIN [3] p. 141) and possibly also *Ginkgo*. There are of course, also important differences, f. inst. that *Dioon* produces motile sperms which are discharged directly into the pollen chamber, *Pseudotsuga* non-motile male nuclei which are discharged through a special pollen tube into the nucellus top. The position of the pollination mechanism of *Pseudotsuga* seems therefore to be somewhere between the *Cycads* (and perhaps *Ginkgo)* and conifers like f. inst. *Picea* and *Pinus*. According to GOEBEL (6) the pollen grains of *Cycadales* and *Ginkgoales* discharge motile sperms from the proximal pole of the pollen grain, those of the *Coniferales* and *Gnetales* non-motile male nuclei at the distal pole. The fact that *Pseudotsuga* discharges non-motile male nuclei at the pole, which happens to be in contact with the nucellus top, also points to an intermediary position.

The former assumption that the pollen grains of *Pseudotsuga* germinate on the stigmatic flap has hitherto been considered a separating factor in the relationship *Larix — Pseudotsuga* (cf. 4c). The present investigation has, however, shown that the main discernible difference between the pollination mechanism of the two genera lies in the shape and size of the germinating pollen grains. It is possible that the formation of the pollen tubes is also somewhat different, but the important point is, that the pollen grains of both genera are caught on the stigmatic flap and later on transferred to the nucellus top where they germinate.

The Shedding of Pollen and Pollination

The shedding of pollen and pollination within the clones V. 659, V. 661 and V. 847 took place during the period April 29 — May 16, i. e. about one week later than in *Larix*. It was tried to force the male inflorescences, but with scant success; for this reason we had to abandon an attempt to find out whether pollen, brought into the female inflorescences before they are receptive, may remain fertile and perhaps get on the stigmatic flap when the latter opens. There is, however evidence that the answer will be in the affirmative (cf. below).

There seems to be marked differences as to the time of emergence of male and female inflorescences on different trees. ROHMEDER and SCHÖNBACH (12) state that the time of flowering and pollen dissemination depends inter alia on the environment and on the local conditions, but it is also to a high degree genetically conditioned; early flowering trees and trees flowering extremely late were found in the same stand. ORR-EWING (11a) found that on one of two trees located within a few hundred feets of each other, the female inflorescences had completely emerged about ten days before there was general pollen dissemination from the male inflorescences of the same tree. On the second tree, pollen dissemination and complete emergence of the female inflorescences occurred almost simultaneously, but both took place about a week after the pollen dissemination of the first mentioned tree had become general. ORR-EWING also found that the bagging not only accelerates the development of the female inflorescences, but also reduces the time from pollination to fertilization.

A detailed description of the pollination mechanism is given above, to which the reader is referred. As mentioned the pollen grains are carried by the wind to female inflorescences. If the inflorescences are receptive, the pollen grains are caught on the protuberances of the stigmatic flap of the ovule *(Fig. 58, 59)*. If the bracts are open but the stigmatic flap not receptive, the pollen grains my lie in a heap close to the stigmatic flap and are probably caught on the latter when it opens. In view of the fact that pollen grains found outside on the epidermis of the ovule 5—6 weeks after pollination were at the same stage of development as the pollen grains inside the canal, there seems no reason to doubt the viability of the pollen waiting a few days inside the cone for the stigmatic flaps to open. It is therefore important that female inflorescences are bagged very early before the bud scales rupture (cf. also [12] p. 229).

The pollen grains remain on the stigmatic flap or in the canal for 5—7 weeks. They are thereafter transferred to the nucellus top where they germinate (cf. p. 96).

Test of the Effectiveness of Controlled Pollination During the Resting Period

During the resting period the effectiveness of the pollinations may be tested by examining and counting the number of pollen grains embedded on the stigmatic flap or lying in the micropylar canal. The technique is the same as described in respect of *Larix* (cf. [2]).

The Most Favourable Time for Controlled Pollination

The most favourable time for controlled pollinations is the period when the highest possible number of ovules are receptive i. e. the stigmatic flaps are swollen 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 bagging, pollination and removal of the bags, but also for the utilization of the whole period of receptiveness for pollination. It is the aim of the present investigation to try to find simple criteria for these stages and thereby help the worker to decide when the inflorescenses are to be bagged, the pollination most effectively executed, and when it is safe to remove the bags.

The experiments were conducted along the same lines as described in respect of *Larix* (2).

Three clones of *Pseudotsuga Menziesii,* V. 659, V. 661 and V. 847 were used in the experiments; they were all derived from graftings in 1943 at the Hoersholm Arboretum of the Royal Veterinary and Agricultural College.

In each clone 50 baggings were made on April 23, 1960, each bag containing 2—4 female inflorescences.

A series of controlled pollinations were carried out on the three clones as soon as pollen was available and continued with intervals of 1—2 days during a period of 3 weeks.

At each pollination date an average of 3 bags per clone, i. e. 5—10 female inflorescences, were pollinated. A number of bags were left unpollinated as a control, none of them yielded viable seeds. Furthermore a couple of female inflorescences typical of the stage of receptiveness prevalent at the date and clone in question were fixed in 70% alcohol. The fixed inflorescences were photographed, dissected and examined. By this examination the size and position of the ovuliferous scales and bracts together with the condition of the stigmatic flaps were registered for each pollination date. Later the relationship between these particulars on one side and the germination percentage of seeds derived from pollination at the corresponding dates on the other side was determined. It follows as a matter of course that the pollinated and the examined inflorescences were not identical, and although inflorescences most typical of the pollination date in question were carefully selected for examination, complete accordance with the germination percentage cannot be expected. An example of this is seen *Fig. 43.* The shown inflorescence, which was fixed as typical, was covered by the inner bud scales, and its stigmatic flaps were not receptive. Nevertheless other inflorescences of the same clone and same date yielded 13% viable seeds. This discrepancy seems to be more marked in *Pseudotsuga* than in *Larix,* no doubt because even female inflorescences of *Pseudotsuga* situated on the same twig vary much more in size and development than do the inflorescences of *Larix.*

During the autumn the cones derived from the above mentioned pollinations were collected separately from each clone and for each pollination date. — After extraction the total weight of seeds derived from each pollination, the weight of 1000 seeds and the germination percentage were determined. The latter was determined after 6 weeks of stratification and 3 weeks germination on JACOBSEN's apparatus. The results are shown in *table 1.* The total weight of the seeds obtained and the weight of thousand seeds are shown together with the germination percentages. By means of the weight of thousand seeds, the number of seeds in each sample may be computed. The main cause of the much variable weight of thousand seeds is that in *Pseudotsuga,* like f. inst. in *Larix* and *Abies,* normal appearing but empty seeds are produced even if fertilization has not taken place. *Table 1* shows that the highest weight of thousand seeds in a clone coincides with the highest germination percentage, and that the lowest weight is found were the germination percentage is 0. Furthermore the weight of thousand seeds varies considerably from clone to clone.

Table 1. — Weight and germination test of seeds emanating from different pollination dates.

Dates of Pollination 1960	Clone no. V. 659			Clone no. V. 661			Clone no. 847		
	Weight of seeds g	Weight of 1000 seeds g	Germination percentage	Weight of seeds g	Weight of 1000 seeds g	Germination percentage	Weight of seeds g	Weight of 1000 seeds g	Germination percentage
April 27	1 36	4.56	6	0.25	9.01	64	3.41	7.16	14
April 29	0.80	4.10	13	1.85	9.13	53	4.30	7.79	17
May 1	1.35	5.58	30	2.45	6.23	13	3.91	9.11	32
May 3	1.70	7.60	63	1.83	8.62	53	2.57	6.23	17
May 6	1.74	5.80	34	2.56	6.94	29	3.08	5.72	26
May 9	0.48	4.62	0	1.04	6.08	0	2.75	6.32	0
May 11	0.88	3.85	0	1.48	6.09	0	2.43	6.97	0
May 14	1.57	4.05	0	1.37	6.60	0	1.71	6.38	0
May 16	—	—	—	0.12	4.77	0	1.21	6.21	0
May 18	—	—	—	0.32	5.68	0	0.53	4.75	0

On account of difficulties in procuring the necessary amount of pollen it proved impossible to start pollination early enough to cover the beginning of the period of receptiveness. This is particularly conspicuous as regards clone V. 661 which shows a germination percentage of 64 on April 27, the date when the first pollination took place. As the inflorescences in clone V. 661 had already emerged 2—3 mm on April 23 when the bagging was done *(Table 2),* the beginning of the receptive period in this clone could not be determined, and the possibility of contamination cannot be

Table 2. — Relationship between the development of male and female inflorescences of *Pseudotsuga Menziesii.*

Dates of pollination 1960 (*cf. Table 1*)	Clone V. 659			Clone V. 661			Clone V. 847		
	Pollen dissemination	Length in mm of exposed part of female inflorescenses	Germination percentage (*cf. Table 1*)	Pollen dissemination	Length in mm of exposed part of female inflorescences	Germination percentage (*cf. Table 1*)	Pollen dissemination	Length in mm of exposed part of female inflorescences	Germination percentage (*cf. Table 1*)
April 23		0		÷	2—3		÷	0	
April 27	÷	2—3	6	÷	4—5	64	÷	0	14
April 29	÷	1—2	13	+	7—8	53	÷	1—2	17
May 1	+	5—6	30	+	10—11	13	÷	5—6	32
May 3	++	10—11	63	++	15—16	53	+	10—11	17
May 6	++	17—18	34	++	22—23	29	++	16—17	26
May 9	++	28—29	0	++	30—33	0	++	25—26	0
May 11	++	28—29	0	++	30—31	0	++	26—27	0
May 14	+	30	0	+	32—33	0	+	32—33	0
May 16	+		0	+	36—37	0	+	33—34	0
May 18	÷		0	÷	36—37	0	÷	33—34	0

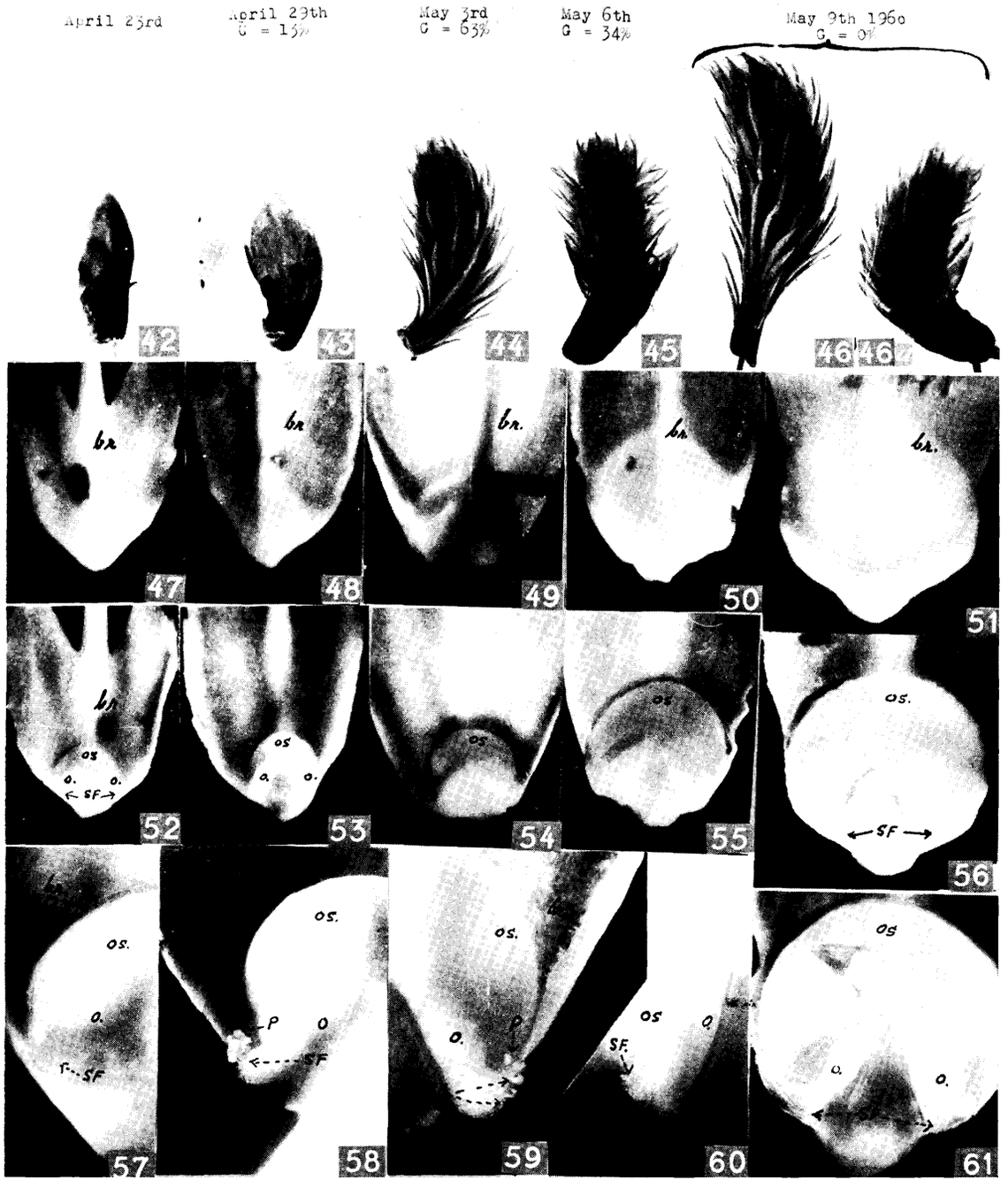

Fig. 42—61. — Criteria for receptiveness in *Pseudotsuga Menziesii* (expl. see text). — *Figs. 42—46 a.* — Female inflorescences of clone V. 659; fixed on dates heading photos. Under each date is shown the germination percentages of seeds originating in the corresponding experimental pollination on date in question. — *Fig. 42.* — Inflorescence not open; — *Fig. 43.* — outer bud scales thrown off, inner scales intact (cf. text); — *Figs. 44, 45.* — inflorescence receptive; — *Fig. 46.* — stigmatic flaps closed; — *Fig. 46 a.* — fixed same date as *Fig. 46*, part of stigmatic flaps receptive, part collapsing. — *Figs. 47—51.* — Abaxial view of basal part of bract from inflorescence shown in *Figs. 42—46* (*br* in *Fig. 51* is from inflorescence shown in *Fig. 46*), note the change in shape from inverted heartshaped in *Fig. 47* to roughly rectangular in *Fig. 51.* — *Figs. 52—56.* — Adaxial view of bracts shown in *Figs. 47—51;* the area of the ovuliferous scale in *Fig. 56* has increased to about 6—7 times the area in *Fig. 52.* — *Fig. 57.* — Part of bract and ovuliferous scale with left stigmatic flap *(SF); SF* not receptive; fixed April 23. — *Fig. 58.* — Part of bract and ovuliferous scale with pollen on left *SF*, receptive; fixed May 1. — *Fig. 59.* — Part of bract and ovuliferous scale with pollen on right *SF*, receptive; fixed May 3. — *Fig. 60.* — Collapsing *SF*, not receptive; fixed May 9. — *Fig. 61.* — Ovuliferous scale with two ovules and collapsed stigmatic flaps, not receptive; fixed May 11. — *Figs. 42—46;* × ca. 1; — *Figs. 47—56, 61;* × ca. 7; — *Figs. 57—60;* × ca. 18. —

Abbreviations: br bract; *G* germination percentage; *infl* inflorescence(s); *o* ovule; *os* ovuliferous scale; *P* pollen; *SF* stigmatic flap. —

entirely excluded; the control bags, however, yielded no viable seeds.

As criteria for the different stages of receptiveness, the position, size and shape of bracts and ovuliferous scales are of course, together with the condition of the stigmatic flap, of special interest. — These characteristics were carefully examined on inflorescences from the three clones under investigation. *Figs. 42—61* show the details for some of the pollination dates of clone V. 659. In the heading pollination dates and germination percentages are shown. Under each date is shown 1) a photograph of the inflorescence(s) fixed on the date in question: *Figs. 42—46;* 2) abaxial view of a bract from each of these inflorescences: *(Figs. 47—51);* 3) adaxial view of the same bracts with ovuliferous scale: *Figs. 52—56. Figs. 57—61* show photographs of stigmatic flaps on different dates and at different stages of receptiveness.

A comparison of the position, size and shape of bracts, ovuliferous scales and of the condition of the stigmatic flap on one side with germination percentages for each pollination date *(Table 1)* on the other side gave the following results as regards clone V. 659:

On April 23 the fixed inflorescence was closed, the stigmatic flaps not receptive and according to *table 2* no inflorescences with ruptured bud scales were found. The shape of the bracts was inverted heart shaped *(Fig. 47* and *52).* On this date the inflorescenses were not receptive.

On April 27—29 part of the bud scales had ruptured (the inflorescence fixed April 29, *Fig. 43,* had still the inner bud scales intact). The stigmatic flaps of the fixed inflorescence seemed not receptive but loose pollen was often found near the stigmatic flaps. As the pollinated inflorescences yielded respectively 6 and 13% viable seeds, the beginning of the receptive period was probably about April 25—26.

On May 1—3 the bracts were free *(Fig. 44).* The breadth of the basal part of the bracts and the size of the ovuliferous scales were slowly increasing *(Fig. 49* and *54).* The stigmatic flaps were receptive *(Fig. 58—59).* The germination percentages were 30 and 63 respectively, and the receptiveness reached maximum.

On May 6 the exterior of the inflorescences was practically unaltered *(Fig. 45).* The breadth of the lower part of the bracts and the size of the ovuliferous scales had increased considerably *(Fig. 50* and *55).* Some of the stigmatic flaps were still receptive, others were beginning to collapse. The germination percentage was 34, i. e. decreasing.

On May 9 the exterior of the inflorescences was still practically unchanged *(Figs. 46, 46a).* The breadth of the lower part of the bracts and the size of the ovuliferous scales had increased, the basal part of the bracts was now almost rectangular, and the size of the ovuliferous scales seven times the size on April 23rd *(Figs. 51* and *56; 47* and *52).* The stigmatic flaps had collapsed and were not receptive. Although single stigmatic flaps (perhaps at the boundaries of the sterile zone at the top and bottom of the inflorescences) may still be receptive, the receptiveness terminated practically sometimes between May 6 and May 9. — Consequently *the receptive period was approximately April 25—26 to May 7—8, i. e. 12—14 days, with maximum receptiveness about April 30 to May 4—5, i. e. 5—6 days.*

The duration of the period of receptiveness in clones V. 661 and V. 847 were approximately the same as that found in V. 659 and quoted above.

As to the *position of the bracts,* there is, after the rupture of the bud scales and apart from a certain increase in the length of the inflorescences, not much change during the period of receptiveness and the first week thereafter. The bracts are no doubt more spread during the receptive period than after, but the difference is too small to be of practical value as a criterion.

The beginning of the receptive period is easily discernible as it coincides with the rupture and casting off of the bud scales, which is, of course important. The beginning of the receptive period is further characterized by the basal part of the bracts being of inverted heartshape and by the receptiveness of the stigmatic flap *(Figs. 58—59). — Fig. 57* shows a stigmatic flap before the beginning of the receptive period. The flap was not yet stigmatic, and in the same inflorescence small heaps of pollen grains were often found in the corner formed by the flap and the edge of the bract. It seems probable that these pollen grains will be caught by the stigmatic protuberances when the flap becomes receptive.

As to the end of the receptive period the only reliable criteria found are the almost rectangular shape of the basal part of the bracts and the collapse of the stigmatic flaps, and the use of these characteristics is by no means as difficult as it may seem (cf. later in text). The reason why there is no immediate change in the spreading of the bracts seems to be, that the closing of the inflorescences after the period of receptiveness is caused more by the tremendous growth of the ovuliferous scales than by movement of the bracts. The closing of the inflorescences and the collapse of the stigmatic flaps occur irrespective of whether pollination has taken place or not. — *Fig. 60* shows a collapsing, *Fig. 61* collapsed stigmatic flaps.

The ovules and stigmatic flaps of *Pseudotsuga* are, apart from certain minor differences (cf. 4b, p. 199), almost identical with those of *Larix,* but when they are receptive the stigmatic flaps do not as a rule protrude outside the bracts as do the stigmatic flaps of *Larix,* in which this protrusion is a valuable criterion for receptiveness.

As a guidance to the execution of controlled pollinations in *Pseudotsuga,* the present experiments and other information available indicate:

1. That the receptiveness of the inflorescences of different trees may occur at different times. It is therefore necessary to keep each tree under close observations and follow the development of its inflorescences.
2. The inflorescences must be bagged early before the bud scales rupture.
3. The beginning of the receptive period is characterized by rupture of the bud scales covering the inflorescence, spreading of the bracts and by the opening of the stigmatic flaps. All stigmatic flaps of an inflorescence do not open simultaneously, but it is probable that pollen arriving at a flap before it opens may later be caught on the protuberances. The bracts at this stage are inverted heart shaped *(Figs. 47* and *52).*
4. During the middle and most receptive part of the period, which lasted 5—6 days, most of the stigmatic flaps were open. During this period the lower part of the bracts becomes more rectangular and the size of the ovuliferous scales is increasing *(Figs. 49* and *54).*
5. At the end of the receptive period the basal part of the bracts is roughly rectangular and the ovuliferous scales have increased in size till about seven time their size at the beginning of the period. The stigmatic flaps are

collapsing or have collapsed, *(Figs. 60—61)*, and no viable seeds were obtained after this stage. The end of the period of receptiveness occurred about 12—14 days after the beginning of the period.

6. The first pollination should be executed as soon as the bud scales start to rupture and pollination must be repeated as often as possible until the inflorescences close.
7. In order to follow the development of bracts, ovuliferous scales and stigmatic flaps it is recommended to examine a couple of female inflorescences from each tree at each pollination date. From the middle of each inflorescence a couple of bracts with ovuliferous scales are dissected out, fixed in 70% alcohol and stored in a glass marked with tree number and date. By comparison of the bracts, scales and stigmatic flaps fixed on the different dates it is easy to follow the development, especially if a preparation microscope is available. The condition of the stigmatic flap may be examined with the preparation microscope (as the fresh protuberances dry and shrink quickly the examination is best carried out in 70% alcohol). Dry pollen may be dusted on unfixed stigmatic flaps and if pollen grains stick to the protuberances, the flap is receptive. These tests are most conveniently carried out without removing the stigmatic flaps from the ovuliferous scales.

It appears from the above that *the beginning* of the receptive period coincides with the rupture of the bud scales of the female inflorescences, and the determination of this stage therefore presents no difficulties.

The end of the period is not marked by any noticeable change in the exterior of the female inflorescenses. It is characterized by the collapse of the stigmatic flaps, by the increase in volume of the ovuliferous scale, and by the shape of the basal part of the bracts, which is now more or less rectangular.

Frequent, if possible daily, pollinations are particularly important in *Pseudotsuga* because the rupture of the bud scales of the inflorescences does not take place simultaneously, and the optimum of receptiveness of all ovules is not reached at the same time.

ROHMEDER and SCHÖNBACH (12, p. 232) emphasize the necessity of protecting the cones against birds, rodents and — particularly — against the wasp Megastigmus. During flowering the isolation bags offer sufficient protection, but when they are removed, they must *immediately* be replaced by loose woven cotton bags thoroughly impregnated with an insecticide. This is important advice; especially in bad or mediocre crop years the hungry Megastigmus may, without such precautions, not leave a single seed intact.

The relationship between the development of the female and male inflorescences: During the pollination period the progress of pollen dissemination in the three clones was registered together with the stage of receptiveness of the female inflorescences as determined by the germination percentages obtained. — The results are shown in *table 2.* — For each clone the pollen dissemination is denoted as follows: - = no dissemination. + = slight dissemination. ++ = full dissemination.

In respect of the female inflorescences the lengths in mm of the part of the inflorescence which was free of the bud scales is shown. The table shows that in all three clones full pollen dissemination did not occur untill the receptiveness of the female inflorescences had culminated, and that the dissemination continued after the closing of the female inflorescences.

The dissemination continued about 13—16 days, which is somewhat longer than the period of receptiveness of the female inflorescences. The results show a clear tendency to protogyny. But as well known the climatic conditions may influence the issue, and there is normally considerable individual variation of this character.

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Summary

A brief description is given of the development of the male and female inflorescences of *Pseudotsuga Menziesii* var. *viridis*, from the formation of buds in the year preceding the flowering year till fertilization. The most important stages are illustrated by photos. An idiogram of the chromosomes of Pseudotsuga is shown. The four shortest chromosomes are telocentric. Irregularities of meiosis and mitosis were found. The structure of the pollen grains and the development of the male gametophyte is described. A pore in the intine at the proximal pole of the pollen grain is described, and it is shown that the pollen grains elongate to a length of about five times their original diameter and that they germinate, not on the stigmatic flap as hitherto assumed, but on the nucellus top as in *Larix*. It is suggested that the elongation of the pollen grains and the rupture of the exine is a purely mechanical process due to uptake of water and of no value as a criterion of viability. Actual germination of the pollen *in vitro* proved unattainable.

Attention is drawn to the resemblance between the elongated grains of *Pseudotsuga* and those of certain *Cycads*, and it is suggested that the finding of the pollination mechanism described removes a hitherto separating factor in the relationship *Larix* — *Pseudotsuga*.

Experiments with controlled pollinations to find reliable criteria for receptiveness of the female inflorescences are described. On account of the variation in the development of the inflorescences not only of different trees but of the same tree, it is much more difficult to find the most favourable time for pollination in *Pseudotsuga* than in *Larix*. It was found that the beginning of the receptive period coincides with the rupture of the bud scales and that the end of the period can be determined by the shape of the bracts and the conditions of the stigmatic flaps. — Details as to bagging, pollination and observation of the development of the inflorescences are given. It is particularly emphasized that the inflorescences should be pollinated as often as possible, preferably daily, during the receptive period.

Zusammenfassung

Titel der Arbeit: *Pollenbildung, Bestäubungsmechanismus und Feststellung der günstigsten Zeit für die kontrollierte Bestäubung bei Pseudotsuga Menziesii.*

Die Entwicklung der männlichen und weiblichen Infloreszenzen von *Pseudotsuga Menziesii* var. *viridis* von der Knospenbildung im Jahr vor der Blüte bis zur Befruchtung wird kurz beschrieben. Die wichtigsten Stadien werden mit Fotos erläutert. Es wird ferner ein Idiogramm der Chromosomen von *Pseudotsuga* gezeigt. Die vier kürzesten Chromosomen sind telozentrisch. Unregelmäßigkeiten wurden

in der Meiosis und in der Mitose gefunden. Die Struktur der Pollenkörner und die Entwicklung des männlichen Gametophyten werden beschrieben. Es wird weiter ein Porus in der Intine am proximalen Pol des Pollenkornes beschrieben und gezeigt, daß die Pollenkörner sich zu einer Größe von etwa 5mal ihrer Originaldurchmesser verlängern und daß sie nicht am Narbenläppchen, wie sonst angenommen, sondern an der Nuzellusspitze, wie bei *Larix,* keimen. Es wird vermutet, daß die Verlängerung der Pollenkörner und der Bruch der Exine nur rein mechanische Prozesse sind, die durch die Wasseraufnahme verursacht werden und keinen Wert als Kriterium der Lebensfähigkeit besitzen. Die tatsächliche Pollenkeimung *in vitro* war nicht erreichbar.

Es wird ferner auf die Ähnlichkeit zwischen der Verlängerung der Pollenkörner von *Pseudotsuga* und der von gewissen *Cycadinae* aufmerksam gemacht. Es wird vermutet, daß der Befund des beschriebenen Bestäubungsmechanismus einen bisher vorhandenen Trennungsfaktor in der Verwandtschaft *Larix — Pseudotsuga* beseitigt.

Experimente mit kontrollierten Bestäubungen zur Auffindung zuverlässiger Kriteria über die Empfängnisbereitschaft weiblicher Infloreszenzen werden beschrieben. Infolge der Variation in der Entwicklung der Infloreszenzen nicht nur verschiedener Bäume, sondern sogar beim gleichen Baum, ist es bei *Pseudotsuga* viel schwieriger, den günstigsten Zeitpunkt für die Pollination zu finden, als das bei *Larix* der Fall ist. Es wurde erkannt, daß der Beginn der Empfängnisperiode mit dem Aufbrechen der Knospenschuppen übereinstimmt und daß das Ende der Periode durch die Brakteenform und den Zustand der Narbenläppchen ermittelt werden kann. — Einzelheiten über Eintüten, Bestäubung, sowie Beobachtungen über die Infloreszenzenentwicklung werden mitgeteilt. Es wird dabei besonders betont, daß die Infloreszenzen so oft wie möglich, am besten täglich, während der Empfängnisperiode bestäubt werden sollten.

Résumé

Titre de l'article: *Formation du pollen, mécanisme de pollinisation et détermination de l'époque la plus favorable pour les pollinisations contrôlées chez le Douglas.*

On donne une brève description du développement des inflorescences mâles femelles de *Pseudotsuga menziesii* var *viridis,* depuis la formation des bourgeons l'année avant la floraison jusqu'à la fertilisation. Les stades les plus importants sont illustrés par des photographies, et les chromosomes du douglas représentés par un diagramme. Les quatre chromosomes les plus courts sont télocentriques. On a observé des méioses et des mitoses irrégulières. On décrit la structure des grains de pollen et le développement du gamétophyte mâle. On a observé un pore dans l'intine au pôle proximal du grain de pollen, et on a montré que les grains de pollens s'allongent jusqu'à cinq fois leur diamètre initial et qu'ils germent non sur le stigmate comme on le croyait jusqu'ici, mais sur le sommet du nucelle comme chez les mélèzes. On pense que l'élongation des grains de pollen et la rupture de l'exine sont des processus purement mécaniques dus à l'absorption d'eau et sans valeur comme critères de viabilité. Il n'est pas possible d'obtenir *in vitro* une véritable germination du pollen.

On attire l'attention sur la ressemblance entre les grains de pollen de douglas après l'élongation et ceux de certains *Cycas* et on pense que la découverte du mécanisme de pollination décrit ici lève une objection à la parenté *Larix-Pseudotsuga.*

On décrit des expériences de pollinisation contrôlées destinées à trouver des critères valables pour la réceptivité des fleurs femelles. Tenant compte de la variation dans le développement des inflorescences, non seulement entre arbres différents mais sur le même arbre, il est beaucoup plus difficile pour le douglas que pour le mélèze de déterminer l'époque favorable à la pollinisation. Le début de la période de receptivité coïncide avec la rupture des écailles du bourgeon et la fin de la période peut être déduite de la forme des bractées et de l'état des stigmates. On donne des détails sur l'ensachage, la pollinisation et l'observation du développement des inflorescences. On montre en particulier que les inflorescences doivant être pollinisées aussi souvent que possible, de préférence tous les jours, pendant la période de réceptivité.

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